Salt-Induced Plasticity in Cardiopulmonary Baroreceptor Reflexes in Salt-Resistant Hypertensive Patients

Bruno Trimarco, Giuseppe Lembo, Bruno Ricciardelli, Nicola De Luca, Virgilio Rendina, Gianluigi Condorelli, and Massimo Volpe

To investigate the effects of salt loading on cardiopulmonary and arterial baroreceptor reflexes, 34 hypertensive patients underwent two 4-day periods with different dietary sodium intakes (70 and 370 meq/day). The patients were classified as salt-sensitive or salt-resistant depending on whether the mean arterial pressure value obtained on day 4 of high salt intake did or did not increase by 8% or more. In 22 patients cardiopulmonary and carotid baroreceptor reflexes were assessed during each dietary period by measuring the reflex responses to the application of −10 mm Hg lower body negative pressure and of +60 mm Hg increase in neck tissue pressure. Salt-resistant patients (n=16) retained less sodium than salt-sensitive patients (n=6) and showed a reduction in plasma norepinephrine and forearm vascular resistance during high sodium intake, whereas the salt-sensitive patients did not. During low sodium diet, no significant differences could be detected in the reflex responses to cardiopulmonary and carotid baroreceptor unloading between the two groups. High salt diet, however, potentiated the gain of cardiopulmonary baroreceptor reflex, which was expressed as the increase in plasma norepinephrine or forearm vascular resistance per millimeter of mercury decrease in pulmonary capillary wedge pressure, only in the salt-resistant hypertensive patients. In addition, the atrial natriuretic factor response to changes in pulmonary capillary wedge pressure was significantly enhanced by high salt intake only in the salt-resistant hypertensive patients. The reflex responses to carotid baroreceptor unloading were unaffected by salt loading in either group. In the remaining 12 patients, the hemodynamic effects of graded lower body negative pressure (−5, −10, −15 mm Hg) and neck tissue positive pressure (+30, +45, +60 mm Hg) were tested for both diets. Again, high salt intake significantly potentiated the cardiopulmonary baroreceptor reflex gain, expressed as the slope of the linear correlation between the changes in forearm vascular resistance (mm Hg/ml/min/100 g) and pulmonary capillary wedge pressure (mm Hg), in salt-resistant (from 3.8±0.9 to 7.2±1.0, p<0.05) but not in salt-sensitive patients (from 4.2±0.9 to 3.2±0.6, NS). In conclusion, the present study demonstrates that high salt diet potentiates cardiopulmonary baroreceptor reflexes and enhances atrial natriuretic factor response in salt-resistant but not in salt-sensitive hypertensive patients. The salt-induced plasticity of cardiopulmonary baroreceptor reflexes may exert a protective effect against the development of salt-induced hypertension by augmenting the reflex vasodilatory response to volume expansion. Conversely, the lack of this compensatory potentiation in cardiopulmonary baroreceptor reflex function in salt-sensitive hypertensive patients might contribute to salt sensitivity. (Hypertension 1991;18:483–493)
the recent demonstration that in salt-sensitive hypertensive patients, high sodium intake does not suppress plasma norepinephrine. This finding may also account for inadequate decrease in vascular resistance in response to the increased cardiac output, which in turn may be responsible for the rise in blood pressure elicited by an increase in sodium intake. The mechanisms underlying these abnormalities, however, are still unclear.

In experimental animals it has been demonstrated that high salt diet sensitizes cardiopulmonary and aortic baroreceptors in salt-resistant but not in salt-sensitive Dahl rats, suggesting that the inadequate reduction in sympathetic discharge during sodium loading may be secondary to a dysfunction of compensatory baroreceptor reflex mechanisms. Victor et al speculated that salt-induced plasticity in baroreceptor reflex function might help protect against salt-induced hypertension.

In the present study, to clarify whether abnormalities of the baroreceptor reflex mechanisms contribute to salt sensitivity of blood pressure in humans, we evaluated the effects of sodium loading on cardiopulmonary and arterial baroreceptor reflexes in salt-sensitive and salt-resistant hypertensive patients.

