Impaired Inactive to Active Kallikrein Conversion In Human Salt-Sensitive Hypertension

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ABSTRACT

Active and inactive urinary kallikrein excretion rates were evaluated in 43 essential hypertensive men (45.4 ± 5.6 yr) after normal- (120 mmol/day), low- (20 mmol/day), and high- (240 mmol/day) NaCl diets were given for 2 wk each. Patients were classified as salt-sensitive, salt-resistant, or counterregulating, on the basis of their blood pressure responses to the different NaCl intakes. Resulting data show that active and inactive kallikrein excretion rates were lower (P < 0.001) in salt-sensitive (active, 0.59 ± 0.27 U/24 h; inactive, 3.45 ± 1.31 U/24 h) than in salt-resistant (active, 1.41 ± 0.35 U/24 h; inactive, 6.93 ± 2.68 U/24 h) and in counterregulating hypertensive patients (active, 1.37 ± 0.39 U/24 h; inactive, 6.32 ± 2.58 U/24 h) after the normal NaCl diet. Salt-sensitive hypertensive patients showed also higher plasma digoxin-like substance (P < 0.001), atrial natriuretic peptide (P < 0.001), and fasting insulin (P < 0.005) levels than the other subgroups. Active kallikrein decreased after high and increased after low-NaCl intake in all groups. Inactive kallikrein varied similarly to active one in salt-resistant patients and counterregulating patients, whereas it increased during salt-loading in salt-sensitive patients. Consequently, the active/total kallikrein ratio decreased in salt-sensitive patients (from 20.2 ± 3.5 to 5.82 ± 1.02%, P < 0.05) when they switched from low- to high-NaCl intake, and the ratio was lower in these patients than in the other subgroups (P < 0.0001) after the high-NaCl diet. In conclusion, active and inactive kallikrein excretions after normal-NaCl intake are reduced in salt-sensitive hypertensive patients. The divergent active and inactive kallikrein responses to dietary NaCl changes in salt-sensitive patients could indicate an impairment of inactive to active kallikrein conversion during NaCl loading as a new mechanism in human salt-sensitive hypertension.

Key Words: Sodium, blood pressure, kallikrein-kinin system, kidney

The renal kallikrein-kinin system promotes vasodilation, diuresis, and natriuresis (reviewed in Reference 1). Because of its biological properties, a heritable abnormality of the kallikrein-kinin system has been often suggested as a contributor to the development of human essential hypertension through reduced kallikrein production, decrement of kinin generation, and impaired renal sodium handling (1).

In agreement with this theory, segregation analysis provided evidence that a large proportion of urinary active kallikrein levels is determined by a major gene (2), and homozygosity for the allele responsible for a low kallikrein excretion is associated with a positive family history of hypertension (2).

In this context, the possibility that environmental factors, such as an "inappropriate" NaCl intake, may lead to hypertension in subjects with an inheritable defect of renal kallikrein production was further supported by the significant association between low active kallikrein excretion and salt-sensitivity of blood pressure, which has recently been described in both normotensive (3) and hypertensive subjects (4). In particular, none of salt-resistant and 55% of salt-sensitive hypertensive patients had a kallikrein excretion that was <0.5 U/24 h. Among salt-sensitive patients having a kallikrein excretion below this cutoff value, 80% had a positive family history of hypertension (4).

The reduced active kallikrein excretion in salt-sensitive individuals was interpreted as the direct consequence of a primary defect in enzyme production, without considering the possibility that an impaired inactive to active kallikrein conversion might cooperate in reducing the kidney's ability to excrete the active form of the enzyme. Indeed, about 60 to 70% of total kallikrein is excreted in urine in an inactive pro-form (5), which can be activated in vivo by at least three renal trypsin-like enzymes (6), and in vitro by trypsin (7,8).

In a recent report (9), we showed that active kal-

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llikrein excretion response to dietary NaCl-loading and depletion was similar in both salt-sensitive and salt-resistant hypertensive patients, although active kallikrein levels were lower in salt-sensitive patients during each different NaCl diet. On the other hand, in unselected essential hypertensive patients, the active-to-inactive kallikrein ratio significantly decreased during a high-NaCl intake, because of simultaneous active kallikrein decrement and inactive kallikrein increment (7). In our opinion, this finding suggests that the decrease in kallikrein excretion might reflect the existence of an abnormal conversion of inactive to active kallikrein.

To verify this hypothesis, we planned this randomized crossover double-blind study, in which measurements of 24-h urinary excretion rates of active and inactive kallikrein were made in a group of essential hypertensive patients characterized as salt-sensitive or salt-resistant on the basis of their blood pressure response to changes in dietary NaCl intake. To exclude all factors that may alter kallikrein excretion (1,10), the study was conducted in a selected cohort of nonobese, nondiabetic essential hypertensive men with normal renal function, on a constant K+ intake (10).

A reduced excretion rate of active kallikrein in human urine is combined with the highest prevalence of familial hypertension (2.4), body mass index (10), and fasting insulin levels (4). On the other hand, an atherogenic pattern of serum lipoproteins (11), fasting (12) and post-load hyperinsulinaemia (13), familial prevalence of hypertension (4,14), and cardiovascular accidents (12,15) have been often described in salt-sensitive subjects. Taken together, these findings suggest a possible linkage among salt-sensitivity, reduced urinary kallikrein excretion, and metabolic disturbances. Therefore, in the study presented here, we also investigated the relationships among salt-sensitivity, urinary kallikrein excretion, serum lipoproteins, glucose tolerance, and family histories of hypertension and myocardial infarction.

METHODS

Patients

Informed consent to participate in this study was obtained from 55 Caucasian never-treated hypertensive patients ranging in age from 34 to 56 yr (mean, 46.7 ± 6.3 yr). They were affected by uncomplicated essential hypertension and had no personal history of alcohol abuse or smoking. They had body mass indexes of between 19 and 28 kg/m².

Recruitment Phase

Patients were recruited if their diastolic blood pressure (DBP) was between 95 and 114 mm Hg at four consecutive weekly visits. Secondary causes of hypertension were excluded by requiring normal levels of BUN, creatinine, potassium, norepinephrine, epinephrine, dopamine, and 24-h urine vanillylmandelic acid and urinalysis. Proteinuria was absent in two consecutive 24-h urine collections. Fasting glycemia was <5.6 mmol/L, postprandial glycemia was <8.0 mmol/L, glycosuria was absent, and HbAlc was <7%. Cardiac, hepatic, and renal diseases were excluded by clinical and laboratory assessments.

