Endothelial Dysfunction in Salt-Sensitive Essential Hypertension

Ernesto Bragulat, Alejandro de la Sierra, María Teresa Antonio, Antonio Coca

Abstract—The aim of this study was to evaluate endothelium-dependent and -independent vasodilation, as well as endothelium biochemical markers, in a group of essential hypertensive patients classified on the basis of salt sensitivity. Changes in forearm blood flow in response to acetylcholine, sodium nitroprusside, and $N^G$-monomethyl-L-arginine (L-NMMA) infusion were determined by means of strain-gauge plethysmography. Moreover, plasma and urinary concentrations of nitrates, cGMP, and endothelin were measured during low (50 mmol/d) and high (250 mmol/d) salt intake. Salt-sensitive hypertension was diagnosed in 26 patients who exhibited a significant increase in 24-hour mean blood pressure assessed by ambulatory blood pressure monitoring after 1 week of high salt intake. Nineteen patients were considered salt resistant. Compared with salt-resistant hypertensives, salt-sensitive patients presented a significant lower ($P = 0.005$) maximal acetylcholine-induced vasodilation (21±6.3 versus 28±7.5 mL · 100 mL$^{-1}$ · tissue · min$^{-1}$). On the contrary, maximal sodium nitroprusside–induced vasodilation did not significantly differ between groups (22.4±4.5 versus 23.9±5.3 mL · 100 mL$^{-1}$ · tissue · min$^{-1}$). The decrease in maximal acetylcholine-induced vasodilation promoted by the coadministration of L-NMMA was significantly more pronounced in salt-resistant compared with salt-sensitive patients ($P = 0.003$). Finally, high salt intake promoted a significant decrease in 24-hour urinary nitrate excretion in salt-sensitive patients (from 443±54 to 312±54 μmol/d; $P = 0.033$) compared with salt-resistant hypertensives (from 341±50 to 378±54 μmol/d). We conclude that salt-sensitive hypertension is associated with endothelial dysfunction characterized by a defective endothelium-dependent vasodilation. Impairment of the L-arginine–nitric oxide pathway may be responsible for this abnormal endothelial response. (Hypertension. 2001;37[part 2]:444-448.)

Key Words: endothelium ■ salt sensitivity ■ hypertension ■ nitric oxide ■ dietary salt

Several epidemiological and interventional studies have demonstrated a clear relationship between salt intake and hypertension. However, blood pressure (BP) response to increase the maintenance of vascular tone. Abnormalities in endothelial function, in the pathogenesis of salt sensitivity.

With respect to salt-induced hypertension, animal studies have demonstrated a clear relationship between dietary salt and hyper- tension. Although salt sensitivity is well established in experimental and human hypertension, the pathophysiological mechanisms leading to such individual susceptibility remain unresolved. It has been suggested that abnormalities in the renin-angiotensin system, sympathetic nervous system, and transmembrane sodium transport are all involved in the pathogenesis of salt sensitivity.

The vascular endothelium seems to play a critical role in the maintenance of vascular tone. Abnormalities in endothelium-derived factors, especially the nitric oxide (NO) system, are implicated in both experimental and essential hypertension. With respect to salt-induced hypertension, animal studies have demonstrated that blockade of NO production favors the development of salt-sensitive hypertension. Studies in essential hypertensive patients have suggested that high salt intake and/or salt sensitivity is associated with impaired endothelial function. In fact, high salt intake is able to decrease both plasma levels and urinary excretion of nitrates, indirect measurements of NO production. Two previous studies measured endothelium-dependent vasodilation (EDV) in the forearm of normotensive and hypertensive individuals in relation to salt intake. Whereas Stein et al found no effect of salt intake in methacholine-induced vasodilation in a group of healthy subjects, Miyoshi et al reported a decrease in acetylcholine-induced forearm vasodilation in salt-sensitive hypertensive subjects regardless of the level of salt intake.

The aim of the present study was to evaluate forearm EDV and endothelium-independent vasodilation (EIV), assessed by strain-gauge plethysmography, as well as several indirect endothelium biochemical markers, in a group of essential hypertensive patients classified on the basis of salt sensitivity.

Methods

Selection of Patients

The study population included 45 never-treated essential hypertensive patients consecutively recruited from the Hypertension Unit, Hospital Clinic, Barcelona, Spain. There were 25 men and 20 women.

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with a mean age of 44 years (range, 27 to 59 years). The diagnosis of essential hypertension was considered if seated arterial BP (after 10 minutes of rest) measured by mercury sphygmomanometer 3 times at 1-week intervals was consistently >140/90 mm Hg. Secondary forms of hypertension were excluded by routine diagnostic procedures. Subjects with hypercholesterolemia (total cholesterol >6.5 mmol/L [250 mg/dL]), diabetes mellitus, impaired renal function (serum creatinine >132 μmol/L [1.5 mg/dL]), or previous history of coronary or cerebrovascular diseases were excluded from the study. Moreover, patients who drank >40 g/d of ethanol, as well as women taking oral contraceptives or estrogen replacement therapy, were excluded.