We have recently reported a possible functional interaction between atrial natriuretic factor and cardiopulmonary baroreceptors showing that in hypertensive patients with left ventricular hypertrophy, changes in the plasma concentration of this peptide compensate at least partially for the hemodynamic consequences of cardiopulmonary receptor impairment. Therefore, we also investigated the effects of salt loading on plasma levels of atrial natriuretic factor in control conditions and during cardiopulmonary receptor unloading.

Methods

Study Population

The study was performed in 34 patients (22 men and 12 women, mean age 31±2 years) with established mild-to-moderate, uncomplicated essential hypertension. In these patients, diastolic blood pressure readings were above 95 mm Hg and below 115 mm Hg in at least five consecutive readings obtained in the outpatient clinic. None of the patients had received any treatment for at least 3 weeks before the study. Blood pressure was measured with the patient in the sitting position, after a 10-minute rest in a darkened room, by means of a standard sphygmomanometer with a cuff of appropriate size, and following the recommendations of the American Heart Association. Secondary hypertension had been previously ruled out in all patients by laboratory and x-ray studies. Also, the existence of major diseases other than hypertension was excluded. All patients were fully informed about the procedures and the aim of the study, and informed written consent consistent with the rules of the ethical committee on clinical investigation of our institution was obtained in all cases. Since it has been demonstrated that the development of hypertension-induced left ventricular hypertrophy is associated with an impairment in cardiopulmonary receptor responsiveness, we excluded from the study the patients who fulfilled the echocardiographic criteria for left ventricular hypertrophy.

The patients were hospitalized and were given a daily diet containing 70 meq sodium, 70 meq potassium, 65 g protein, 50 g fat, 270 g carbohydrate, and 100 mg phosphorus as meat, eggs, bread, vegetables, and fruit. Personal food preferences were allowed as much as possible. Water intake was allowed as desired. Sodium balance was verified daily on the basis of sodium intake and urinary sodium output. In particular, the achievement of sodium balance was demonstrated by the lack of significant differences in sodium excretion between day 3 and 4 of each sodium regimen. On day 4 the patients underwent the first study session. Two days before the study session, the patients were familiarized with the lower body negative pressure (LBNP) device and the neck chamber. Patients were requested to refrain from cigarette smoking and coffee drinking during the 12 hours immediately before the study session. After the first study session, all patients continued on the diet for a second 4-day period and received a 300 meq/day sodium supplementation (divided in three doses of 100 meq crystalline sodium chloride each wrapped in wafers). During this period all measurements were repeated as previously described, and on the last day a second study session was performed following the same protocol used for the first session.

Procedures

The studies were performed in a quiet room with the temperature kept between 22° and 24°C, after an overnight fast, on days 4 and 8 of hospitalization. No premedication was administered. On arrival at the laboratory, forearm volume was measured by water displacement. Then the patients assumed the supine position with the right arm supported at the midhumerus level, relaxed and opened in the control environment, and had electrocardiographic (ECG) leads attached for ECG monitoring. An LBNP chamber, similar to that described by Mark and Kerber, was placed over the lower portion of each subject’s body, from the iliac crest down, to apply LBNP to unload cardiac baroreceptors. Under local anesthesia with 2% lidocaine, a heparinized arterial catheter was introduced percutaneously into the left brachial artery for direct measurement of systemic blood pressure. Mean arterial pressure was obtained by the integration of the pulsatile trace over periods of 5 seconds. A three-lumen Swan-Ganz thermodilution catheter was introduced through an antecubital vein and positioned under fluoroscopy with the distal tip into the pulmonary artery and the proximal hole in the right atrium for pressure monitoring. Heart rate was taken from an ECG lead monitored continuously during the study, and the patients were asked to breathe regularly. Systemic arterial pressure as well
as right atrial and pulmonary capillary wedge pressures (PCWP) were continuously measured with Statham P23Db (Spectramed Inc., Oxnard, Calif.) pressure transducers and were recorded simultaneously with the ECG on a multichannel polygraph at a paper speed of 100 mm/sec. Forearm blood flow (expressed in ml/min/100 g) was measured by strain-gauge plethysmography using a Digimatic DM2000 (Medimatic, Copenhagen, Denmark) with a mercury-in-Silastic strain gauge applied around the arm. The strain gauge was placed 4–5 cm below the antecubital crease. Forearm blood flow was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating the cuff at the upper arm. The pressure in the venous occlusion or congesting cuff at the upper arm was 60 mm Hg. To eliminate hand blood flow, a wrist cuff was inflated both before determining blood flow and continuously throughout the measurements. Forearm vascular resistance (FVR) (expressed in mm Hg/ml/min/100 g) was calculated by dividing mean arterial pressure (in mm Hg) by forearm blood flow.

