Active Kallikrein Response to Changes in Sodium-Chloride Intake in Essential Hypertensive Patients

Claudio Ferr, Cesare Bellini, Antonio Carlonagno, Giovambattista Desideri, and Anna Santucci

ABSTRACT

To evaluate the behavior of active kallikrein excretion in salt-sensitive and salt-resistant hypertensive patients during changes in sodium-chloride (NaCl) intake, 61 male, nonobese, nondiabetic outpatients affected by uncomplicated essential hypertension were given a diet that contained 140 mmol NaCl per day for 2 wk. Patients then received either a low- (20 mmol NaCl/day) or a high- (320 mmol NaCl/day) sodium diet for 2 wk, according to a randomized, double-blind, cross-over protocol. Hypertensive patients were classified as salt sensitive when their diastolic blood pressure rose by at least 10 mm Hg after the high-sodium diet, and decreased by at least 10 mm Hg after the low-sodium diet, considering as baseline blood pressure values those that were taken at the end of the 140 mmol NaCl/day intake period. The remaining patients were classified as salt resistant or, when diastolic blood pressure increased by 10 mm Hg or more after low-sodium intake, as counterregulating. Twenty-three patients were therefore classified as salt sensitive, 28 as salt resistant, and 10 as counterregulating. The baseline active kallikrein excretion was significantly lower (P < 0.0001) in salt-sensitive (0.62 ± 0.31 U/24 h) patients than in salt-resistant (1.39 ± 0.44 U/24 h) and counterregulating patients (1.27 ± 0.38 U/24 h). Surprisingly, the kallikrein response to changes in sodium intake was similar in all subgroups, although enzyme excretion was always at the lowest level in salt-sensitive hypertensive patients. This latter group also showed the highest plasma atrial natriuretic peptide levels (28.2 ± 8.5 fmol/mL, P < 0.0001 versus salt-resistant and counterregulating patients), and the greatest peptide increment with sodium load (P < 0.0001 versus salt-resistant and counterregulating patients). Counterregulating patients showed the steepest increase in plasma renin activity (from 0.24 ± 0.18 to 0.83 ± 0.21 ng/L per s, P < 0.001) and decrease of plasma atrial natriuretic peptide (from 26.1 ± 6.3 to 6.8 ± 3.1 fmol/mL, P < 0.001) when switched from a high to a low-sodium intake. In conclusion, salt-sensitive hypertensive patients excrete less active kallikrein than do salt-resistant and counterregulating patients, but maintain a normal enzyme response to changes in dietary sodium intake. The exaggerated response of atrial natriuretic peptide to high-sodium intake that was observed in the same patients could be compensating for an impaired renal capability to excrete a sodium load.

Key Words: Sodium, hypertension, renin, atrial natriuretic peptides, kidney

Salt sensitivity is defined as a significant rise in blood pressure when individuals switch from a low- to a high-sodium (NaCl) intake and/or vice versa (1–6). The frequency of salt sensitivity is strongly influenced by age (2–4,7–9), body weight (2,3,8–13), race (2,3,8,9,14–16), gender (6,17), and family history of hypertension (2–4,18).

In middle-aged Caucasian populations, approximately 50% of hypertensive patients can be included within the salt-sensitive subgroup (1–3,5,6,17,19). In the remaining patients, blood pressure does not change or may even increase during low-sodium intake (1–3,20–22), making the potential efficacy of health care programs that are directed toward indiscriminate sodium restriction debatable (2,3,21–26). As a consequence, a number of studies attempted to define genetic (8,27), familial (8), racial (8,9,14–16), environmental (28), renal (14,29,30), endocrine (19,27,31–33), and cell-membrane (34–36) characteristics related to salt sensitivity, and to indicate one or more parameters that would be able to predict the individual blood-pressure response to sodium.

In this regard, the work of Bönner et al. with normotensive patients (37,38) and our group’s work with hypertensive patients (39) have recently suggested that a low-kallikrein excretion might determine the salt sensitivity of blood pressure. In particular, we demonstrated that a reduced kallikrein excretion at baseline was present in 84% of salt-sensitive and 22% of salt-resistant patients (39). Therefore, we suggested an inability of the kidney to modulate kallikrein production during changes in sodium intake as the cause for sodium retention and the consequent blood-pressure rise (39).
Although fascinating, this theory was merely speculative, because we did not evaluate the kallikrein response to changes in dietary sodium intake (39). Furthermore, we excluded from the study the so-called "counterregulating hypertensives," i.e., those patients whose blood-pressure levels increase with sodium restriction (and/or decrease with sodium load) (20-22,39-44). This fact limited the pathogenetic as well as therapeutic implications of our findings. Indeed, counterregulating hypertensive patients are those patients who could report a potential increment of cardiovascular risk after severe sodium restriction (3,20-22).