At the first screening visit, a questionnaire was given to each patient to check for familial history of hypertension and myocardial infarction, according to a previously described methodology (4,9,15). The patient’s physician, wife, and, if possible, other relatives (brothers and sisters, sons) were also interviewed to confirm the information provided by the patients. This procedure allowed us to identify the presence of a positive family history of hypertension and myocardial infarction as the presence of at least one first-degree relative who had been afflicted by one or both of these conditions before the age of 60. This part of the study was performed by researchers who were unaware of the study purpose and results.

All patients were placed on a isocaloric diet containing 120 mmol NaCl per day (Figure 1). The diet was prepared by our dietitians and contained (per 100 kcal) approximately 4.5 g proteins, 13.5 g carbohydrates, and 3.1 g fats. The diet was rigorously “tailored” to each patient to avoid weight loss. The total number of calories remained identical during all the study period and was calculated on the basis of individual needs. Standard diet contained 20 mmol NaCl and 75 mmol K+ per day. A supplement of 100 mmol NaCl was added to the diet by means of five capsules per day (1 capsule = 20 mmol NaCl). Oral and written instructions and detailed information on how to cook without added NaCl were given to each patient. To simulate as closely as possible the Italian way of eating, patients took one capsule at breakfast, two capsules at lunch, and two capsules at dinner. Patients were advised

![Figure 1. Schematic representation of the study protocol.](image-url)

**Figure 1.** Schematic representation of the study protocol. After definitive enrollment in the study, participants followed a normal-NaCl diet (120 mmol NaCl per day) for 2 wk to obtain the same baseline conditions. At the end of this period, baseline blood pressure measurement and blood and urine samples were taken and patients and control subjects were randomized to one of two opposite sequences: some persons followed the sequence HIGH-NORMAL-LOW NaCl diet, some others the sequence LOW-NORMAL-HIGH NaCl diet. Blood pressure measurements for salt-sensitivity assessment and blood and urine samples for hormonal evaluation were repeated at the end of each different diet period.
to drink 1.5 L of tap water/day. Adherence to the diet was assessed by measuring the 24-h urinary Na" excretion on the last 2 consecutive days of each week. Na" excretion was required to be > 100 and < 140 mmol/24 h.

Of the initial cohort of 55 patients, nine did not enter the salt-sensitivity assessment phase because of noncompliance with the diet (N = 2), DBP < 95 mm Hg (N = 3) or > 114 mm Hg (N = 1), systolic blood pressure (SBP) > 180 mm Hg (N = 1), or lack of return to follow-up visits (N = 2). Thus only 46 patients were definitively enrolled.

Salt-Sensitivity Assessment Phase

After enrollment, patients followed the normal NaCl diet for further 2 wk (Figure 1). Blood samples for plasma renin activity (PRA), aldosterone (PAC), atrial natriuretic peptide (ANP), norepinephrine (NA), digoxin-like substance (DLS), serum lipids levels, erythrocyte Na" /K" /2Cl⁻ cotransport, Na" /Li" countertransport, and Na" /K" ATPase activities were then taken after the patients rested for 1 h in a supine position. An oral glucose tolerance test (75 g glucose) was also performed (with blood samples for plasma glucose and insulin measurements taken at times 0, 30, 60, 90, 120, and 180 min). Two consecutive 24-h urine collections were requested from each patient to determine urinary active and inactive kaliurein and albumin excretion rates. After blood and urine samples were taken, patients were randomly assigned to a high- (240 mmol NaCl per day for 2 wk) or a low- (20 mmol NaCl per day for 2 wk) NaCl intake, according to a crossover, double-blind procedure (Figure 1). Twenty-four patients were assigned first the high- and then the low-NaCl diet, whereas the remaining ones (N = 22) followed the reverse order. During the crossover phase, a normal-NaCl diet was administered to reset all hormonal mechanisms before varying NaCl assumption (Figure 1).

Different NaCl diets were obtained by changing the 20 mmol NaCl content of each capsule to 44 mmol NaCl (on a high-NaCl diet) and 0 mmol NaCl (on a low-NaCl diet). To eliminate the influence of changes in K⁺ intake on active kaliurein excretion (10), each diet contained always the same amount of K⁺ (75 mmol per day).

Also during the evaluation of salt-sensitivity, compliance was controlled by measuring the 24-h sodium excretion on the last 2 days of each wk. Patients were considered to be compliant when their 24-h sodium excretion rate was > 230 and < 40 mmol/24 h during the high- and low-NaCl intake periods, respectively. Three additional patients revealed as not compliant. Thus, the salt-sensitivity assessment phase was successfully completed in 43 patients.

Blood samples, including those for oral glucose tolerance tests, and urine collections were taken again at the end of the low- and high-sodium diet periods.

Patient Classification

Considering as baseline blood pressure the measurement taken at the end of the 120 mmol NaCl diet period, a patient was classified as salt-sensitive when DBP increased 5 mm Hg or more after the high-NaCl intake and decreased 5 mm Hg or more after the low-NaCl intake (3). Patients having a DBP increase of at least 5 mm Hg after salt restriction and a DBP decrease of at least 5 mm Hg after salt loading were considered as "counterregulating patients" (14).

Reproducibility

To verify the reproducibility of our procedure to determine salt-sensitivity, 20 patients were randomly recalled 3 months after the end of the study. The reproducibility evaluation was not feasible in four patients (three because of loss at follow-up, one for compliance problems during changes in NaCl intake), although it was successfully performed in the remaining patients (N = 16). Only urine collections, necessary for measuring 24-h Na" excretion, were repeated during this phase.

Blood Pressure and Laboratory Evaluations

Blood pressure and heart rate were controlled during each visit in our outpatient unit. Blood pressure was measured after the patients rested for 10 min in a sitting position, in a comfortable 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 (16), a normal-size cuff completely circled the nondominant arm, which was supported at the heart level. Systolic blood pressure was taken at Korotkoff's phase I, and DBP was taken at Korotkoff's phase V (16). 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 measured by researchers who were unaware of the study purpose, design, and results.