Blood losses and fluid administration did not exceed 250 ml.

Protocols

In 22 patients (14 men and eight women, mean age 30±4 years), we assessed the hormonal and hemodynamic effects of one level (−10 mm Hg) of LBNP and the hemodynamic response to +60 mm Hg increase in neck-tissue pressure.

The cardiopulmonary baroreceptor reflex sensitivity is commonly assessed by the slope of the relationship between changes in atrial filling pressure and the corresponding changes in FVR induced by the application of graded LBNPs. To achieve steady-state hormonal responses, each level of LBNP should be maintained for at least 15 minutes. Since the application of multiple sustained levels of LBNP would have made our protocol exhausting for the patients, we decided to adopt a more tolerable procedure for studying cardiopulmonary baroreceptor reflexes. In preliminary experiments, we assessed the linearity of the forearm reflex response to LBNP by evaluating the hemodynamic changes induced by −5, −10, and −15 mm Hg LBNP each applied for 15 minutes, and we found that the slope of this regression was significantly correlated with the ratio between the change in FVR induced by a single LBNP level of −10 mm Hg and the corresponding change in PCWP (r=0.92, n=8, p<0.001). Thus, in this section of our study a single level of LBNP (−10 mm Hg) was applied for 15 minutes.

Baseline hemodynamic measurements included systemic arterial pressure, right atrial pressure, PCWP, heart rate, and forearm blood flow.

Twenty minutes after catheter placement, baseline measurements were obtained. Blood samples for determination of plasma renin activity (PRA) and norepinephrine as well as plasma atrial natriuretic factor (ANF) concentrations were obtained from an antecubital vein. Blood sampling and hemodynamic measurements were performed during the last 3 minutes of LBNP. Systemic hemodynamic responses induced by reduction in carotid transmural pressure were evaluated by applying an increase in external neck pressure (60 mm Hg) in about 1 second by means of a pneumatic chamber similar to that previously described by Ludbrook et al.13 This stimulus was maintained for 120 seconds. The 60 mm Hg increase in neck tissue pressure was adopted to avoid changes in cerebral blood flow and the involvement of chemoreceptors in the reflex effect.13 Blood pressures and heart rate were monitored continuously during the stimulus. Forearm blood flow was measured once before the stimulus and again during the last 30 seconds of positive neck pressure. Interventions were performed in random order.

To further investigate the interactions between salt intake and cardiopulmonary and arterial baroreceptor reflexes in the remaining 12 hypertensive patients (eight men and four women, mean age 32±3 years), we assessed the hemodynamic effects of graded LBNPs (−5, −10, −15 mm Hg each applied for 5 minutes) and progressive increases in neck tissue positive pressure (+30, +45, +60 mm Hg each applied for 2 minutes) during both low (70 meq/day) and high (370 meq/day) dietary salt intakes.

Systemic arterial pressure, right atrial pressure, PCWP, heart rate, and forearm blood flow were measured in control conditions and during the last 3 minutes of each level of LBNP. The reflex responses to graded carotid baroreceptor unloading were assessed by changes in RR interval, systemic blood pressure, and forearm blood flow, recorded as described above.