Herewith we present a randomized double-blind cross-over study that aims to investigate the behavior of urinary active kallikrein during changes in dietary sodium-chloride intake in a large number of nonobese, nondiabetic hypertensive men, classified according to individual blood-pressure response to changes in sodium intake as salt-sensitive, salt-resistant or counterregulating hypertensive patients. In this study, we also analyzed the relationship between kallikrein and other sodium-related hormonal changes. Because reproducibility is a crucial point in salt-sensitivity evaluation (3,6,18,45), and the entire existence of salt sensitivity has been attributed to artifacts and/or casual variations of blood pressure (2,6,45), we also tested the reproducibility of our procedure 6 months after the end of the study.

PATIENTS AND METHODS

Patients

The study was approved by the Ethics Committee of the Andrea Cesalpino Foundation and started in 79 never-treated outpatients who had a body mass index > 19 and < 27 kg/m². Patients were between the ages of 30 to 55 years (mean 47.8 ± 6.3 yr), and were affected by uncomplicated essential hypertension. All patients were white Caucasians. Because the menstrual cycle strongly influences the behavior of several of the hormones that are involved in renal sodium handling (46), and because urine collection for kallikrein and electrolyte determinations is not feasible in menstruating women, only male patients were recruited.

In all patients, diastolic blood pressure (DBP) was required to be >95 mm Hg and <114 mm Hg at three consecutive visits performed at 1-wk intervals before definitive enrollment in the study (Figure 1). Serum creatinine concentration was <100 μmol/L; proteinuria was absent, microalbuminuria was <20 μg/min on two consecutive 24-h urine collections, 121-I-Orthodihypurate scintrenographies were normal. All patients had a normal glucose tolerance, as evaluated by oral glucose tolerance tests (75 g D-glucose), and had no cardiovascular alterations as evaluated on the basis of clinical and ultrasound studies. The secondary forms of hypertension were excluded by clinical and laboratory assessments.

At the first of the three screening visits, patients answered standard questions concerning family history of hypertension, according to a previously described methodology (39,47). In brief, family information was first obtained by each patient and then confirmed by the family physician and, when possible, by other patient's relatives (wife, brethren, children, etc.). A positive family history was defined as the presence of at least one first-degree relative who was affected by essential hypertension. To avoid bias, the family history information was collected by a separate group of researchers, who were unaware of the study purpose and results.

During the recruitment period, all patients were on a constant sodium intake (140 mmol NaCl per day). Each patient received a scheme of a diet prepared by our dietitians, and containing approximately 1 g/kg protein, 2.5 g/kg carbohydrates, 0.7 g/kg fat, 35 mmol calcium, 20 mmol NaCl, and 70 mmol potassium per day. A total amount of 120 mmol NaCl was added to the diet by means of six capsules per day (each capsule containing 20 mmol of NaCl), to be taken orally. Each patient was given oral and written instructions and detailed information on the way to cook their meals without added salt. To simulate as closely as possible the absorption of salt with food, according to the Italian habit, patients were advised to take three capsules at lunch and three at dinner.

The total number of calories remained identical during the entire study period and was calculated on the basis of individual needs. All patients were advised to drink 1.5 L water/day. Adherence to the diet was assessed by 24-h urine collections on the last two consecutive days of each week. Participants were instructed by our medical staff on how to collect the 24-h urine sample. Sodium-chloride excretion was required to be between 100 mmol/24 h and 140 mmol/24 h. On these same occasions, potassium and calcium excretions were also measured. In particular, urinary potassium was required to be between 50 and 70 mmol/24 h to allow patients' continued participation in the study.
Of the initial cohort of 79 patients, 14 did not enter the salt-sensitivity assessment phase because of noncompliance with the diet (N = 7), DBP < 95 mm Hg (N = 4), or > 114 mm Hg (N = 1) requirements, and because of lack of return to follow-up visits (N = 2). Therefore, only 65 patients were definitively enrolled in the following phase of the study.

Salt-Sensitivity Assessment Phase. At the end of the recruitment phase, blood samples for plasma renin activity (PRA), aldosterone (PAC), atrial natriuretic peptide (ANP), and digoxin-like substance (DLS) levels were taken after 1 h at rest in a supine position. Two consecutive 24-h urine collections were requested from each patient, to determine the urinary active kallikrein excretion. After blood and urine samples were taken, patients were assigned in a random double-blind manner to a high- (320 mmol NaCl per day for 2 wk) or a low- (20 mmol NaCl per day for 2 wk) sodium intake (Figure 1). Thirty-two patients received first the high and then the low-sodium diet. The remaining patients (N = 33) followed the reverse order. To have a similar baseline diet before switching to low or high-sodium intake, a period of 2 wk on a diet that contained 140 mmol NaCl per day was interposed during the cross-over phase (Figure 1).

Individual sodium intake was obtained by changing the NaCl content of the capsules. During the high-sodium intake period, each capsule contained 50 mmol NaCl. During the low-sodium intake period, each capsule contained a placebo (dextrose). Throughout the study, potassium intake remained unvaried (70 mmol potassium per day).