Blood samples for PRA, PAC, ANP, NA, DLS, serum Na" and K⁺, erythrocyte Na" /K" /2Cl⁻ cotransport, Na" /Li" countertransport, and Na" /K" ATPase activities were taken from an antecubital vein after the patients rested for 1 h in a supine position. For hormonal measurements, blood samples were collected on ice and immediately spun. Plasma was then separated and frozen at -80°C until assayed. All of the assays were performed no later than 8 days after storage. Plasma ANP was measured as described elsewhere (12,15). In brief, Amprep c-18 columns (Amersham, Buckinghamshire, U.K.) were first activated with 4 mL methanol and 12 mL distilled water, then loaded with 2.5 mL acidified plasma. The eluate was collected in 1 mL ethanol, dried under vacuum, and reconstituted in 1 mL neutral phosphate buffer. A fraction of 100 µL in duplicate was assayed by a commercially available human-ANP (99–126) RIA kit (Peninsula Laboratories Inc., Belmont, CA). Synthetic human-ANP (99–126) (Bisssendorf GmbH Peptide, Wedemark, Germany) was used as standard. Mean recovery for ANP in this study was 84% (range 78 to 106), inter- and intra-assay variations were less than 10%. The RIA sensitivity was 1 fmol/tube. PRA and PAC were assayed by commercially available RIA kits (Sorin Biomedica, Vercelli, Italy). Plasma NA was assayed by reverse-phase HPLC (LKB, Pharmacia, Uppsala, Sweden) with electrochemical detection (ESA, Bedford, MA) after extraction and concentration onto activated alumina (15). Plasma DLS was measured after extraction from plasma. In brief, Amprep c-18 columns (Amersham) were activated with 5 mL methanol and 20 mL distilled water, 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 buffer solution (NaCl130 mM, sucrose 20 mM, glucose 10 mM, tris-HCl 10 mM, pH 7.4). For DLS RIA, a solid-phase system was used (Diagnostic Products Corporation, Los Angeles, CA). Digoxin (Wellcome, Pomezia, Italy) was used as standard.

For erythrocyte studies, peripheral blood was collected in heparin-containing tubes. Red blood cells were separated within 3 h by centrifugation for 10 min at 300 g. Plasma and
buffy coat were separated by suction. Erythrocytes were washed three times in Na"-containing washing solution (magnesium chloride 75 mM, sucrose 85 mM, glucose 10 mM, tris-MOPS 10 mM, pH 7.4) at 4°C, and the maximal velocity (V_{max}) of erythrocyte Na"/Li" countertransport was then assayed from the external sodium-stimulated lithium efflux after lithium loading. The V_{max} of erythrocyte Na"/K"/2Cl" cotransport was evaluated by determining furosemide-sensitive sodium and potassium efflux into choline media from cells loaded with sodium by means of nystatin as previously described by our laboratory (17). The V_{max} of erythrocyte Na"/K" ATPase was evaluated by determining ouabain-sensitive sodium efflux from cells loaded with sodium by means of nystatin (17). For oral glucose tolerance tests, plasma glucose was evaluated by the glucose oxidation method and a Glucose Analyzer II (Beckman, Fullerton, CA). Plasma insulin levels were assessed by a commercial RIA kit (Ares-Serono, Milano, Italy). Serum lipid levels (total cholesterol, triglycerides, and high-density lipoprotein [HDL] cholesterol levels) were evaluated by standard enzymatic methods. Plasma concentrations of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were assessed by the Friedewald method (18). The other laboratory measurements were performed by standard laboratory methods.

The 24-h urinary excretion rate of active kallikrein was measured on fresh urine samples by hydrolysis of the peptide-nitroanilide chromogenic substrate H-D-V-L-R-pNA (S-2226) (KabiVitrum, Mölndal, Sweden) according to the amidolytic method of Amundsen et al. (19). The urinary excretion rate of total kallikrein was determined after trypsin-activation of inactive kallikrein by the method of Maddi et al. (7). Inactive kallikrein was then calculated by subtracting active kallikrein from kallikrein activity obtained after trypsin activation (i.e., total kallikrein) (7). In random urine samples (N = 40), the results obtained by the chromogenic method were compared with those obtained by kininogenase assay. Kininogenase activity was considered as μg of kinins generated in 20 min incubation of 10 μL urine with 450 μg low-molecular-weight kinogen (Seikagaku Kogyo Co., Tokyo, Japan) (8).

Microalbuminuria was evaluated by a commercial RIA kit (Ares-Serono, Milano, Italy).

Control Group

Ten healthy male volunteers with no family history of hypertension and myocardial infarction participated as the control group and underwent the same testing protocol as the hypertensive patients.

Statistical Analyses

The statistical evaluation was performed by a PC Olivetti M-380×P1 (Olivetti, Ivrea, Italy). The statistical software PRIMIT 3.03 (McGraw-Hill, New York, NY; 1992) was used. To evaluate intragroup statistical significances, we used the analysis of variance for repeated measures. To establish differences between groups, we used the 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 result of each statistical test was considered significant when the associated P value was <0.05. Data are given as mean ± SD.

RESULTS

The general characteristics of hypertensive patients and normotensive subjects are given in Table 1. With regard to the hormonal data, hypertensive patients showed significantly higher levels of plasma ANP and DLS than normotensive subjects (Table 2). Hypertensive patients were also characterized by a lower urinary active kallikrein excretion rate than normotensive subjects, whereas the urinary inactive kallikrein excretion rate was not significantly reduced (Table 2). Thus, the active-to-total kallikrein ratio was significantly lower in hypertensive patients than in control subjects (Table 2). Similarly, hypertensive patients showed accelerated erythrocyte Na"/K"/2Cl" cotransport and Na"/Li" countertransport activities than normotensive subjects (Na"/K"/2Cl" cotransport).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypertensive Subjects</th>
<th>Normotensive Subjects</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>43</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>45.7 ± 5.9</td>
<td>44.2 ± 3.6</td>
<td>0.45</td>
</tr>
<tr>
<td>Family History of Hypertension (yes/no)</td>
<td>28/15</td>
<td>9/9</td>
<td>0.03</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>25.6 ± 1.4</td>
<td>24.8 ± 1.7</td>
<td>0.12</td>
</tr>
<tr>
<td>Waist/Hip Ratio</td>
<td>0.94 ± 0.06</td>
<td>0.90 ± 0.04</td>
<td>0.051</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mm Hg)</td>
<td>159.8 ± 15.2</td>
<td>134.9 ± 20.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>103.6 ± 4.5</td>
<td>87.6 ± 4.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>73.2 ± 4.1</td>
<td>71.4 ± 3.1</td>
<td>0.2</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>5.15 ± 0.80</td>
<td>5.33 ± 0.78</td>
<td>0.52</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dL)</td>
<td>85.5 ± 9.2</td>
<td>81.4 ± 10.7</td>
<td>0.22</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.77 ± 0.25</td>
<td>1.81 ± 0.51</td>
<td>0.72</td>
</tr>
<tr>
<td>Clearance (mL/min)</td>
<td>15.8 ± 2.0</td>
<td>14.9 ± 2.9</td>
<td>0.24</td>
</tr>
<tr>
<td>Plasma Total Cholesterol (mmol/L)</td>
<td>5.06 ± 0.61</td>
<td>4.75 ± 0.53</td>
<td>0.15</td>
</tr>
<tr>
<td>HDL Cholesterol (mmol/L)</td>
<td>1.41 ± 0.33</td>
<td>1.63 ± 0.29</td>
<td>0.058</td>
</tr>
<tr>
<td>LDL Cholesterol (mmol/L)</td>
<td>3.30 ± 0.53</td>
<td>2.89 ± 0.21</td>
<td>0.021</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.34 ± 0.20</td>
<td>0.23 ± 0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Serum Triglycerides (mmol/L)</td>
<td>1.74 ± 0.23</td>
<td>1.15 ± 0.16</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>4.87 ± 0.12</td>
<td>4.82 ± 0.20</td>
<td>0.3</td>
</tr>
<tr>
<td>Fasting Insulin (pmol/L)</td>
<td>104.5 ± 38.9</td>
<td>89.6 ± 10.7</td>
<td>0.24</td>
</tr>
</tbody>
</table>

*HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very-low-density lipoprotein.
TABLE 2. Hormonal data of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypertensive Subjects</th>
<th>Normotensive Subjects</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>43</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Plasma Renin Activity (ng/L per s)</td>
<td>0.45 ± 0.17</td>
<td>0.43 ± 0.11</td>
<td>0.7</td>
</tr>
<tr>
<td>Plasma Aldosterone (pmol/L)</td>
<td>316.4 ± 52.2</td>
<td>315.7 ± 52.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Atrial Natriuretic Peptide (fmmol/mL)</td>
<td>20.7 ± 8.8</td>
<td>12.6 ± 5.1</td>
<td>0.007</td>
</tr>
<tr>
<td>Digoxin-Like Substance (pg/mL)</td>
<td>32.5 ± 14.2</td>
<td>18.4 ± 10.6</td>
<td>0.004</td>
</tr>
<tr>
<td>Noradrenaline (pmol/L)</td>
<td>1308.6 ± 325.1</td>
<td>1284.6 ± 212.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Urinary Total Kallikrein Excretion (U/24 h)</td>
<td>6.69 ± 2.16</td>
<td>8.23 ± 2.01</td>
<td>0.045</td>
</tr>
<tr>
<td>Urinary Active Kallikrein Excretion (U/24 h)</td>
<td>1.12 ± 0.34</td>
<td>1.51 ± 0.29</td>
<td>0.002</td>
</tr>
<tr>
<td>Urinary Inactive Kallikrein Excretion (U/24 h)</td>
<td>5.56 ± 2.84</td>
<td>6.72 ± 2.18</td>
<td>0.1</td>
</tr>
<tr>
<td>Active/Total Ratio (%)</td>
<td>16.3 ± 2.75</td>
<td>18.4 ± 3.49</td>
<td>0.044</td>
</tr>
</tbody>
</table>

port: 498.5 ± 97.8 versus 397.8 ± 72.7 μmol/L cell per h, P < 0.004; Na⁺/Li⁺ countertransport: 449.6 ± 98.8 versus 301.5 ± 72.1 μmol/L cell per h; P < 0.001). On the contrary, the Na⁺/K⁺ ATPase activity was similar between the two groups (4.87 ± 1.88 versus 5.21 ± 1.65 mmol/L cell per h; not significant [n.s.]).

Salt-Sensitive Patients (N = 19; mean age, 46.1 ± 6.4 yr)

At the end of the normal-NaCl diet, SBP was 163.8 ± 15.1 mm Hg, and increased to 176.6 ± 18.3 mm Hg (P < 0.0001) after the high-NaCl diet. After the low-NaCl diet, SBP decreased to 151.3 ± 10.8 mm Hg (P < 0.0001 versus normal-NaCl diet SBP). After the high-NaCl intake, SBP was significantly higher than after the low-NaCl intake (P < 0.0001). Diastolic blood pressure was 104.6 ± 4.7 mm Hg after the normal-NaCl intake, increased to 115.8 ± 8.2 mm Hg (P < 0.0001) after the high-NaCl diet, and decreased to 92.6 ± 5.7 mm Hg (P < 0.0001 versus normal-NaCl diet SBP) after the low-NaCl intake period. After the high-NaCl intake, DBP was significantly higher than after the low-NaCl intake (P < 0.0001).

Urinary Na⁺ excretion rate was 110.7 ± 38.7 mmol/24 h after the normal-NaCl diet, 231.8 ± 43.7 mmol/24 h (P < 0.0001) after the high-NaCl diet, and 15.6 ± 8.3 mmol/24 h (P < 0.0001) after the low-NaCl diet. Body weight was 75.6 ± 2.8 kg after the normal-NaCl diet, increased to 78.8 ± 3.6 kg after the high-NaCl diet (P < 0.05), and decreased to 72.2 ± 4.2 kg at the end of the low-NaCl intake period (P < 0.05 versus normal- and high-NaCl intake).

At the end of the period of normal-NaCl intake, mean PRA level was 0.36 ± 0.10 ng/L per s, PAC level was 285.6 ± 47.8 pmol/L, ANP level was 32.7 ± 12.4 fmol/mL, NA level was 1327.8 ± 368.3 pmol/L, and DLS level was 47.8 ± 18.3 pg/mL. Sodium-related hormonal variations are shown in Table 3. Urinary active and inactive kallikrein excretion rates were 0.59 ± 0.27 U/24 h and 3.45 ± 1.31 U/24 h, respectively. The inactive kallikrein response to high-NaCl intake was opposite that of active kallikrein one (Figure 2). Accordingly, the active-to-total kallikrein ratio was significantly lower (P < 0.05) after high- than after low-NaCl intake (Figure 2). Microalbuminuria was 7.5 ± 3.1 μg/min on a normal-NaCl, 3.67 ± 2.51 μg/min on a low-NaCl, and 12.4 ± 3.8 μg/min on a high-NaCl diet (P < 0.001 low- versus high-NaCl intake period).

The behavior of serum lipoprotein concentrations is given in Table 4. Salt-sensitive patients had a significant tendency to an "atherogenic" lipid pattern, showing low HDL and high LDL cholesterol levels. Interestingly, serum triglyceride levels significantly increased after the high-NaCl diet. With regards to glucose tolerance, salt-sensitive patients had significantly higher fasting and post-load insulin levels than did control subjects (Figure 3). Also, in this case, hyperinsulinemia became more evident after the high-NaCl load, whereas it was not observable when patients received a low-NaCl intake.

As far as the erythrocyte sodium transport systems are concerned, salt-sensitive patients showed an accelerated activity of erythrocyte Na⁺/Li⁺ countertransport (Table 5).