**Study Design**

All patients gave informed consent. The protocol was approved by the Ethics Committee of the Hospital Clinic and by the Spanish Health Authority (protocol FIS 00/0435). Essential hypertensive patients were placed on a baseline low-salt diet containing 50 mmol Na+ during 14 days. This diet was supplemented in a single-blind fashion by placebo tablets during the first 7 days (low-salt period) and by NaCl tablets 200 mmol/d (high-salt period) during the following 7 days. Thus, the total NaCl intake during the high-salt period was 250 mmol/d. The amount of nitrates was also adjusted to a total daily intake of <400 mmol/d. This was achieved by excluding food items that contain a high concentration of nitrate, ie, cured meat, cheese, and green leafy vegetables, as has been previously described. Compliance with the diet was assessed twice a week by measurement of 24-hour urinary Na+ excretion.

On the last day of both the low- and high-salt periods, 24-hour ambulatory BP monitoring was performed with an automated, noninvasive oscillometric device (SpaceLabs 90207, SpaceLabs Inc). BP was registered automatically at 15-minute intervals for 24 hours. Salt-sensitive hypertension was defined by a significant increase (P<0.05; >4 mm Hg) of 24-hour mean BP from low to high salt intake.5 7

**Laboratory Measurements**

A venous blood sample was obtained on the last day of both the low- and high-salt periods after 12 hours of fasting and 1 hour of bedrest with the patient in the recumbent position. This prolonged fasting period has been shown to be necessary for a meaningful measurement of plasma nitrate concentration.14 Serum and urine concentrations of nitrates and nitrites (NOx) were determined by the fluorometric method of Misko et al.19 The fluorescent signal was measured in a fluorometer (Perkin Elmer) at excitation and emission wavelengths of 365 and 425 nm, respectively. Plasma and urine concentrations of cGMP were measured in a fluorometer (Perkin Elmer) at excitation and emission wavelengths of 365 and 425 nm, respectively. Plasma and urine concentrations of cGMP were measured by Pearson’s correlation coefficient.

**Results**

**General Characteristics of Essential Hypertensive Patients Studied**

Table 1 shows the clinical characteristics of the 45 essential hypertensive patients included in the study. No differences were observed in terms of age, sex distribution, body mass index, and baseline office systolic and diastolic BPs between salt-sensitive and salt-resistant hypertensive patients. The effect of L-NMMA on baseline FBF, maximal response to acetylcholine, and sodium nitroprusside in the whole group of patients was analyzed by means of paired Student’s t test. The correlation between changes in 24-hour mean BP and different laboratory parameters was assessed by Pearson’s correlation coefficient.

**Diagnosis of Salt-Sensitive Hypertension**

Salt-sensitive hypertension was diagnosed in 26 patients in whom 24-hour mean BP significantly increased (P<0.05) when switched from low to high salt intake. The mean increase in 24-hour mean BP was 8.8±4.5 mm Hg (from 103±13 to 112±14 mm Hg at the end of the low-salt period; Table 2). The remaining 19 essential hypertensive patients were diagnosed as having salt-resistant hypertension. The change in 24-hour mean BP was −0.9±4.1 mm Hg (from 104±13 to 103±12 mm Hg). Table 2 also shows systolic and diastolic BPs during low and high salt intakes in salt-sensitive and salt-resistant essential hypertensive patients.

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**Table 1. Baseline Clinical Characteristics of Salt-Resistant and Salt-Sensitive Essential Hypertensive Patients**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Salt Resistant (n=19)</th>
<th>Salt Sensitive (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>44.7±11.3</td>
<td>43.2±11.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75±12</td>
<td>79±12</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.6±2.4</td>
<td>26.8±4.7</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>160±12</td>
<td>163±17</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>93±5</td>
<td>96±7</td>
</tr>
</tbody>
</table>

BMI indicates body mass index.
Effect of Salt Intake on Endothelium-Derived Factors in Salt-Sensitive and Salt-Resistant Essential Hypertensive Patients

Table 3 shows plasma levels and 24-hour urinary excretion of endothelium-derived factors at low and high salt intakes in salt-sensitive and salt-resistant essential hypertensive patients. As shown, 24-hour urinary excretion of nitrates significantly decreased with high salt intake only in salt-sensitive patients (from 443±54 to 312±54 μmol/d) but not in salt-resistant hypertensives (from 341±50 to 378±54 μmol/d; P=0.033 for salt-induced variation between groups). Salt-induced changes on the remaining endothelium derived factors (plasma nitrates, cGMP, endothelin, and 24-hour urinary excretion of cGMP) did not significantly differ between the 2 groups.