Hormonal Measurements

PRA was measured by radioimmunoassay, according to the method described by Menard and Catt14 (sensitivity 50 pg/tube angiotensin I, intra-assay and interassay variability coefficients 6% and 10%, respectively). Plasma norepinephrine assay was performed with reverse-phase, high-performance liquid chromatography with electrochemical detection.8 The samples were previously extracted and concentrated by adsorption onto activated alumina15 and the catecholamines were eluted with perchloric acid 0.1 M. Analytical recovery, using dihydroxybenzylamine (Aldrich Chemical Co., Milwaukee, Wis.) as internal standard, ranged from 63% to 75%. The chromatograms were obtained with a 30 cm×4 mm column of reverse-phase material (Bondapak C18 Waters Chromatography Division, Milford, Mass.) and the ESA 5100A model (Environmental Sciences Associates, Bedford, Mass.) as electrochemical detector. The mobile phase, delivered by a Powerline 600 pump (Waters), was a citrate buffer. The detection limit of the assay was 10 pg. Intra-assay and interassay variation coefficients for norepinephrine were 6.3% and 12.1%, respectively. Plasma immunoreactive ANF was determined by radioimmunoassay as previously.
Described, using rabbit antisera (RAS 8798, Peninsula Laboratories, Belmont, Calif.), iodinated human ANF-(99-126) (2,000 Ci/mmol, Amersham, Buckinghamshire, Great Britain), and human ANF-(99-126) (Bissendorf, Wedemark, FRG) as a standard. ANF was extracted from plasma by using Sep-Pak C18 cartridges (Waters). The recoveries, determined on each plasma sample by adding to it a minimal amount of radiolabeled ANF, ranged from 71% to 90%. The peptide retained on the column was eluted with 80% acetonitrile in 0.1% trifluoroacetic acid. The eluates were dried overnight in a speed-vac evaporator (Savant Instrument, Farmingdale, N.Y.) and reconstituted in radioimmunoassay buffer (phosphate buffer 0.1 M, pH 7.4, containing 0.3% bovine serum albumin, 0.1% Triton X-100, and 0.1% sodium azide). The assay was a sequential radioimmunoassay with delayed addition of tracer. Bound/free separation was carried out by using ice-cold, dextran-coated charcoal 1.5% in radioimmunoassay buffer. Results were calculated from standard curves of bound/free peptide counts per minute versus log ANF standard and were corrected for internal recoveries. Intraassay and interassay variation coefficients were 6.6% and 10.5%, respectively. The radioimmunoassay sensitivity was 3 pg/tube.

Data Analysis

To analyze the hemodynamic effects of the increase in neck tissue pressure, we measured systolic and diastolic blood pressure, RR interval, and forearm blood flow in control conditions, that is, in the 30 seconds preceding the change in neck tissue pressure, and during the late or steady-state response, that is the average value in the last 30 seconds of the increase in neck tissue pressure.

The individual values of each parameter obtained in basal conditions and in response to the single level of LBNP before and after sodium loading were compared by Student's t test for paired observations. Also the responses to the single level of increase in neck tissue pressure were compared by paired t tests.

Comparisons between the study groups were performed by using unpaired t tests. To evaluate the ability to release ANF in response to salt loading in either group of patients, we plotted the absolute change in plasma ANF induced in each patient by the increase in salt intake against the corresponding change in PCWP, that is, the main determinant of ANF secretion. The comparison of the regression lines obtained in the two groups was performed by standard analysis. In the study performed with graded LBNP levels and progressive increases in neck tissue pressure, the responses obtained in each group during both low and high sodium dietary regimens were analyzed by two-way analysis of variance. Whenever F ratios were significant at 5%, Duncan's multiple range test was used for a posteriori comparisons of the responses. Individual cardiopulmonary baroreceptor reflex gains were estimated by the linear regression obtained by plotting the changes of FVR against the corresponding changes of PCWP. Chronotropic and vascular carotid sinus baroreceptor reflex gains were assessed by the linear regressions obtained by plotting the changes in RR interval and FVR, respectively, against the level of positive pressure applied to neck tissues. In all instances, the correlation coefficient was greater than 0.80. The mean slopes obtained in each group during low sodium diet was compared with that obtained in the same group during high salt intake by paired t test. Comparisons between groups were performed by unpaired t test. Data are presented as mean±SEM.

