Elevated plasma and urinary endothelin-1 levels in human salt-sensitive hypertension

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1. The behaviour of the potent vasoconstrictive endothelium-derived peptide endothelin-1 was evaluated in salt-sensitive hypertension.

2. Circulating and urinary endothelin-1 levels were evaluated in 30 men (mean age 44.6 ± 3.1 years) with uncomplicated essential hypertension after three consecutive 2-week periods on an intermediate (120 mmol), low (20 mmol) and high (240 mmol) NaCl diet. On the same occasions, blood pressure was measured to identify salt-sensitive patients (n = 16), i.e. those patients showing a mean blood pressure increase > 10 mmHg when switching from a low to a high NaCl diet, and salt-resistant patients (n = 14), i.e. those who did not show such mean blood-pressure variations.

3. Plasma endothelin-1 levels were higher (P < 0.005) in salt-sensitive than in salt-resistant hypertensive patients after intermediate-, low- and high-NaCl diets. Urinary endothelin-1 excretion was similar in both groups after an intermediate-NaCl diet, whereas it was significantly higher in salt-sensitive than in salt-resistant hypertensive subjects after low (P < 0.002) and high (P < 0.007) NaCl diets. High NaCl intake induced a significant increase in plasma endothelin-1 levels (P < 0.002) as compared with intermediate and low NaCl diet levels in salt-sensitive patients, but did not in salt-resistant subjects. No significant NaCl intake-related variations of urinary endothelin-1 excretion were observed in either group.

4. Salt-sensitive hypertensives are characterized by increased levels of endothelin-1 in both plasma and urine. This fact suggests that blood-pressure sensitivity to NaCl intake could be associated with an increased risk of developing both renal and cardiovascular damage.

INTRODUCTION

Salt-sensitive hypertensive patients often manifest increased and poorly-suppressible activities of the sympathetic nervous system [1], fasting [2] and post-load [3] hyperinsulinaemia, elevated urinary albumin excretion [3, 4], serum low-density lipoprotein cholesterol and lipoprotein(a) levels [4], accelerated erythrocyte Na+/Li+ countertransport activity [5], decreased urine kallikrein [6] and serum high-density lipoprotein cholesterol concentrations [4].

Taken together, these abnormalities suggest that salt-sensitive hypertensive patients could be characterized by the cluster of renal, hormonal and metabolic derangements that might lead to greater cardiovascular and renal morbidities. In this context, functional and morphological endothelial changes are fundamental in the development of hypertension-related vascular damage [7].

According to this hypothesis, an elevation of the circulating levels of the potent endothelium-derived vasopressor peptide endothelin-1 (ET-1) [8] has already been described in conditions that are often characterized by salt sensitivity, such as non-insulin-dependent diabetes mellitus [9] and obesity [10]. Moreover, a preliminary report by our group showed increased basal levels of ET-1 in salt-sensitive hypertensive patients compared with salt-resistant ones [11]. However, the impact of different NaCl intakes on plasma and urinary ET-1 is unknown. The aim of this study was to evaluate plasma and urinary ET-1 levels in a selected cohort of non-diabetic, non-obese, never-treated essential hypertensive patients, after low, intermediate and high NaCl intake periods.

METHODS

Patients

Fifty-six never-treated caucasian men affected by mild to moderate essential hypertension [12] (age 30–60 years, mean 47.5 ± 4.6 years), who were seen in our outpatient clinic over a period of 2 years, gave their informed consent to participate in the study. Fifteen patients also participated in a prelimi-
nary study by our group [11]. Female patients were excluded to avoid fluctuation of plasma ET-1 levels due to the menstrual cycle [13], and because repeated urine collections (see study design) are not possible during menstruation. In a similar way, to exclude the influence of obesity [10] or impaired carbohydrate metabolism [9] on circulating ET-1, outpatients were recruited among those having a body mass index >19 and <27 kg/m² and a normal glucose tolerance (HbA1c <6.5%, absence of glycosuria and fasting and post-prandial plasma glucose levels <5.5 mmol/l and <9.8 mmol/l respectively). Patients with serum creatinine >110 \mu mol/l and/or proteinuria were excluded.