During the evaluation of salt sensitivity, compliance was also controlled by measuring 24-h sodium excretion levels. Patients were considered compliant when their 24-h sodium excretion level was > 270 mmol and < 40 mmol in all urine collections obtained during the high- and low-sodium intake periods, respectively. Urinary potassium levels were required to be between 50 mmol/24 h and 70 mmol/24 h. Four additional patients were noncompliant, all during the high-sodium diet phase, and the salt-sensitivity assessment phase was therefore successfully completed in 61 patients.

Patient Classification
Patients were classified as salt sensitive when a change of 10 mm Hg or more of DBP occurred after each period of either low- or high-sodium intake (i.e., blood pressure decreases during the low-sodium intake period, and increases during the high-sodium intake period) (39). Changes in blood pressure were evaluated with baseline blood pressure considered to be the measurement taken at the end of the 140-mmol NaCl diet. In contrast to our previous report (39), patients who had an increase in blood pressure with sodium restriction were not excluded from the study, and were admitted to a third group of patients, i.e., "counterregulators."

Reproducibility
The assessment of reproducibility of the above procedure was scheduled for 32 patients, who were randomly chosen among those patients who initially participated in the study. In all cases, patients were recalled after 6 months from the last visit of the salt-sensitivity assessment phase. The reproducibility evaluation was not feasible in six patients (four for loss at follow-up, two for compliance problems during the changes in sodium intake), although it was successfully performed in the remaining ones (N = 26).

Blood Pressure and Laboratory Evaluations
Throughout the study, blood-pressure levels were measured on the last two days of each week in the Outpatient Unit of the Institute of I Clinica Medica, after 15 min in a seated position in an air-conditioned room (22 to 24°C), by a standard Riva-Rocci sphygmomanometer (Zenith, Rome, Italy), and a stethoscope placed over the brachial artery. As recommended for nonobese adults (48), a normal-size cuff was used to encircle the nondominant arm, which was supported comfortably at heart level. Systolic blood pressure (SBP) was taken at Korotkoff's Phase I, and DBP was taken at Korotkoff's Phase V (48). The first measurement of blood pressure and heart rate was excluded, and the average of the following three measurements, taken at 3-min intervals, was considered. Blood pressure was always taken by the same researchers, who were unaware of the study purpose, design, and results.

After blood-pressure measurement, patients rested comfortably in bed for 1 h. Blood samples for PRA, PAC, ANP, DLS, serum Na⁺ and K⁺ were then withdrawn from an antecubital vein. Plasma samples were immediately stored at −80°C. All of the assays were performed no later than 8 days after storage. Plasma ANP was assayed as described elsewhere (47). In short, ANP was extracted from plasma by Amprep c-18 columns (Amersham, Amersham, Buckinghamshire, U.K.), activated with 4 mL methanol and 12 mL distilled water. Each column was loaded with 2.5 mL acidified plasma. The eluate was collected in 1 mL ethyl alcohol, dried under vacuum, reconstituted in 1 mL neutral phosphate buffer, and then measured by a commercially available human-ANP (99-126) RIA kit (Peninsula Laboratories, Belmont, CA). Synthetic human-ANP (99-126) was used as a standard. Mean recovery for ANP in this study was 80%. Interassay and intra-assay variations were less than 10%.

PRA and PAC were assayed by commercially available RIA kits (Sorin Biomedica S.p.A., Vercelli, Italy). Plasma DLS was measured by the method of Balzan et al. (49) with slight modifications. In brief, plasma samples were purified as follows: Amprep c-18 columns (Amersham) were activated with 5 mL methanol and 20 mL distilled water. Each column was loaded with 2.5 mL plasma, washed with 20 mL distilled water, and then eluted with 2 mL methanol. The eluates of four 2.5-mL aliquots of plasma were collected, dried under vacuum, and reconstituted in 1 mL of a buffer solution (NaCl 130 mmol/L, sucrose 20 mmol/L, glucose 10 mmol/L, Tris-HCl 10 mmol/L, pH 7.4). For DLS radioimmunoassay, a solid-phase system was used (Diagnostic Products Corporation, Los Angeles, CA). Digoxin (Wellcome, Pomezia, Italy) was used as a standard. The other laboratory measurements were performed by standard laboratory methods.

The 24-h urinary excretion level of active kallikrein was measured on fresh urine samples by hydrolysis of the chroomagen tryptide substrate H-o-V-L-R-pNA (S-2266; KabiVitrum, Mölndal, Sweden) according to the method of Amundsen et al. (50).

Statistical Analyses
The statistical evaluation was performed by a PC Olivetti M-380×P1 (Olivetti, Ivrea, Italy). The statistical software SPSS (SPSS Inc., Chicago, IL) was used. To evaluate intra-group statistical significances, we used the paired t test and analysis of variance for repeated measures. To establish the differences among groups, we used unpaired t test and one-way analysis of variance followed by post hoc analysis (Bonferroni's test). Linear regression and correlation were used to evaluate the relationship between two variables. Descriptive parameters were analyzed by the chi-squared method.
The results of each statistical test were considered significant when the associated probability value (P) was <0.05. Unless otherwise stated, data are given as mean ± SD.

RESULTS

General Characteristics

The general characteristics of the study population are given in Table 1.