Salt-Resistant Patients (N = 16; mean age, 44.5 ± 5.8 yr)

After the normal-NaCl intake, SBP was 158.3 ± 16.4 mm Hg and did not change after both the high-(162.9 ± 19.2 mm Hg, n.s.) and the low-(160.1 ± 15.8 mm Hg, n.s.) NaCl diets. Similarly, DBP was 102.5 ± 4.1 mm Hg after the normal-NaCl intake and was not significantly influenced by NaCl intake variations, being 104.7 ± 5.1 mm Hg at the end of the high-NaCl diet and 100.8 ± 5.6 mm Hg at the end of the low-NaCl intake (n.s.). Body weight was 76.2 ± 3.1 kg after the normal-NaCl diet, 77.8 ± 3.5 kg after the high-NaCl diet (n.s. versus normal-NaCl diet), and 75.4 ± 2.9 kg after the low-NaCl diet (n.s. versus normal, P < 0.05 versus high-NaCl diet).

Urinary Na⁺ excretion rate was 112.7 ± 28.7 mmol/24 h after the normal-NaCl diet, increased to 235.4 ± 42.5 mmol/24 h after the high-NaCl diet (P < 0.001 versus normal), and decreased to 18.4 ± 9.7 mmol/24 h after the low-NaCl diet (P < 0.001 versus normal- and high-NaCl diet). After the period on a normal-NaCl intake, the mean PRA level was 0.45 ± 0.18 ng/L per s, PAC level was 301.7 ± 41.5 pmol/L, ANP was 16.1 ± 6.3 fmol/mL, DLS was 25.4 ± 11.8 pg/mL, and NA was 1309.7 ± 285.4 pmol/L. Hormonal variations during different diet periods are
shown in Table 3. Urinary active and inactive kallikrein excretion rates of 1.41 \pm 0.35 U/24 h and 6.93 \pm 2.68 U/24 h, respectively, resulted. At variance with the salt-sensitive patients, in these patients urinary inactive kallikrein excretion did not change with salt-loading (Figure 2). Microalbuminuria was 8.2 \pm 3.7 \mu g/min on a normal-NaCl diet, 7.5 \pm 3.2 \mu g/min on a low-NaCl diet, and 9.1 \pm 4.3 \mu g/min on a high-NaCl diet (n.s.).

Changes in serum lipoprotein concentrations induced by different NaCl diets are given in Table 4. As it is shown, salt-resistant patients had no evident abnormalities in serum lipids levels, and no significant changes were observable after the high-NaCl intake. In a similar way, both glucose and insulin responses to oral glucose load (Figure 3) were similar to those observed in control subjects, and no marked modifications of glucose tolerance were inducible by changing NaCl intake.

### Counterregulating Patients (N = 8; mean age, 45.8 \pm 5.3 yr)

The blood pressure variations in counterregulating patients during different NaCl intake periods were different from those shown by salt-sensitive and salt-resistant patients. Indeed, SBP was 157.2 \pm 14.2 mm Hg after normal-NaCl diet, 152.3 \pm 11.8 mm Hg after high-NaCl intake (n.s.), and 162.4 \pm 12.1 mm Hg after the low-NaCl intake in counterregulating patients. DBP varied from 103.8 \pm 4.7 mm Hg after the normal-NaCl intake period to 98.9 \pm 3.8 mm Hg after the high-NaCl diet (n.s.), and to 109.3 \pm 4.8 mm Hg after the low-NaCl diet period (P < 0.01 versus high-NaCl diet).

The behavior of these patients mimicked that of salt-resistant patients, with regard to the hormonal (Table 3), the lipids (Table 4), and the erythrocyte sodium transport systems (Table 5) data. Interestingly, the kallikrein behavior, for active enzyme as well as for the inactive one, was very close to that observed in salt-resistant patients (Figure 2).

### Kallikrein Behavior in Control Subjects

Changes in NaCl intake divided the control population in four salt-sensitive and six salt-resistant subjects. Although to a lesser extent than hypertensive patients, inactive kallikrein increased with salt load in salt-sensitive (from 2.87 \pm 0.85 to 4.72 \pm 0.79 U/24 h, P < 0.05) but not in salt-resistant control subjects (from 11.77 \pm 5.81 to 6.96 \pm 2.93 U/24 h, n.s.). As a consequence, active-to-total ratio decreased with NaCl load in salt-sensitive (from 23.46 \pm 5.71 to 13.52 \pm 4.8%, P < 0.05) but not in salt-resistant subjects (from 19.82 \pm 6.71 to 20.18 \pm 6.38%, n.s.).
Intergroup Comparison

No differences were found between salt-resistant and salt-sensitive patients with regard to baseline blood pressure, PRA, PAC, and NA levels (Table 3). On the contrary, plasma ANP and DLS levels were significantly higher ($P < 0.001$) in salt-sensitive than in salt-resistant and counterregulating patients and in healthy subjects (Table 3). Moreover, the salt-sensitive patients were characterized by lower ($P < 0.001$) urinary kallikrein excretion levels than were salt-resistant patients (Figure 2).

With regard to the hormonal modifications after different dietary NaCl variations, the sodium-related PRA changes that resulted were clearly less evident in salt-sensitive hypertensive patients than in salt-resistant ones (Table 3). Opposite results were found for plasma ANP and DLS levels (Table 3). Plasma NA markedly increased after the low-NaCl diet in salt-sensitive ($P < 0.05$), but not in salt-resistant patients (Table 3). With regard to active kallikrein, variations versus baseline because of changes in NaCl intake were similar in salt-resistant and salt-sensitive hypertensive patients (Figure 2). Inactive kallikrein increased with NaCl load only in salt-sensitive patients, while it remained unchanged in the remaining ones (Figure 2).

As far as the metabolic parameters are concerned, salt-sensitive patients displayed higher serum LDL cholesterol and triglyceride levels, and lower HDL cholesterol levels than other groups. This "atherogenic" pattern was evident on both a low- and a high-NaCl diet, although serum triglyceride levels showed a clear tendency to further increment with dietary NaCl loading (Table 4). In a similar way, comparing patient subgroups on a low-NaCl intake, the glucose and insulin responses to oral glucose load resulted substantially identical (Figure 3). On the contrary, when patients received the high-NaCl diet, marked hyperinsulinemic and hyperglycemic responses to oral glucose load became evident in salt-sensitive patients (Figure 3). Interestingly, the behavior of microalbuminuria paralleled the insulin response to NaCl intake. Indeed, salt-resistant and salt-sensitive patients excreted similar amounts of albumin on a low-NaCl diet, but a significant increment of albumin excretion was detectable only in salt-sensitive patients after the high NaCl intake.