With regard to blood pressure levels, diastolic levels were required to be >95 and <114 mmHg for four different measurements performed at 1-week intervals, in sitting position. Systolic blood pressure levels were required to be >140 and <180 mmHg, under the same conditions. Five patients who did not fit one or both criteria were screened out. During the screening visits, patients answered standard questions concerning family history of hypertension and myocardial infarction according to a previously described methodology [6]. In brief, family information was firstly obtained from each patient and then confirmed in all cases by the primary care physician and, when possible, by other relatives of the patient. A positive family history was considered. In particular, echo-Doppler examinations of the limb and neck vessels testified the absence of atherosclerotic lesions in these areas. Twelve normal subjects participated as control group. Their inclusion criteria were the same as for the hypertensive patients.

Assessment of salt sensitivity and ET-1 response to changes in dietary NaCl intake

Salt sensitivity was assessed according to a randomized, double-blind, cross-over protocol. After the blood and urine samplings described above a standard hydrosalorhic diet with fixed NaCl content (20 mmol) was given to each participant. Dietary NaCl intake variations were performed by adding to the standard diet a daily supplement of 10 NaCl capsules. During the intermediate NaCl intake period, each capsule contained 10 mmol NaCl, so that the daily NaCl intake was of 120 mmol. Also in this case, patients were asked to provide 24-h urine collections obtained on the last 3 days of the week, for the evaluation of individual compliance. After 1 week on the above diet, 3 patients proved to be uncompliant, i.e. had a urinary NaCl excretion >130 mmol/24 h and were screened out. The remaining 34 patients were randomly, double-blindly assigned a high (220 mmol NaCl per day for 2 weeks) or a low (20 mmol NaCl per day for 2 weeks) NaCl intake, according to a previously described methodology [6], i.e. by changing the NaCl content of the capsules (0 mmol in the case of low and 20 mmol in the case of high NaCl intake). NaCl intake was the only variable introduced on the patients' diet, and other nutrients were maintained constant throughout the salt-sensitivity assessment phase. In particular, evaluation of patient compliance to each assigned diet was made by measurements of 24-h urinary calcium, potassium magnesium and sodium excretions on the last three days of the second week. With regard to sodium, patients were considered compliant when 24 h sodium excretion was >200 mmol and <30 mmol in all urine collections obtained during the high and
the low sodium intake regimes respectively. Four other patients were revealed as not compliant. Thus, the salt sensitivity assessment phase was successfully completed in 30 patients. The same urine collections as used for urinary NaCl were used for urinary albumin and ET-1 evaluations. On the last day of each different diet period, baseline blood samplings for plasma ET-1 were also repeated, as described above.

A patient was classified as salt-sensitive when a mean blood pressure difference of 10 mmHg or more between the low and the high NaCl intake was detected. Blood pressure measurements were made on the last two days of each diet by a Riva-Rocci sphygmomanometer and a stethoscope, after patients rested in a supine position for 15 min. Korotkoff phase I and V were used for systolic and diastolic blood pressures respectively. The first blood pressure measurement was not included, and the average of four consecutive mesurements, taken at 2 min intervals, was recorded.