Results

The 34 patients were classified as salt-sensitive or salt-resistant on the basis of the mean arterial pressure response when their dietary sodium intake varied from "low" (i.e., 70 meq/day) to "high" (i.e., 370 meq/day). The blood pressure response to salt was evaluated throughout the hospitalization by serial sphygmomanometric measurements of blood pressure according to the procedures reported in the previous section. The measurement of blood pressure obtained on day 4 of each diet was used to determine blood pressure response to salt. The values for the outpatient screening of blood pressure, creatinine clearance, urinary sodium excretion, and body weight obtained during the patients' ad libitum diet were similar in the two groups (Table 1).

<table>
<thead>
<tr>
<th>Measured variable</th>
<th>Salt-sensitive (n=10)</th>
<th>Salt-resistant (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>76±2</td>
<td>73±2</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>161±7</td>
<td>155±4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>101±1</td>
<td>101±1</td>
</tr>
<tr>
<td>Urinary sodium excretion (meq/day)</td>
<td>191±10</td>
<td>168±11</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>119±8</td>
<td>103±6</td>
</tr>
</tbody>
</table>

Studies Performed With -10 mm Hg Lower Body Negative Pressure and +60 mm Hg Increase in Neck Tissue Pressure

The salt-sensitive group had a mean age of 25±3 years (range, 18–35 years) and a female/male ratio of 2:4. The salt-resistant patients had a mean age of 32±2 years (range, 18–48 years) and female/male ratio of 4:12. No significant difference in clinical and laboratory characteristics could be detected between the two groups.

During low sodium intake, mean cumulative sodium loss, calculated as the sum of daily urinary sodium excretion minus sodium intake, was similar for salt-sensitive and salt-resistant patients: −81±37 and −53±7 meq, respectively, NS. During high sodium intake, mean cumulative sodium retention, the sum of sodium intake minus daily urinary sodium excretion, was significantly higher in salt-sensitive as
TABLE 2. Hemodynamic and Hormonal Effects of -10 mm Hg Lower Body Negative Pressure in Salt-Sensitive Hypertensive Patients During Low or High Sodium Intake

<table>
<thead>
<tr>
<th>Measured variable</th>
<th>Low sodium intake</th>
<th>High sodium intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>LBNP</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>101±5</td>
<td>103±4</td>
</tr>
<tr>
<td>PP (mm Hg)</td>
<td>40±5</td>
<td>39±5</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>72±4</td>
<td>75±4</td>
</tr>
<tr>
<td>RAP (mm Hg)</td>
<td>2.5±0.1</td>
<td>0.5±0.1†</td>
</tr>
<tr>
<td>PCWP (mm Hg)</td>
<td>6.3±0.7</td>
<td>3.2±0.5†</td>
</tr>
<tr>
<td>FBF (ml/min/100 g)</td>
<td>2.7±0.4</td>
<td>2.3±0.3†</td>
</tr>
<tr>
<td>FVR (mm Hg/ml/min/100 g)</td>
<td>40±5</td>
<td>50±7†</td>
</tr>
<tr>
<td>NE (pg/ml)</td>
<td>286±40</td>
<td>346±39†</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>2.3±0.3</td>
<td>3.2±0.7†</td>
</tr>
<tr>
<td>ANF (pg/ml)</td>
<td>34±5</td>
<td>28±5†</td>
</tr>
</tbody>
</table>

Each value represents mean±SEM. n=6. LBNP, lower body negative pressure; MAP, mean arterial pressure; PP, pulse pressure; HR, heart rate; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; FBF, forearm blood flow; FVR, forearm vascular resistance; NE, plasma norepinephrine concentration; PRA, plasma renin activity; ANF, plasma atrial natriuretic factor concentration.

*p<0.01 as compared with the corresponding value during low sodium intake.

compared with salt-resistant patients (298±32 and 191±23 meq, respectively, p<0.01). Finally, in both groups the rise in sodium intake was accompanied by an increase in body weight, which tended to be greater in salt-sensitive as compared with salt-resistant patients, although statistical significance was not achieved (+1.5±.2 versus +1.0±0.3 kg).