When all patients were considered (N = 61; mean age, 47.1 ± 2.9 yr), baseline SBP and DBP were 160.7 ± 5.4 mm Hg and 104.8 ± 2.9 mm Hg, respectively. Urinary Na\(^+\) excretion was 123.9 ± 14.6 mmol/24 h, whereas urinary K\(^-\) excretion was 53.1 ± 9.5 mmol/24 h (mean urinary Na/K ratio, 2.3 ± 0.6). PRA and PAC levels were 0.40 ± 0.18 ng/L per s and 302.9 ± 52.3 pmol/L, respectively. Plasma ANP and DLS levels were 21.2 ± 9.1 fmol/mL and 34.2 ± 10.5 pg/mL, respectively. Urinary active kallikrein excretion was 1.09 ± 0.37 U/24 h and were negatively correlated with plasma ANP levels (r = -0.677, P < 0.0005).

Changes in dietary sodium intake induced minor changes in SBP and DBP levels (Table 2), although the investigated hormones showed significant variations during the different diets. In particular, PRA, PAC and urinary kallikrein levels significantly increased with sodium restriction and decreased with sodium load (Table 2). The opposite behavior was demonstrated for circulating ANP and DLS levels (Table 2).

At the end of the salt-sensitivity evaluation, 23 (37.7%) men from a total of 61 hypertensive men, were classified as salt sensitive, 28 (45.9%) as salt resistant, and 10 (16.4%) as counterregulating. Their general characteristics are given in Table 1. As shown in Table 1, salt-sensitive patients had higher fasting insulin (P < 0.05) and triglyceride (P < 0.05) levels than did salt-resistant and counterregulating patients. Counter-regulating patients were younger than salt-resistant and salt-sensitive patients (P < 0.05) (Table 1). The latter group showed higher baseline blood-pressure levels than the other two groups (P < 0.02) (Table 1). A positive history for hypertension occurred more frequently in salt-sensitive patients than in both of the remaining groups (P < 0.02) (Table 1).

Blood Pressure and Hormonal Data

Salt-Sensitive Group (N = 23 patients; mean age, 48.1 ± 3.1 yr). SBP was 163.7 ± 4.9 mm Hg at the end of the normal-sodium diet, increased to 171.8 ± 7.4 mm Hg (P < 0.0001) after the high-sodium diet and decreased to 158.7 ± 8.2 mm Hg (P < 0.0001) after the low-sodium diet (Figure 2). After the high-sodium intake, SBP was significantly higher than after the low-sodium intake (P < 0.0001) (Figure 2). DBP was 106.8 ± 2.7 mm Hg after the normal-sodium intake, increased to 116.3 ± 5.8 mm Hg (P < 0.0001) after the high-sodium diet, and decreased to 94.7 ± 4.5 mm Hg (P < 0.0001) after the low-sodium intake period (Figure 2). After the high-sodium intake period, DBP was significantly higher than after the low-sodium intake (P < 0.0001) (Figure 2).

Urinary Na\(^+\) excretion was 118.7 ± 12.8 mmol/24 h after the normal-sodium diet, 288.7 ± 10.7 mmol/24 h (P < 0.0001) after the high-sodium diet, and 25.3 ± 8.6 mmol/24 h (P < 0.0001) after the low-sodium diet. Body weight was 77.5 ± 2.4 kg after the normal-sodium diet, increased to 79.2 ± 2.3 kg after the high-sodium diet (P < 0.05), and decreased to 75.7 ± 2.1 kg at the end of the low-sodium intake period (P < 0.05 versus normal-sodium intake; P < 0.0005 versus high-sodium intake).

At the end of the normal-sodium intake phase, PRA levels were 0.32 ± 0.15 ng/L per s, PAC levels were

<table>
<thead>
<tr>
<th>TABLE 1. General characteristics of the study population (mean ± SD)</th>
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<tr>
<td>Characteristic</td>
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<tr>
<td>----------------</td>
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<tr>
<td>Number (yr)</td>
</tr>
<tr>
<td>Family history of hypertension (Yes/No)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
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<tr>
<td>Diastolic blood pressure (mm Hg)</td>
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<tr>
<td>Heart rate (bpm)</td>
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<tr>
<td>Body mass index (kg/m(^2))</td>
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<tr>
<td>Serum cholesterol (mmol/L)</td>
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<td>Serum triglycerides (mmol/L)</td>
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<tr>
<td>Plasma glucose (mmol/L)</td>
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<td>Plasma insulin (pmol/L)</td>
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<tr>
<td>Blood urea nitrogen (mmol/L)</td>
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<tr>
<td>Serum creatinine (µmol/L)</td>
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<td>Creatinine clearance (mL/s)</td>
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<tr>
<td>Serum sodium (mmol/L)</td>
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<td>Serum potassium (mmol/L)</td>
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</table>