Laboratory measurements

Circulating ET-1 levels were assessed as previously described [18]. Briefly, each plasma sample was injected onto a C18 octyloecylsilane column (Pharmacia, Uppsala, Sweden), activated with trifluoroacetic acid. The eluate (derived from two aliquots of 2.5 ml of plasma) was freeze-dried, reconstituted in starting HPLC buffer (15% acetonitrile in distilled HPLC water) and eluted by reverse-phase HPLC over 70 min using a linear gradient of 15–75% acetonitrile/0.1% trifluoroacetic acid in distilled water. The chromatographic separation of plasma eluates identified a single peak of ET-1, perfectly corresponding to the elution position of a human ET-1 standard and of 125I-ET-1. Fractions corresponding to ET-1 were collected at 1 min intervals and evaporated before reconstitution in assay buffer (50 mmol/l phosphate buffer, pH 7.4, containing 0.9% NaCl, 0.04% NaN3 and 0.3% BSA). Endothelin-1 immunoreactivity was assayed on reconstituted samples by RIA, using a rabbit antiendothelin-1 antibody (Peninsula Laboratories, Belmont, CA, U.S.A.), 125I-ET-1 (Peninsula Laboratories) and human endothelin-1 (Peptide Institute, Osaka, Japan) as standard. Inter-assay and intra-assay variations were <10%. Sensitivity was 0.1 pg/ml. Cross-reactivity of the ET-1 antibody with ET-2 and ET-3 was 7%, and with big-ET-1 (the precursor of ET-1) 17%, according to the supplier statement. Preliminary laboratory tests on urine samples from volunteers allowed us to establish that the RIA procedure for ET-1 determination on urine eluates gave the same results as the procedure performed after HPLC purification of urine. Thus, urine ET-1 levels were assessed as for plasma, without the HPLC procedure.

Urinary albumin excretion was measured by nephelometry.

All the above laboratory procedures, as well as all blood samplings during each test, were performed by separate groups of researchers, who were unaware of the study design, purpose and results.

**Statistical analysis**

Data were stored in a PC and analysed by the software Primit (McGraw Hill Co, New York, NY, U.S.A., 1992). Differences among groups were tested for significance by one-way analysis of variance followed by the Newman–Keuls test for pairwise comparisons. For multiple comparisons, the analysis of variance followed by post hoc analysis for adjusting the significance level was used. Linear regression and correlation were used to test relations between two variables. Descriptive parameters were tested for significance by the X2 method. Statistical significance was considered as a P value <0.05. Unless otherwise stated, data are presented as mean ± SD.

**RESULTS**

**Baseline data**

The general characteristics of the study population are given in Table 1. As is shown, 16 patients proved to be salt-sensitive and 14 salt-resistant. A family history of hypertension was more frequent in salt-sensitive than in salt-resistant patients, while no other significant differences were found.

On the initial NaCl diet, i.e. 120 mmol NaCl per day, plasma and urinary ET-1 levels were similar in hypertensive and in normotensive subjects [plasma, 0.87±0.36 and 0.65±0.16 pg/ml respectively; not significantly different (n.s.); urine, 82.34±20.65 and 73.21±19.31 pg/min respectively (n.s.)]. However, when data were analysed separately for salt-sensitive and salt-resistant hypertensive patients, the former manifested the greater levels of both plasma and urinary ET-1 (Fig. 1). Similarly, urinary albumin levels were not different between the whole hypertensive group and normotensive subjects (11.81±4.01 compared with 9.53±2.74 µg/min respectively; n.s.), but when hypertensive patients were divided according to the blood pressure response to dietary NaCl changes, urinary albumin excretion was higher (P<0.007) in salt-sensitive (13.43±4.0 µg/min) than in salt-resistant (9.97±3.23 µg/min) individuals, and correlated with plasma ET-1 levels (Fig. 2).

**Effects of NaCl intake changes**

Salt-sensitive patients showed the widest variations of both systolic (intermediate NaCl diet, 161.7±11.1 mmHg; low NaCl diet, 155.4±12.3 mmHg; high NaCl diet, 168.7±14.8 mmHg;
Table 1. General characteristics of the study population (mean ± SD). *P<0.05 compared with other groups; †P<0.01 compared with normotensive subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypertensive subjects</th>
<th>Hypertensive subjects</th>
<th>Normotensive subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salt sensitive</td>
<td>Salt resistant</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>16</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.3 ± 2.1</td>
<td>43.7 ± 3.9</td>
<td>44.6 ± 2.4</td>
</tr>
<tr>
<td>Family history of hypertension</td>
<td>12/4*</td>
<td>5/9</td>
<td>2/10</td>
</tr>
<tr>
<td>(yes/no)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.1 ± 1.1</td>
<td>24.7 ± 1.4</td>
<td>23.8 ± 1.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>161.7 ± 11.1†</td>
<td>155.3 ± 9.7†</td>
<td>131.7 ± 7.4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>104.8 ± 2.1†</td>
<td>101.2 ± 3.2†</td>
<td>78.9 ± 3.0</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>74.7 ± 5.3</td>
<td>71.6 ± 5.4</td>
<td>72.1 ± 3.5</td>
</tr>
<tr>
<td>Blood urea nitrogen (mmol/l)</td>
<td>5.3 ± 0.7</td>
<td>5.5 ± 0.4</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>86.2 ± 8.3</td>
<td>84.9 ± 7.2</td>
<td>80.2 ± 9.7</td>
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<td>Fasting glucose (mmol/l)</td>
<td>4.89 ± 0.21</td>
<td>4.81 ± 0.20</td>
<td>4.72 ± 0.25</td>
</tr>
<tr>
<td>Fasting insulin (mmol/l)</td>
<td>102.3 ± 27.3</td>
<td>98.5 ± 28.9</td>
<td>88.9 ± 15.7</td>
</tr>
</tbody>
</table>