Reproducibility

The reproducibility of our procedure was assessed in a small patient cohort ($N = 16$, 35% of the initial entire population: eight salt-sensitive, six salt-resistant, and two counterregulating hypertensive patients). Individual response to changes in dietary NaCl intake was found to be different in three hypertensive patients compared with the first study. Two salt-sensitive patients became salt-resistant, while of the previously counterregulating hypertensive patients, one became salt-resistant. NaCl-related changes in blood pressure in the first and the second studies were highly significantly correlated (systolic: $r = 0.567$, $P < 0.001$; diastolic: $r = 0.772$ $P < 0.0005$).

DISCUSSION

In this investigation, we present a randomized crossover double-blind study aiming to evaluate active and inactive kallikrein excretion in essential hypertensive patients divided into salt-sensitive or salt-resistant groups according to the individual blood pressure response to changes in dietary NaCl intake. The resulting data in salt-sensitive patients demonstrated divergent active and trypsin-activated kallikrein responses to a high-NaCl intake (i.e., active decreased while inactive increased). Because trypsin should activate the inactive form of kallikrein by mimicking the in vivo action of endogenous renal proteases, i.e., releasing an N-terminal epitope and thus activating the inactive pro-enzyme (20), it
TABLE 4. Plasma lipoprotein behavior during NaCl intake variations in the study populationa

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Salt-Sensitive (N = 19)</th>
<th>Salt-Resistant (N = 16)</th>
<th>Counterregulating (N = 8)</th>
<th>Normotensive Subjects (N = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal-NaCl Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>5.15 ± 0.55</td>
<td>5.03 ± 0.61</td>
<td>5.01 ± 0.67</td>
<td>4.75 ± 0.53</td>
</tr>
<tr>
<td>HDL Cholesterol (mmol/L)</td>
<td>1.23 ± 0.19</td>
<td>1.49 ± 0.20</td>
<td>1.50 ± 0.30</td>
<td>1.63 ± 0.29</td>
</tr>
<tr>
<td>LDL Cholesterol (mmol/L)</td>
<td>3.56 ± 0.48c</td>
<td>3.22 ± 0.52</td>
<td>3.16 ± 0.58</td>
<td>2.89 ± 0.21</td>
</tr>
<tr>
<td>VLDL Cholesterol (mmol/L)</td>
<td>0.36 ± 0.19</td>
<td>0.33 ± 0.16</td>
<td>0.33 ± 0.25</td>
<td>0.23 ± 0.09</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.83 ± 0.19</td>
<td>1.68 ± 0.21</td>
<td>1.71 ± 0.28</td>
<td>1.15 ± 0.16b</td>
</tr>
<tr>
<td>High-NaCl Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>5.19 ± 0.48c</td>
<td>5.01 ± 0.33</td>
<td>5.10 ± 0.42</td>
<td>4.63 ± 0.48</td>
</tr>
<tr>
<td>HDL Cholesterol (mmol/L)</td>
<td>1.25 ± 0.20c</td>
<td>1.46 ± 0.18</td>
<td>1.45 ± 0.27</td>
<td>1.68 ± 0.31</td>
</tr>
<tr>
<td>LDL Cholesterol (mmol/L)</td>
<td>3.53 ± 0.51c</td>
<td>3.20 ± 0.58</td>
<td>3.31 ± 0.46</td>
<td>2.74 ± 0.27</td>
</tr>
<tr>
<td>VLDL Cholesterol (mmol/L)</td>
<td>0.41 ± 0.18c</td>
<td>0.36 ± 0.20</td>
<td>0.34 ± 0.20</td>
<td>0.21 ± 0.10</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.07 ± 0.25d,e</td>
<td>1.77 ± 0.19</td>
<td>1.67 ± 0.22</td>
<td>1.09 ± 0.11b</td>
</tr>
<tr>
<td>Low-NaCl Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>5.18 ± 0.51c</td>
<td>4.97 ± 0.25</td>
<td>5.07 ± 0.38</td>
<td>4.58 ± 0.41</td>
</tr>
<tr>
<td>HDL Cholesterol (mmol/L)</td>
<td>1.24 ± 0.25c</td>
<td>1.45 ± 0.19</td>
<td>1.46 ± 0.19</td>
<td>1.65 ± 0.25</td>
</tr>
<tr>
<td>LDL Cholesterol (mmol/L)</td>
<td>3.57 ± 0.43d</td>
<td>3.18 ± 0.47</td>
<td>3.30 ± 0.42c</td>
<td>2.73 ± 0.23</td>
</tr>
<tr>
<td>VLDL Cholesterol (mmol/L)</td>
<td>0.37 ± 0.12c</td>
<td>0.34 ± 0.18</td>
<td>0.32 ± 0.18</td>
<td>0.20 ± 0.09</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.85 ± 0.31</td>
<td>1.67 ± 0.16</td>
<td>1.64 ± 0.24</td>
<td>1.11 ± 0.09b</td>
</tr>
</tbody>
</table>

a HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

b P < 0.05 versus other groups.
b P < 0.05 versus normotensive subjects.
b P < 0.05 versus salt-resistant hypertensive subjects.
b P < 0.01 versus other diets.

seems conceivable that an impaired inactive to active kallikrein conversion acts as a contributor to the reduced excretion of active kallikrein in salt-sensitive individuals. As a consequence, the impaired activation of the inactive pro-form of kallikrein should cooperate in determining the salt-sensitivity of blood pressure.

A pathogenic role in determining the individual blood pressure response to NaCl load has been proposed in humans for several genetic, familial, racial, sexual, ethnic, environmental, endocrine, cell membrane, and renal characteristics (reviewed in Reference 21). With regard to these factors, Bönnner et al. (3) and our group (4,9) have already indicated that a low urinary excretion rate of active kallikrein is combined with salt-sensitivity of blood pressure in normotensive (3) and hypertensive subjects (4,9). These reports interpreted the low kallikrein excretion as the cause of salt-sensitivity, and the reduced rate of kallikrein excretion as the direct consequence of an impaired renal production of active kallikrein.

According to this explanation, a low kallikrein excretion has been reported in salt-sensitive compared with salt-resistant Dahl rats (8). Furthermore, the reduction of active kallikrein excretion preceded salt-loading and the consequent development of hypertension (8). Oral administration of taurine, a low-molecular-weight organic constituent that is known to increase kallikrein synthesis in vivo (23), augmented active kallikrein excretion and prevented salt-induced hypertension in Dahl rats (23). Therefore, also considering that a simple transposition of these data to human salt-sensitive hypertension is not possible, a primary abnormality in kallikrein production seemed to us to be the most likely cause for both active kallikrein reduction and salt-sensitivity of blood pressure.