\(^a\) P < 0.05 Counterregulating hypertensive patients versus salt-sensitive and salt-resistant hypertensive patients.
\(^b\) P < 0.02 Salt-sensitive hypertensive patients versus salt-resistant and counterregulating hypertensive patients.
\(^c\) P < 0.05 Salt-sensitive hypertensive patients versus salt-resistant and counterregulating hypertensive patients.
TABLE 2. Primary clinical and endocrine changes after different sodium intake periods in a group of 61 hypertensive patients (mean ± SD)\(^a\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal NaCl diet</th>
<th>Low NaCl diet</th>
<th>High NaCl diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>76.8 ± 4.5</td>
<td>75.6 ± 3.3(^b)</td>
<td>78.2 ± 4.1</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>160.7 ± 5.4</td>
<td>161.3 ± 8.5(^b)</td>
<td>168.7 ± 8.9(^c)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>104.8 ± 2.9(^b)</td>
<td>104.2 ± 5.6(^d)</td>
<td>107.7 ± 6.1</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>73.2 ± 5.1</td>
<td>73.5 ± 4.9</td>
<td>73.3 ± 4.9</td>
</tr>
<tr>
<td>Plasma renin activity (ng/L per s)</td>
<td>0.40 ± 0.18</td>
<td>0.62 ± 0.22(^c)</td>
<td>0.26 ± 0.15(^c)</td>
</tr>
<tr>
<td>Plasma aldosterone (pmol/L)</td>
<td>302.9 ± 52.3</td>
<td>306.3 ± 65.5(^d)</td>
<td>239.8 ± 73.6(^c)</td>
</tr>
<tr>
<td>Plasma ANP (fmol/mL)</td>
<td>21.2 ± 9.2</td>
<td>12.3 ± 4.9(^d)</td>
<td>29.3 ± 7.2(^d)</td>
</tr>
<tr>
<td>Plasma DLS (pg/mL)</td>
<td>34.2 ± 10.5</td>
<td>26.3 ± 8.7(^d)</td>
<td>42.7 ± 16.6(^b)</td>
</tr>
<tr>
<td>Urinary active kallikrein (U/24 h)</td>
<td>1.09 ± 0.37</td>
<td>1.40 ± 0.35(^d)</td>
<td>0.79 ± 0.22(^c)</td>
</tr>
<tr>
<td>Urinary Na(^+) excretion (mmol/24 h)</td>
<td>123.9 ± 14.6</td>
<td>26.9 ± 6.3(^d)</td>
<td>291.4 ± 8.7(^d)</td>
</tr>
<tr>
<td>Urinary K(^+) excretion (mmol/24 h)</td>
<td>53.1 ± 9.5</td>
<td>56.7 ± 8.9</td>
<td>52.3 ± 7.4(^d)</td>
</tr>
</tbody>
</table>

\(^a\) During the normal-sodium diet, patients assumed 140 mmol NaCl per day; during the low-sodium diet, patients assumed 20 mmol NaCl per day; and during the high-sodium diet, patients assumed 320 mmol NaCl per day. ANP, atrial natriuretic peptide; DLS, digoxin-like substance.

\(^b\) \(P < 0.05\) versus high-sodium diet period.

\(^c\) \(P < 0.0005\) versus normal-sodium diet period.

\(^d\) \(P < 0.0005\) versus high-sodium diet period.

278.3 ± 75.2 pmol/L, ANP levels were 28.2 ± 8.5 fmol/mL, and DLS levels were 45.2 ± 12.3 pg/mL. Urinary active kallikrein excretion resulted of 0.62 ± 0.31 U/24 h. The urinary excretion of active kallikrein and plasma ANP levels were inversely correlated (\(r = -0.615\), \(P < 0.002\)). Hormonal parameter changes that occurred with sodium restriction and sodium load are given in Figure 3.

Salt-Resistant Group (\(N = 28\) patients; mean age, 47.9 ± 2.5 yr). With regard to blood pressure, SBP after the normal-sodium intake was 159.6 ± 5.3 mm Hg and did not change after both the high- (162.3 ± 9.4 mm Hg, not significant [NS]) and the low-sodium diets (159.6 ± 9.7 mm Hg, NS) (Figure 2). DBP was 104.2 ± 3.1 mm Hg after the normal-sodium intake and was not significantly influenced by sodium intake variations (105.1 ± 5.3 mm Hg [NS] at the end of the high-sodium diet and 103.8 ± 5.8 mm Hg [NS] at the end of the low-sodium intake) (Figure 2). Body weight was 76.1 ± 3.8 kg after the normal-sodium diet, 77.2 ± 3.5 kg after the high-sodium diet (NS), and 75.2 ± 2.8 kg after the low-sodium diet (NS).

The urinary Na\(^+\) excretion level was 125.9 ± 13.7 mmol/24h after the normal-sodium diet, increased to 296.1 ± 12.5 mmol/24h after the high-sodium diet (\(P < 0.005\)), and decreased to 28.5 ± 7.7 mmol/24h after the low-sodium diet (\(P < 0.005\)). After the normal-sodium intake period, PRA levels were 0.41 ± 0.19 ng/L per s, PAC levels were 308.9 ± 88.7 pmol/L, and ANP levels were 18.6 ± 9.3 fmol/mL. Plasma DLS levels were 30.1 ± 8.9 pg/mL. The urinary active kallikrein excretion level was 1.39 ± 0.44 U/24 h. The hormonal parameter changes induced by different sodium diets are given in Figure 3.