P<0.02) and diastolic (intermediate NaCl diet, 104.8 ± 2.1 mmHg; low NaCl diet, 96.6 ± 2.8 mmHg; high NaCl diet, 107.3 ± 3.1 mmHg; P<0.001) blood pressure. On the other hand, in salt-resistant hypertensive patients NaCl intake variations poorly affected both systolic (intermediate NaCl diet, 155.3 ± 9.7 mmHg; low NaCl diet, 153.4 ± 10.8 mmHg; high NaCl diet, 161.5 ± 11.4 mmHg; n.s.) and diastolic (intermediate NaCl diet, 101.2 ± 5.2 mmHg; low NaCl diet, 99.4 ± 3.8 mmHg; high NaCl diet, 102.5 ± 3.5 mmHg; n.s.) blood pressure. Similar findings were obtained in normotensive subjects (systolic: intermediate NaCl diet, 131.7 ± 7.4 mmHg; low NaCl diet, 128.7 ± 8.9 mmHg; high NaCl diet, 134.6 ± 10.3 mmHg; n.s.; diastolic: intermediate NaCl diet, 78.9 ± 3.0 mmHg; low NaCl diet, 77.2 ± 4.5 mmHg; high NaCl diet, 82.4 ± 5.1 mmHg; n.s.). Low sodium intake induced minor changes of plasma ET-1 in both groups (Fig. 1, upper panel). In contrast, the high NaCl diet increased plasma ET-1 levels in salt-sensitive but not in salt-resistant patients (Fig. 1, upper panel).

Urinary ET-1 excretion did not change after dietary NaCl intake variations in any of the groups (Fig. 1, bottom panel). Urinary albumin excretion tended to increase with NaCl loading in salt-sensitive (low NaCl intake, 12.10 ± 3.60 µg/min; high NaCl intake, 15.26 ± 3.89 µg/min; P<0.001), but not in salt-resistant patients (low NaCl intake, 9.50 ± 3.14 µg/min; high NaCl intake, 10.26 ± 3.33 µg/min; n.s.) and control subjects (low NaCl intake, 8.95 ± 2.42 µg/min; high NaCl intake, 10.08 ± 3.34 µg/min; n.s.).

DISCUSSION

In the present study we demonstrate that both circulating and urinary ET-1 concentrations are higher in salt-sensitive than in salt-resistant non-obese, non-diabetic, essential hypertensive men. The increased levels of a circulating substance which could exert detrimental effects at the vascular level, favouring both atherogenesis [7] and vasoconstriction [19], suggest salt-sensitive hypertensive subjects as a patient subset at increased risk of developing hypertension-related cardiovascular damage.