FBF in Response to Acetylcholine, Sodium Nitroprusside, and L-NMMA

Figure 1 (left) shows the increase in FBF induced by acetylcholine (EDV) in both groups. As shown, the maximal vasodilation induced by acetylcholine was significantly blunted (P=0.005 for dose-response curves between groups) in salt-sensitive essential hypertensive patients (from 4.6±0.9 at baseline to 21.6±3.6 mL · 100 mL⁻¹ · tissue · min⁻¹ with the maximal doses of acetylcholine) with respect to salt-resistant hypertensives (from 4.7±1.2 to 28.7±5.5 mL · 100 mL⁻¹ · tissue · min⁻¹). Moreover, maximal acetylcholine-induced vasodilation inversely correlated with the 24-hour mean BP increase during high salt intake (R=−0.393; P=0.010). On the contrary, as shown in Figure 1 (right), the increase in FBF induced by sodium nitroprusside (EIV) did not display significant differences between salt-sensitive (from 4.4±0.8 to 22.4±4.5 mL · 100 mL⁻¹ · tissue · min⁻¹) and salt-resistant (from 4.6±0.9 to 23.9±5.3 mL · 100 mL⁻¹ · tissue · min⁻¹) hypertensive patients.

EDV and EIV were then measured with the addition of the nitric oxide synthase inhibitor L-NMMA. In the whole group of essential hypertensive patients studied, L-NMMA infusion promoted a significant decrease (P<0.001) in baseline blood flow (from 4.8±1.1 to 3.1±0.7 mL · 100 mL⁻¹ · tissue · min⁻¹). Moreover, the maximal response to acetylcholine decreased in the presence of L-NMMA (from 24.3±7.8 to 19.2±7.2 mL · 100 mL⁻¹ · tissue · min⁻¹; P<0.001). However, the addition of L-NMMA did not significantly modify the maximal response to sodium nitroprusside (from 23.1±5.2 to 22.8±5.3 mL · 100 mL⁻¹ · tissue · min⁻¹; P=0.963).

Figure 2 shows the effect of L-NMMA on EDV in salt-resistant (left) and salt-sensitive (right) essential hypertensive patients. The inhibitory effect of L-NMMA on maximal EDV was significantly lower in salt-sensitive patients (from 21.3±6.4 to 18.6±8.1 mL · 100 mL⁻¹ · tissue · min⁻¹) compared with salt-resistant hypertensives (from 28.4±8 to 20.1±6.5 mL · 100 mL⁻¹ · tissue · min⁻¹; P=0.003 comparing L-NMMA effect on EDV between groups).

Finally, in both salt-resistant and salt-sensitive essential hypertensive patients, contralateral FBF did not significantly change throughout the drug infusion (data not shown).

Discussion

The present results demonstrate a greater impairment in EDV and a lesser effect of L-NMMA on this EDV in salt-sensitive essential hypertensive patients compared with salt-resistant patients. Furthermore, the urinary excretion of nitrates showed a significant decrease with salt loading only in

Table 2. The 24-Hour BPs During Low and High Salt Intakes in Salt-Resistant and Salt-Sensitive Essential Hypertensive Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Salt Resistant (n=19)</th>
<th>Salt Sensitive (n=26)</th>
<th>Total (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP, mm Hg</td>
<td>Low Salt</td>
<td>High Salt</td>
<td>Low Salt</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>137±19</td>
<td>137±17</td>
<td>135±14</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>85±9</td>
<td>85±10</td>
<td>85±13</td>
</tr>
<tr>
<td>Mean BP</td>
<td>104±13</td>
<td>103±12</td>
<td>103±13</td>
</tr>
</tbody>
</table>

Table 3. Endothelium-Derived Factors During Low and High Salt Intakes in Salt-Resistant and Salt-Sensitive Essential Hypertensive Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Salt Resistant (n=19)</th>
<th>Salt Sensitive (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrates, nmol/mL</td>
<td>41.6±14.7</td>
<td>33.1±13.7</td>
</tr>
<tr>
<td>cGMP, nmol/L</td>
<td>5.9±3.4</td>
<td>6.7±4.0</td>
</tr>
<tr>
<td>Endothelin, pg/mL</td>
<td>5.6±2.0</td>
<td>5.1±2.7</td>
</tr>
<tr>
<td>24-h urinary excretion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrates, μmol/d</td>
<td>341±231</td>
<td>378±255</td>
</tr>
<tr>
<td>cGMP, nmol/d</td>
<td>511±295</td>
<td>522±235</td>
</tr>
</tbody>
</table>

*P=0.033 for salt-induced variation between groups.
salt-sensitive patients. These results suggest that the NO system is implicated in the pathogenesis of human salt-sensitive hypertension.