Counterregulating Group (\(N = 10\) patients; mean age, 45.2 ± 3.2 yr). The behavior of these patients mimicked that of the salt-resistant patients. Interestingly, these patients showed the greatest rise in PRA after the low-sodium diet (Figure 3). Indeed, the mean percentage increment of PRA levels was 72.9% in these patients, whereas it was 25% in salt-sensitive (P.
Figure 3. Primary hormonal changes after normal (140 mmol/day), low (20 mmol/day), and high-sodium diets (320 mmol/day) in salt sensitive \( (N = 23; \text{open squares}) \), salt-resistant \( (N = 28; \text{filled squares}) \), and counterregulating hypertensive patients \( (N = 10; \text{diagonal-filled squares}) \). DLS, digoxin-like substance; ANP, atrial natriuretic peptide. Standard deviations were omitted for clarity. * \( P < 0.0001 \) versus normal-sodium diet, ** \( P < 0.0001 \) versus high-sodium diet. Intergroup significant differences are given on the lines in the figure.

< 0.001 versus counterregulating patients) and 53.6% in salt-resistant patients \( (P < 0.001 \) versus counterregulating patients; \( P < 0.01 \) versus salt-sensitive patients) (Figure 4). Counterregulating patients also showed the greatest ANP decrease \( (-59.5\%) \) after the low-sodium diet \( (P < 0.001 \) versus salt-sensitive patients and salt-resistant patients) (Figure 4). As explained in the “Methods” section, counterregulating patients reported a rise in DBP levels after sodium restriction. In this context, it is interesting to note that five tenths of our counterregulators reported also a 10 mm Hg decrease in DBP levels when they followed a high-sodium diet.

Intergroup Comparison. No differences were found between salt-resistant and salt-sensitive patients with regard to baseline PRA and PAC levels. On the contrary, plasma ANP and DLS levels were significantly higher \( (P < 0.0001) \) in salt-sensitve than in salt-resistant and counterregulating patients (Figure 3). Moreover, compared with salt-resistant patients, the salt-sensitive patients were characterized by lower values of urinary active kallikrein excretion \( (P < 0.0001) \) (Figure 3). With regard to the hormonal modifications after the different sodium diets, sodium-related PRA changes were clearly less evident in salt-sensitive patients than in salt-resistant patients (Figure 3). The opposite results were found for plasma ANP and DLS levels. Indeed, although both hormones increased significantly with sodium load in both salt-sensitive and salt-resistant patients, the mean increment was significantly higher in the first than in the second group (Figure 4). Surprisingly, mean active kallikrein excretion changes during different sodium diets were similar in all groups (Figure 4).

Other comparisons, regarding the counterregulating group, have been given in the above paragraph.
Reproducibility

Reproducibility of our procedure was assessed in 26 patients (i.e., 42.6% of the entire initial population: 10 salt-sensitive, 11 salt-resistant, and five counterregulating patients). The individual response to changes in dietary sodium intake was found to be different in three patients, compared with the first study. One salt-sensitive patient became salt resistant. Of the previously counterregulating patients, one was found to be salt resistant, whereas one previously salt-resistant patient became a counterregulating patient. Sodium-related changes in blood pressure in both the first and the second study were significantly correlated (SBP changes after low-sodium diet: \( r = 0.720, P < 0.0005 \); DBP changes after low-sodium diet: \( r = 0.704, P < 0.0005 \). SBP changes after high-sodium diet: \( r = 0.672, P < 0.0005 \); DBP changes after high-sodium diet: \( r = 0.899, P < 0.0005 \).

DISCUSSION

In a previous report (39), we found that a low urinary excretion of active kallikrein was highly predictive of a salt-sensitive behavior of blood pressure, and combined with the highest prevalence of hypertensive relatives. We interpreted the reduced kallikrein excretion as the consequence of an impaired capability of the kidney to modulate the enzyme production during changes in sodium intake.

The study presented here aimed to verify this hypothesis, and to analyze the relationships among kallikrein and other sodium-regulating hormones.

Furthermore, because the influence of sodium intake on blood pressure (51, 52), and the concept of salt sensitivity itself (45) are still debated, we planned to also study the reproducibility of our procedure of evaluating blood-pressure sensitivity to changes in sodium intake.

When one considers the entire study population, blood-pressure levels were not significantly influenced by sodium (Table 2). However, the arbitrary definition of a cut-off value of salt sensitivity as an increment of DBP of at least 10 mm Hg with the high-sodium diet and of an equal decrement with the low-sodium diet identified 37.7% of hypertensive patients (\( N = 23 \)) as salt sensitive and 45.9% (\( N = 28 \)) as salt resistant. Therefore, this study confirmed that the blood-pressure response to salt restriction is markedly heterogeneous (1-4, 6, 15, 17, 43, 44). Furthermore, it supports previous studies done in hypertensive patients (3, 6, 39, 41, 42) and normotensive patients (18, 42), indicating that severe salt restriction may have adverse pressor effects in some individuals, because a low-sodium diet increased DBP levels in 10 of 61 patients (16.4%).