In this context, a gold standard technique for assessing endothelial damage is not currently available, and neither functional assessments of endothelium-dependent vasorelaxation [20] nor vascular biopsies were carried out in this study. Furthermore, the elevated concentrations of both plasma and urine ET-1 in salt-sensitive patients could simply reflect the mildly higher blood pressure levels on a 120 mmol NaCl diet and the increased pressor response to a high sodium diet. Nevertheless, a close correlation among the degree of vascular damage, as evaluated by biopsies of the skin microvessels, local and circulating ET-1 and von Willebrand factor concentrations has been already demonstrated in diabetics, before the onset of overt diabetic vasculopathy [21]. Moreover, identical findings have been obtained for an acute form of endothelial damage, as it occurs in patients with Mediterranean spotted fever [22]. In a similar manner, two studies have indicated elevated levels of ET-1 in plasma from patients with diabetic nephropathy [23] and retinopathy [24] and in atherosclerotic subjects [14]. Thus, it seems reasonable to interpret our data as a clear indication that salt sensitivity is characterized by marked endothelial damage and an increased risk of developing cardiovascular damage.

Therefore, elevated levels of ET-1 should be added to a cluster of renal, hormonal and metabolic derangements [3] that are expected to increase cardiovascular morbidity and mortality of salt-sensitive subjects.
The presence of elevated albumin excretion also suggests that salt-sensitive patients are a patient subset at increased risk of developing hypertension-related renal damage. Indeed, increased urinary albumin excretion is combined with an increased prevalence of cardiovascular events in insulin-dependent diabetes [26] and non-insulin-dependent diabetes [27]. Moreover, a direct correlation among elevated urinary albumin excretion, circulating von Willebrand factor and cardiovascular events have been described in non-insulin-dependent diabetic patients [28]. Furthermore, urinary albumin excretion is a good predictor of progressive renal disease in patients with diabetic nephropathy [29]. Thus, the elevated urinary albumin excretion manifested by salt-sensitive patients could be interpreted as a marker of more severe risk of the progression of renal damage.

In this context, elevated albumin excretion is also combined with a significant increase in circulating ET-1 levels in non-insulin-dependent diabetics [23], suggesting the intriguing hypothesis that the peptide could contribute to the development of hypertension-related renal damage. Consistent with this theory, urinary ET-1 levels were also higher in salt-sensitive than in salt-resistant patients. Indeed, since

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**Fig. 1.** Plasma (upper panel) and urinary (bottom panel) ET-1 levels in salt-sensitive hypertensives (S, n = 16), salt-resistant hypertensives (R, n = 14) and control subjects (C, n = 12) after 2 weeks on an intermediate (120 mmol), low (20 mmol) and high (220 mmol) NaCl diet. Patients were subdivided according to their blood pressure response to dietary NaCl intake variations (see Methods section). a = P < 0.005 compared with other groups; b = P < 0.002 compared with other diets; c = P < 0.002 compared with other groups; d = P < 0.007 compared with other groups.
circulating ET-1 is filtered across the glomerular barrier and destroyed by endopeptidase 24.11 (EC 3.4.24.11) [30], urinary ET-1 is believed to derive from tubular production. Therefore, it is possible that the increased levels of urinary ET-1 manifested by salt-sensitive patients could reflect increased vascular production of the peptide, which in turn could negatively influence the progression of hypertension-related renal damage. Accordingly, plasma and urine ET-1 levels were directly correlated in salt-sensitive but not in salt-resistant patients (Fig. 2). In contrast to this hypothesis, Hoffman et al. [31] recently reported reduced rather than increased urine ET-1 levels in salt-sensitive hypertensive subjects. Nevertheless, several patients in that study were blacks and/or women, and the discrepancy with our findings could simply reflect the different study populations. The increased levels of urinary ET-1 also suggest that locally produced peptide might contribute to salt sensitivity. Indeed, ET-1 has been reported to act in an autocrine fashion to alter renal ET-1 function during different NaCl diets.

On the other hand, as already described by Hoffman et al. [31], we confirmed that urinary ET-1 excretion did not change during different NaCl diets. In our opinion, this finding is not surprising and simply reflects the inability of blood pressure to affect renal ET-1 production. According to this explanation, blood pressure increments due to either angiotensin II or phenylephrine infusions were not accompanied by significant modifications of urinary ET-1 excretion [34].

In conclusion, the current study demonstrates that salt-sensitive hypertensive patients are characterized by increased levels of ET-1 in both plasma and urine, supporting the hypothesis that blood pressure sensitivity to NaCl intake is combined with an increased risk of developing both renal and cardiovascular damage.

REFERENCES