Six of the 22 patients who participated in this protocol showed increases in mean blood pressure greater than 8% (from 9.3 to 15.8%; mean change, 13.1%; p<0.01) and were defined as salt-sensitive; the remaining 16 patients who had mean blood pressure changes less than 8% (from -5.3 to +3.7%; mean change, -1.1%; NS) were defined as salt-resistant.

Hemodynamic Effects of Salt Loading

In salt-sensitive patients the increase in dietary sodium intake induced a rise in systolic and diastolic blood pressure (from 128±8/87±3 to 148±10/96±4 mm Hg; p<0.01) that was associated with an increase in forearm blood flow and no change in FVR (Table 2). In contrast, systolic and diastolic blood pressures were not raised by high salt intake in salt-resistant patients (from 145±4/98±2 to 143±4/97±1 mm Hg; NS) in whom a significant decrease in FVR counterbalanced an increase in forearm blood flow not statistically different from that observed in salt-sensitive patients (Table 3). The increase in sodium intake did not modify heart rate in either group (Tables 2 and 3).

During low sodium diet, there were no significant differences in right atrial pressure and PCWP between the two study groups (Tables 2 and 3). However, in salt-sensitive patients high salt intake caused greater increases in these parameters than those observed in

TABLE 3. Hemodynamic and Hormonal Effects of -10 mm Hg Lower Body Negative Pressure in Salt-Resistant Hypertensive Patients During Low or High Sodium Intake

<table>
<thead>
<tr>
<th>Measured variable</th>
<th>Low sodium intake</th>
<th>High sodium intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>LBNP</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>113±2</td>
<td>113±2</td>
</tr>
<tr>
<td>PP (mm Hg)</td>
<td>47±3</td>
<td>47±3</td>
</tr>
<tr>
<td>HR (beats/min)</td>
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<td>70±3</td>
</tr>
<tr>
<td>RAP (mm Hg)</td>
<td>2.4±0.3</td>
<td>0.2±0.4*</td>
</tr>
<tr>
<td>PCWP (mm Hg)</td>
<td>7.0±0.5</td>
<td>4.5±0.5*</td>
</tr>
<tr>
<td>FBF (ml/min/100 g)</td>
<td>2.5±0.2</td>
<td>2.0±0.1*</td>
</tr>
<tr>
<td>FVR (mm Hg/ml/min/100 g)</td>
<td>48±3</td>
<td>60±4*</td>
</tr>
<tr>
<td>NE (pg/ml)</td>
<td>204±18</td>
<td>259±18*</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>2.4±0.4</td>
<td>3.1±0.5*</td>
</tr>
<tr>
<td>ANF (pg/ml)</td>
<td>46±15</td>
<td>38±13†</td>
</tr>
</tbody>
</table>

Each value represents mean±SEM. n=16. LBNP, lower body negative pressure; MAP, mean arterial pressure; PP, pulse pressure; HR, heart rate; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; FBF, forearm blood flow; FVR, forearm vascular resistance; NE, plasma norepinephrine concentration; PRA, plasma renin activity; ANF, plasma atrial natriuretic factor concentration.

*p<0.01 LBNP vs. base.

†p<0.01 as compared with the corresponding value during low sodium intake.
were compared, the slope obtained in salt-resistant patients (SS) (solid line) and salt-resistant (SR) (dotted line) hypertensive patients. Significant correlation coefficients were obtained in both groups. Slope of regression obtained in SR was significantly higher than in SS patients (p<0.05).