The reexamination of 26 patients indicated a very high reproducibility of our procedure of evaluating blood-pressure response to changes in dietary sodium intake. Indeed, only 3 of 26 patients changed their class, compared with the first study. Therefore, this study further demonstrates that the pressor response to changes in sodium intake is not a result of casual variations of blood pressure, at least within a short
period of time. Furthermore, only one patient changed from the salt-sensitive to the salt-resistant class, whereas the remaining two patients switched from the salt-resistant to the counterregulating group \((N = 1)\)
or vice versa \((N = 1)\), thus confirming the inefficacy of dietary sodium restriction in their management. An excellent study by Overlack et al. (18), in complete agreement with our findings, demonstrated in normoten
tive patients that the blood-pressure response to changes in dietary sodium intake, as assessed by a protocol similar to the current one, was highly reproducib
le in more than 90% of the subjects. Indeed, reexamination of 31 normoten
sive patients revealed different blood-pressure responses to changes in so
odium intake only in three subjects (18).

On the other hand, patient selection allowed us to exclude any confounding factors related to body weight \((10-12)\), disturbances of the carbohydrate me
tabolism \((13)\), and age \((3,15,17)\). With regard to the influence of sex, salt sensitivity has been reported to be more \((53)\) or less marked \((54)\) in women than in men, although Weinberger et al. (3) were not able to observe any difference at all. However, because sev
eral of the hormones that are involved in sodium homeostasis are strongly influenced by the menstrual cycle \((46)\), and because urine collection is not possible in menstruating women, we decided to select only hypertensive men for our study.

Another problem we tried to bypass with the current protocol is related to the sequence. Indeed, the use of the same order in the diet sequence may result in an order effect, limiting the efficacy of the study \((3,5,6)\). Furthermore, each diet must be followed for a period of time that is long enough to achieve sodium balance \((55,56)\), and must be identical for all patients \((6,57)\). Therefore, we designed a study in which a short period of “normal” \(i.e.,\) intermediate sodium intake pre
ceded the randomization to a 2-wk period on either a high- or low-sodium intake. The use of capsules containing different amounts of sodium allowed us to maintain the study in absolute double-blind condi
tions, whereas repeated measurements of 24-h urinary sodium excretion levels assured the perfect ad
herence to each diet.

With regard to the results obtained, we confirmed that a low active kallikrein excretion level during an intermediate sodium intake is linked with salt sensi
tivity of blood pressure. This association does not imply a causal relationship between kallikrein excre
tion and salt sensitivity. Similarly, it must be empha
sized that it does not prove that an impaired kal
likrein-kinin system function determines the salt sensivity of blood pressure. According to this proto
col, we failed to demonstrate an altered kallikrein response to changes in dietary sodium intake in salt
sensitive patients. Indeed, although low active kal
likrein excretion levels always resulted, sodium-re
lated changes in urinary excretion were similar in the salt-sensitive patients, compared with both salt-resis
tant and counterregulating patients.

This kallikrein behavior is in complete agreement with recent findings obtained in normoten
tive patients \((37,38)\), demonstrating identical decreases in kallikrein excretion after a high-sodium diet in sub
jects with either a low or a high-enzyme excretion level at baseline. Interestingly, normoten
sive patients who had a low kallikrein excretion were markedly salt sensitive, whereas the remaining subjects were salt
resistant \((37,38)\). On the other hand, we have recently demonstrated a marked salt sensitivity of blood pres
sure in the presence of a normal kallikrein response to changes in dietary sodium chloride intake in low
renin hypertensive patients with a reduced kallikrein excretion \((36)\).

In this context, experimental studies have already demonstrated that kallikrein production is regulated by potassium rather than by sodium intake \((58)\). In humans, Berry et al. \((59)\) were able to classify 80% of an Utah population into groups with a high or low-kallikrein genotype. In particular, a low-kallikrein excretion level was combined with the highest preva
lence of hypertensive relatives, segregating as a Mendel
ian recessive trait \((59)\). Enzyme excretion was de
pendent on a major gene with strong environmental modula
tion by dietary potassium in heterozygotic but not in homozygotic subjects \((60)\). Therefore, a reduced kal
likrein excretion level, as well as an increased preva
lence of familial hypertension, resulted from the patients’ being homozygous for the low-kallikrein gene or from being heterozygous and having a low potas
sium intake \((60)\). In agreement with this concept, twin
studies showed that urinary potassium more than urinary sodium correlated with urinary kallikrein and blood-pressure levels \((61)\). Moreover, dietary potas
sium supplementation significantly increased kal
likrein excretion, and reduced blood-pressure levels in essential hypertensive patients \((62)\). In the study pres
ented here, potassium intake did not change during the assessment of salt sensitivity. As a consequence, the low kallikrein excretion we found in salt-sensitive hypertensive patients as well as the normality of the enzyme response to changes in sodium intake should simply reflect the casual allelic distribution and the consequent presence of both homozygotic and heterozygotic subjects for the low-kallikrein gene. Never
theless, a genetic influence on kallikrein excretion does not imply a causal role for kallikrein in determin
ing a progressive sensitization of susceptible individ
uals to an “inappropriate” sodium intake. Further
more, we cannot exclude that the observed kallikrein behavior has been influenced by other factors, such as RBF \((63)\). Indeed, unfortunately, we have not mea
sured this parameter in our patients.