In contrast, at least in part, to our own explanation, no differences are detectable in trypsin-activated kallikrein levels when both salt-sensitive and salt-resistant Dahl rats were fed a 4% NaCl diet (8). Moreover, a report in hypertensive patients (7) and a recent study in Dahl salt-sensitive rats (24) indicated that active kallikrein excretion decreased, whereas inactive kallikrein resulted either unchanged (24) or significantly increased (7) after a high-NaCl diet.
kallikrein ratio. In particular, during the high-NaCl diet, active kallikrein decreased whereas trypsin-activated kallikrein increased in salt-sensitive patients. On the contrary, inactive and active kallikrein excretion rates varied in similar manner in salt-resistant patients during changes in individual NaCl intake. To further support our hypothesis, data obtained in a small control population of normotensive subjects showed similar results. In this context, the fact that the abnormal increment of trypsin-activated kallikrein becomes evident only when an individual receives a high-NaCl diet impiles that an impaired inactive to active kallikrein conversion cooperates in determining the susceptibility of blood pressure to NaCl intake in predisposed subjects.

The reasons leading to the divergent active and inactive kallikrein responses to a high-NaCl diet displayed by salt-sensitive patients are unclear. As is already known, human renal kallikrein is synthesized by renal tubular cells bound to a signal peptide that is cleaved off to produce an inactive precursor (1,20). The latter is secreted in urine and finally transformed into active kallikrein by renal proteases, which cleave the N-terminal activating eptapeptide at the Arg-Ile bond (6,19). On the other hand, active but not inactive kallikrein can be inactivated by kallistatin, a protein belonging to the serpin superfamily, and other kallikrein binding proteins (1). Thus, both an inactive pro-enzyme and an inactivator/active enzyme complex are present in human urine (1), but only the pro-enzyme is trypsin-activated in vitro (8), because of the proteolytic action of trypsin at the same Arg-Ile bond that is cleaved by endogenous renal proteases (20). As a consequence, the most likely explanation of our findings should be related to a defect in renal trypsin-like activating enzymes or to kallikrein gene polymorphisms and the consequent synthesis of abnormal enzyme precursors.

Although a kallikrein gene polymorphism has been described in spontaneously hypertensive rats (25) and differences in the isoelectric focusing pattern of kallikrein have been identified in urine samples from Dahl salt-sensitive rats (26), we have no data to support such intriguing hypotheses. In a similar way, we cannot exclude the possibility that one or more kallikrein inhibitor(s) can be secreted in exaggerated amount in salt-sensitive hypertensive patients having low kallikrein levels, as already demonstrated in low-renin and renoparenchymal hypertension (27). Indeed, we did not investigate the kallikrein-inhibitory activity of urine extracts from our patients, and we do not know whether or not the reduced excretion of active kallikrein is also a result of an abnormal production of kallikrein inactivator(s).

With regard to possible bias, our study design allowed us to exclude any confounding factor that could potentially alter the assessment of salt-sensitivity (21,22) and/or urine samples and kallikrein evaluation (1,11). Indeed, all aspects of the study were conducted after careful patient selection (i.e., exclu-
TABLE 5. Erythrocyte sodium transport system behavior during NaCl intake variations in the study population

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Normal-NaCl Intake</td>
<td>Na+/K+/2Cl− Cotransport (μmol/l cell per h)</td>
<td>505.2 ± 82.3</td>
<td>483.9 ± 90.7</td>
<td>506.6 ± 88.5</td>
</tr>
<tr>
<td></td>
<td>Na+/K+ Countertransport (μmol/l cell per h)</td>
<td>537.9 ± 102.7a</td>
<td>401.7 ± 82.3</td>
<td>409.3 ± 92.7</td>
</tr>
<tr>
<td></td>
<td>Na+/K+ ATPase (μmol/l cell per h)</td>
<td>5.12 ± 1.42</td>
<td>4.73 ± 2.13</td>
<td>4.75 ± 1.67</td>
</tr>
<tr>
<td>High-NaCl Intake</td>
<td>Na+/K+/2Cl− Cotransport (μmol/l cell per h)</td>
<td>524.6 ± 93.6b</td>
<td>478.9 ± 101.2</td>
<td>495.6 ± 97.2</td>
</tr>
<tr>
<td></td>
<td>Na+/K+ Countertransport (μmol/l cell per h)</td>
<td>568.9 ± 96.5a</td>
<td>421.5 ± 96.7</td>
<td>415.7 ± 96.4</td>
</tr>
<tr>
<td></td>
<td>Na+/K+ ATPase (μmol/l cell per h)</td>
<td>5.19 ± 1.55</td>
<td>4.23 ± 1.25</td>
<td>4.58 ± 1.85</td>
</tr>
<tr>
<td>Low-NaCl Intake</td>
<td>Na+/K+/2Cl− Cotransport (μmol/l cell per h)</td>
<td>513.2 ± 101.4</td>
<td>498.3 ± 98.7</td>
<td>503.7 ± 97.5</td>
</tr>
<tr>
<td></td>
<td>Na+/K+ Countertransport (μmol/l cell per h)</td>
<td>549.7 ± 100.2a</td>
<td>416.9 ± 98.4</td>
<td>411.3 ± 95.9</td>
</tr>
<tr>
<td></td>
<td>Na+/K+ ATPase (μmol/l cell per h)</td>
<td>5.12 ± 0.98</td>
<td>4.36 ± 1.01</td>
<td>4.61 ± 1.28</td>
</tr>
</tbody>
</table>

a P < 0.001 versus other groups.
b P < 0.05 versus normotensive subjects.

ing obesity, renal diseases, etc.), using a double-blind method, and by the use of a randomized crossover protocol, which excluded the influence of an order effect (22). Moreover, after each change in NaCl intake, blood and urine samples for hormonal assays as well as blood pressure measurements for salt-sensitivity assessment were taken after the achievement of sodium balance (23). In this context, although tailored for individual needs, each diet was rigorously isocaloric, and the NaCl content was controlled by repeated urinary NaCl evaluations (23). To exclude incorrect urine samples resulting from the menstrual cycle, and also taking into consideration the influence of the menstrual cycle on several hormones involved in renal sodium handling (28), hypertensive women were excluded from this study.

According to this rigorous approach, re-examination of 16 patients indicated a very high reproducibility of our procedure of evaluating blood pressure response to changes in dietary NaCl intake. Indeed, only 3 of 16 patients changed their class, compared with the first study.

To evaluate active kallikrein, we used the chromogenic method of Amundsen et al. (19), which is based on the cationization of urine with the artificial chromogenic substrate H-D-V-L-R-pNA and the following evaluation of the kallikrein’s ability to cleave amide bonds and release paranitroanilide. The good correspondence of this substrate method with the effective kininogenase activity of urine has already been demonstrated (8). According to this, we obtained a high correlation between the chromogenic and the kininogenase methods and the correspondence was similar for low, intermediate, and high values. As a consequence, we think our data were not the results of bias in patient classification, study design, or evaluation of kallikrein secretion.