It is known that endothelial cells play a critical role in the maintenance of vascular wall tone. Studies performed in essential hypertensive patients have consistently shown an impairment in EDV. This impaired response is probably due to a decrease in NO availability, as suggested by the fact that the addition of the NO synthase inhibitor L-NMMA has no or only a minimal effect on EDV. Furthermore, compared with normotensive control subjects, hypertensive patients present a decrease in plasma concentration and urinary excretion of nitrates and cGMP, which can be considered indirect markers of NO production.

Animal studies have also suggested that salt-induced hypertension may be linked to a defective EDV. A blunted increase in NO production in response to dietary salt has been observed in genetically determined salt-sensitive rats. Moreover, chronic NO inhibition enhances the pressor effect of dietary salt. However, studies performed in humans at different levels of salt intake or in salt-induced hypertension have yielded conflicting results.

Campese et al. first studied a group of 27 essential hypertensive and 7 normotensive African Americans during low and high salt intake. They measured plasma nitrate and found a decrease in this NO metabolite after high salt intake. No differences were observed between salt-sensitive and salt-resistant hypertensives in plasma nitrate at either low or high salt intakes or changes induced by a high-salt diet. In contrast to these results, 2 other studies demonstrated a relationship between urinary nitrate excretion and salt-induced BP elevation. Facchinetti et al. studied a group of 19 healthy subjects during low and high salt intakes. Although urinary nitrate excretion did not significantly change with high salt intake in the whole group of subjects, they found an inverse correlation between BP elevation and the change in urinary nitrate excretion induced by a high-salt diet. Furthermore, Fujisawa et al. have recently confirmed this relationship. In a group of Japanese essential hypertensive patients classified on the basis of salt sensitivity, high salt intake significantly decreased urinary nitrate excretion. Moreover, BP change during salt loading inversely correlated with the change in urinary nitrate excretion, and salt-sensitive hypertensive patients displayed significantly lower values of urinary nitrate at the end of high salt intake. Our results confirm these previous observations in a larger group of white essential hypertensives. Patients classified as salt sensitive showed significantly decreased urinary nitrate excretion at the end of high-salt period, in contrast to salt-resistant hypertensives. It is important to note, however, that measurements of plasma or urinary NO metabolites are only indirect indicators of NO production and that their alterations do not necessarily reflect endothelial dysfunction. In this sense, we found no differences between salt-sensitive and salt-resistant patients in other endothelium-derived factors, such as endothelin or cGMP.

Changes in FBF in response to acetylcholine have become the gold standard method to measure endothelial function in humans. Two previous studies have addressed the effect.
of salt intake on endothelium-dependent and -independent response. Stein et al\textsuperscript{15} studied 7 healthy subjects during low and high salt intakes. Forearm vasodilation in response to the endothelium-mediated methacholine did not significantly change at the end of the high-salt diet. Surprisingly, however, sodium nitroprusside vasodilation, a response not mediated by endothelial cells, was stimulated by dietary salt. In contrast, Miyoshi et al\textsuperscript{16} reported an impairment in acetylcholine-induced vasodilation in 6 salt-sensitive essential hypertensive patients compared with 9 salt-resistant hypertensives, although the addition of L-NMMA on baseline FBF showed no differences between groups. Our results also demonstrate an impairment of EDV in salt-sensitive compared with salt-resistant essential hypertensive patients. However, in contrast to the results of Miyoshi et al\textsuperscript{16} we also observed significant differences between groups in the acetylcholine-induced vasodilation after the infusion of L-NMMA. In fact, L-NMDA infusion attenuated the maximal EDV in salt-resistant patients 29\%, whereas salt-sensitive patients 36\%, with the attenuated effect of L-NMMA in salt-sensitive hypertensives, although the addition of L-NMMA on baseline FBF without examining its effect on maximal acetylcholine response. The decreased acetylcholine response, together with the attenuated effect of L-NMMA in salt-sensitive hypertensive patients, observed in the present study strongly suggests an impairment in the l-arginine–NO pathway.

We have also observed a modest but significant inverse relationship between acetylcholine-induced vasodilation and BP response to salt intake. As described in animal models with salt-sensitive hypertension,\textsuperscript{11} the partial inability to increase NO production in response to high salt intake could be partially linked to the BP increase with salt and thus with the development of salt-sensitive hypertension. Although we also observed an inverse correlation between acetylcholine-induced increase in FBF and the absolute BP value obtained at the end of high salt intake (data not shown), this correlation may be due not only to salt sensitivity but also to the severity of high BP.

In conclusion, the present study reports a defective EDV in salt-sensitive hypertension. This observation, together with an attenuated effect of the NO synthase inhibitor L-NMMA on maximal endothelium-mediated increase in FBF, suggests that the l-arginine–NO pathway is implicated in the development of salt-sensitive hypertension in humans.

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References
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