With regard to the other findings of this study, mean PRA levels were lower in salt-sensitive than in salt
resistant patients. Furthermore, PRA levels increased slightly with low sodium intake, confirming previous data that showed a marked salt sensitivity of blood pressure in patients who have a PRA unresponsive to
low sodium intake (19,31,33,43,64–66), furosemide administration (3), and upright posture (47,68).

Counterregulating hypertensive patients showed a marked overstimulation of the renin system after sodium restriction. Even more interestingly, the same patients showed the most evident reduction of plasma ANP with the low-sodium diet. Because ANP can inhibit renin release in man (67,68), it seems intriguing to speculate that a marked decrease of ANP may have favored PRA overstimulation. This latter phenomenon could be the primary contributor to the paradoxical behavior of blood pressure that was shown by counterregulating patients.

In agreement with previous findings (18,65,69), the ANP increment after the high-sodium diet was more pronounced in salt-sensitive than in salt-resistant hypertensive patients. In this regard, some previous reports (65,70,71) have also demonstrated that the ANP response to sodium load is a function of the renin status, being more evident in hypertensive patients with a low renin activity than in those with a high renin activity. Therefore, because atrial secretion of ANP is primarily a result of volume expansion (70,72,73), we think our data support the hypothesis by Overback et al. (18), who suggested that an impaired ability of the kidney to excrete a sodium load might induce a compensatory increase in ANP release from the atrial myocytes. In a similar way, differences in extracellular fluid volume between salt-resistant and salt-sensitive patients may easily explain the inverse relationship between the kallikrein and ANP concentrations that exist in salt-sensitive hypertensive patients, as well as their tendency to display suppressed PRA levels.

In keeping with this interpretation, ANP behavior was paralleled by that of plasma DLS. Indeed, on a normal sodium-intake diet, plasma DLS levels were higher in salt-sensitive than in salt-resistant patients. Moreover, the increase of this substance with the high-sodium diet was particularly evident in the first group, supporting the hypothesis that an impaired ability to modulate a sodium load may induce the secretion of an endogenous substance(s) cross-reacting with digoxin antibodies (74,75). This finding could be particularly interesting because of the suggested natriuretic action of this substance(s) (47,76). However, the effective role of DLS as a natriuretic agent is not undisputed (76). Therefore, we can state that salt-sensitive patients showed the highest sodium-related increment of a circulating substance(s) cross-reacting with digoxin antibodies. On the contrary, any speculative hypothesis that is based on the renal actions of DLS seems absolutely arbitrary.

Another interesting finding of this study is related to the higher triglyceride and fasting insulin levels we found in salt-sensitive patients, compared with salt-resistant and counterregulating patients. In this context, Williams et al. (77) have already suggested that punctiform mutations may explain the segregating single-gene effects for low urinary kallikrein excretion, high sensitivity to environmental “hypertensinogenic” agents, and fasting hyperinsulinemia and hyperglycemia.

According to this hypothesis, Sharma et al. (78) have recently described a hyperinsulinemic response to oral glucose load in salt-sensitive normotensive patients. Furthermore, we have recently described (65,71) the presence of fasting hyperinsulinemia in a patient subset defined as “non-modulators” because of abnormal angiotensin II-mediated control of PAC release and the renal blood supply, abnormal sodium handling, and salt-sensitive hypertension (19,31,33).

In conclusion, we presented herewith a randomized, double-blind, cross-over study that confirmed a marked salt sensitivity of blood pressure in hypertensive patients who have a reduced excretion of active kallikrein. Compared with salt-resistant hypertensive patients, salt-sensitive hypertensive patients excreted less active kallikrein when they ingested either 20 mmol, 140 mmol, or 320 mmol NaCl per day. However, the kallikrein response to different sodium diets was similar in both groups, suggesting that the enzyme production is reduced, although its sodium-related regulation is normal in patients who have a deleterious blood pressure sensitivity to high-sodium intake.

Sodium restriction induced a marked increase of blood pressure in 10 of 61 patients (16.4%). A blood-pressure increment after sodium restriction was linked with an overstimulation of the renin system and suppression of ANP release, suggesting that both of these hormonal changes may have determined the adverse pressor effects.

Finally, we confirmed that salt sensitivity is not a result of casual variations of blood pressure, because identical pressor responses to changes in dietary sodium intake were found in 23 of 26 patients who received again both the low and the high-sodium diets 6 months after the end of the first study.

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