With regard to the other hormonal findings of this study, according to previous data, the lowest baseline PRA concentrations and the smallest PRA increment after the low-NaCl intake were observed in salt-sensitive hypertensive patients (12,15), whereas counter-regulating patients reported the steepest rise in PRA after salt restriction (14). Interestingly, these latter patients also had the most evident reduction of plasma ANP with the low NaCl diet. Because ANP can inhibit renin release in man (29), it seems possible that a marked decrease of circulating ANP concentrations could have favored PRA overstimulation and the subsequent paradoxical behavior of blood pressure during salt restriction. On the contrary, in contrast to an early report by Campese et al. (30) but in agreement with more recent findings (14), we showed that circulating Na levels varied similarly during changes in dietary NaCl intake in each patient subgroup, thus indicating that an impaired suppressibility of the sympathetic nervous system is not relevant in determining either a salt-sensitive or a counterregulating behavior.

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of blood pressure. Nevertheless, evaluation of circulating NA concentrations might not reflect the participation of the sympathetic nervous system to the blood pressure response to changes in NaCl intake. Therefore, the exact role of the sympathetic nervous system in determining the blood pressure response to dietary NaCl intake awaits further clarifications by functional evaluation of its activity.

According to a previous study in normotensive subjects (14), the ANP increment after the high-NaCl diet was more pronounced in salt-sensitive than in salt-resistant hypertensive patients. In this regard, previous data (12) have also demonstrated that the ANP response to sodium load is a function of the renin status, being particularly evident in hypertensive patients with a low renin activity. Therefore, because atrial secretion of ANP is mainly a result of volume expansion (31), our data could support the hypothesis (14) that an impaired ability of the kidney to excrete a sodium load could induce a compensatory increase in natriuretic peptide release from the atrial myocytes.

In keeping with this interpretation, ANP changes were paralleled by those of plasma DLS, supporting the hypothesis that an impaired ability to modulate a sodium load may induce the secretion of an endogenous natriuretic substance(s) crossreacting with digoxin antibodies. From this pathophysiological point of view, the slightly suppressed erythrocyte Na⁺ pump activity manifested by salt-sensitive hypertensive patients could support the in vivo inhibitory activity of DLS on this sodium transport system. Nevertheless, although elevated DLS levels have already been described in salt-sensitive hypertensive patients (32), the effective role of this substance as a natriuretic agent (when not as a true inhibitor of the Na⁺ pump) is not undisputed, making any hypothesis on this argument absolutely arbitrary.

In agreement with previous findings in adult hypertensive patients (33) and in black adolescent patients (34), we found an increased activity of the Na⁺/K⁺/2Cl⁻ cotransport in salt-sensitive individuals. Although the possible mechanisms underlying the linkage between Na⁺/K⁺/2Cl⁻ cotransport and salt-sensitivity are unknown, the presence of the cotransporter also in vascular smooth muscle (35), endothelial (36), and renal tubular cell membranes (37) suggests that its accelerated activity could make an individual susceptible to salt-induced hypertension through increased vascular resistance and sodium reabsorption.

In the study presented here, we also confirmed previous findings by Weder (38), who described an increased activity of the Na⁺/Li⁺ countertransport in erythrocytes from salt-sensitive hypertensive patients. As is already known, the Na⁺/Li⁺ countertransport is a mode of operation of the cell membrane Na⁺/H⁺ antiport (39). Whether Na⁺/Li⁺ countertransport (or Na⁺/H⁺ antiport) acceleration is causally related to hypertension or simply an epiphenomenon of this condition is unclear. However, as for the Na⁺/K⁺/2Cl⁻ cotransport, several studies suggested that the Na⁺/Li⁺ countertransport could be accelerated also in vascular smooth muscle and proximal tubular cells, where it could lead to hypertension by increasing contractility, intracellular pH, cell growth and replication, and sodium reabsorption (39). In this context, our findings also support the relationships between Na⁺/Li⁺ countertransport and individual risk for hypertension and its complications (38,39). Indeed, salt-sensitive subjects showed the highest prevalence of first-degree relatives who were hypertensive patients or who suffered from myocardial infarction, along with the highest fasting and post-load plasma insulin, LDL cholesterol, and triglyceride concentrations, the lowest HDL cholesterol levels, and the greatest rate of albumin excretion. On the other hand, dietary NaCl intake variations did not significantly influence lipoprotein behavior in all groups, whereas triglyceride levels increased after intake of the high-NaCl diet in salt-sensitive patients. Our data are in agreement with a recent report by Bigazzi et al. (11). Contrarily, Ruppert et al. (40) reported an increase of total and LDL cholesterol after 8 days of salt deprivation in counterregulating normotensive subjects. However, Oberman et al. (41) reported no changes of cholesterol levels after a prolonged period of sodium restriction in a large cohort of hypertensive patients. Thus, additional data are required in order to define the lipid response to sodium deprivation in humans.

In this context, reduced levels of kallikrein excretion have been described in subjects who had a positive family history of hypertension (2,4), high body mass index (10), and elevated fasting insulin levels (4). Furthermore, Williams et al. (42) have already suggested that punctiform mutations may explain the segregating single-gene effects for low urinary kallikrein excretion, high sensitivity to environmental "hypertensinogenic" agents, fasting hyperinsulinemia and hypertriglyceridemia. Therefore, it seems possible that a low kallikrein activity may be present in the urine of susceptible individuals, who will develop both hypertension and metabolic disturbances in response to an "inappropriate" NaCl intake.

In conclusion, the study presented here demonstrates that an impaired inactive to active kallikrein conversion cooperates in determining the reduced active kallikrein excretion rate that is present in salt-sensitive individuals. Furthermore, we showed that salt-sensitivity is combined with the common features of insulin-resistant conditions, such as elevated Na⁺ / Li⁺ countertransport, fasting and post-load hyperinsulinemia, high serum concentrations of atherogenic lipoproteins, elevated albumin excretion rate, and increased prevalence of familial hypertension. As a consequence, we propose low active kallikrein and active/total kallikrein ratio on a high-NaCl intake as markers for salt-sensitivity. Moreover, we have speculated on a possible linkage among salt-sensitivity, low urinary excretion of active kallikrein, and increased risk for
renal and cardiovascular complications of hypertension.

Future longitudinal studies are requested in order to demonstrate whether the determination of active kallikrein excretion may represent an easily available marker to individual subjects who may develop salt-induced hypertension and its cardiorenal complications.

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REFERENCES