Hormonal Effects of Salt Loading

During low sodium intake, there was no significant difference in plasma norepinephrine concentrations between the two study groups (Tables 2 and 3). However, plasma norepinephrine levels decreased significantly in salt-resistant but not in salt-sensitive patients while given a high sodium diet. Thus, a significant difference in plasma norepinephrine concentration was detected between the two study groups in this phase of the study (salt-sensitive, 235±25 pg/ml; salt-resistant, 147±11 pg/ml, p<0.01). PRA was comparable in the two groups during the low sodium diet and decreased at a similar extent when sodium intake was increased (Tables 2 and 3). Plasma ANF concentrations were similar in the two groups given both low and high sodium diets (Tables 2 and 3). To relate the ability to suppress PRA and to increase ANF levels in response to volume expansion, we plotted the individual changes in plasma ANF induced by -10 mm Hg LBNP in both groups (salt-sensitive, +9±3; salt-resistant, +11±1). Furthermore, there were significant reductions of plasma ANF levels that were comparable in the two groups (ANF: salt-sensitive, -6±1 pg/ml; salt-resistant, -9±4 pg/ml; NS).

During high sodium intake in salt-sensitive patients, the fall in right atrial pressure and PCWP as well as the responses of plasma norepinephrine, ANF, and calculated FVR to LBNP were comparable with those observed during low sodium diet (Table 2). Due to the marked suppression of PRA induced by salt loading, in both groups the responses of this parameter to -10 mm Hg LBNP were reduced.

In salt-resistant patients the increases in plasma norepinephrine concentration (+69±5 versus +54±5 pg/ml; p<0.05), and FVR (+13±1 versus +11±1 mm Hg/ml/min/100 g; p<0.05) induced by LBNP were potentiated during high sodium intake. Finally, the fall in plasma ANF induced by -10 mm Hg LBNP in salt-resistant patients during high sodium diet was more marked as compared with that observed in response to the same stimulus during low sodium diet (-24±6 versus -9±4 pg/ml; p<0.01 (Table 3). To calculate the cardiopulmonary baroreceptor reflex gain, we used the ratios between changes in plasma norepinephrine or FVR versus the corresponding changes in PCWP. All calculated ratios coherently showed that in salt-sensitive patients no significant changes in cardiopulmonary baroreceptor reflex gain were observed when the patients underwent chronic salt loading (Figure 2). On the contrary, in salt-resistant patients the increase in dietary sodium intake significantly potentiated all indexes of cardiopulmonary baroreceptor reflex sensitivity (Figure 2). Also the ratio between the changes in plasma ANF and in PCWP in response to -10 mm Hg LBNP did not increase significantly when salt-sensitive patients were shifted from low to high salt intake (Figure 2). On the contrary, in salt-resistant patients also this ratio was significantly increased with salt loading (Figure 2). Comparisons of these ratios between the two groups on low salt diet did not show significant differences. In contrast, both the gains of cardiopulmonary responses and of ANF response became significantly greater in the salt-resistant than in salt-sensitive patients during high salt diet (Figure 2).
Figure 2. Graphs show effects of chronic high salt intake on reflex responses to -10 mm Hg lower body negative pressure, expressed as ratios between changes in forearm vascular resistance (FVR) (mm Hg/ml/min/100 g), plasma norepinephrine (NE) (pg/ml), or plasma atrial natriuretic factor (ANF) levels (pg/ml) and the corresponding changes in pulmonary capillary wedge pressure (PCWP) (mm Hg) in salt-sensitive (SS) (solid line) and salt-resistant (SR) (dotted line) hypertensive patients. Each point represents mean ±SEM. SS, n=6; SR, n=16. *p<0.01 low vs. high sodium intake.

Effects of Neck Pressure Increase

When a +60 mm Hg positive neck pressure was applied during low sodium diet, both salt-sensitive and salt-resistant patients showed significant and comparable increases of mean arterial pressure and calculated FVR and a reduction in heart period (Table 4). During high sodium diet carotid baroreceptor unloading caused in both groups changes of mean arterial pressure, heart period, and FVR comparable to those observed while on low sodium diet (Table 4).

Studies Performed With Multiple Levels of Lower Body Negative Pressure and Neck Pressure

This section of the study was designed to further characterize the baroreceptor reflex response and was performed in eight salt-resistant and four salt-sensitive hypertensive patients as defined by the mean arterial pressure response to increased dietary salt intake (mean changes: salt-resistant, -4±4%; salt-sensitive, +12±1%). The outpatient screening values of systolic and diastolic blood pressures, urinary sodium excretion and creatinine clearance were comparable in the two groups (data not shown). The values of mean blood pressure, body weight, urinary sodium excretion, and central and peripheral hemodynamics recorded in the two groups of patients when the daily dietary sodium intake was increased from 70 to 370 meq are shown in Table 5.

In both groups graded LBNP (-5, -10, -15 mm Hg) did not modify heart rate and mean arterial pressure, while right atrial pressure, pulse pressure, and forearm blood flow were significantly reduced during both low and high salt intakes (data not shown). As shown in Figure 3, during low sodium diet, graded LBNP induced comparable changes in PCWP and FVR in the two groups. On the contrary, during high sodium intake, the LBNP-induced increase in FVR was potentiated only in salt-resistant patients. Accordingly, during low salt diet, the mean slope of the FVR/PCWP regression line obtained in salt-sensitive patients during graded LBNP was com-

<table>
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<tr>
<th>Measured variable</th>
<th>Low sodium intake</th>
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<tbody>
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<td></td>
<td>Base</td>
<td>Neck</td>
</tr>
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<td>MAP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>102±4</td>
<td>109±5*</td>
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<tr>
<td>SR</td>
<td>114±2</td>
<td>121±2*</td>
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<td>HP (msec)</td>
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<tr>
<td>SS</td>
<td>903±4</td>
<td>803±38*</td>
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<td>SR</td>
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<td>FBF (ml/min/100 g)</td>
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<td>SS</td>
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<td>SR</td>
<td>2.5±0.1</td>
<td>1.8±0.1*</td>
</tr>
<tr>
<td>FVR (mm Hg/ml/min/100 g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>41±4</td>
<td>64±8*</td>
</tr>
<tr>
<td>SR</td>
<td>48±4</td>
<td>71±6*</td>
</tr>
</tbody>
</table>

Each value represents mean±SEM. Salt-sensitive (SS) patients, n=6; salt-resistant (SR) patients, n=16. MAP, mean arterial pressure; HP, heart period; FBF, forearm blood flow; FVR, forearm vascular resistance.

* p<0.01 LBNP vs. base.

† p<0.01 as compared with the corresponding value during low sodium intake.
TABLE 5. Effects of Increased Dietary Salt Intake (From 70 to 370 meq/day) in Salt-Sensitive and Salt-Resistant Hypertensive Patients

<table>
<thead>
<tr>
<th>Measured variable</th>
<th>Low (n=4)</th>
<th>High (n=8)</th>
<th>Low (n=4)</th>
<th>High (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>100±1</td>
<td>112±1*</td>
<td>114±2</td>
<td>110±5</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>77.2±3</td>
<td>78.6±3*</td>
<td>75.6±2</td>
<td>76.5±3*</td>
</tr>
<tr>
<td>Un,V (meq/24 hr)</td>
<td>65±8</td>
<td>381±15*</td>
<td>55±5</td>
<td>376±9*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>71±1</td>
<td>66±1</td>
<td>73±3</td>
<td>66±2</td>
</tr>
<tr>
<td>PCWP (mm Hg)</td>
<td>8.8±0.4</td>
<td>10.0±0.2*</td>
<td>8.8±1.3</td>
<td>9.9±0.5*</td>
</tr>
<tr>
<td>FBF (ml/min/100 g)</td>
<td>2.3±0.2</td>
<td>3.3±0.5*</td>
<td>2.2±0.2</td>
<td>2.6±0.1*</td>
</tr>
</tbody>
</table>

Each value represents mean±SEM. SS, salt-sensitive; SR, salt-resistant; MAP, mean arterial pressure; Un,V, urinary sodium excretion; HR, heart rate; PCWP, pulmonary capillary wedge pressure; FBF, forearm blood flow.

*p<0.01 as compared with the corresponding value during low sodium intake.

parable to that of salt-resistant patients (Figure 4). In contrast, during high salt diet the mean slope of the FVR/PCWP regression line increased in salt-resistant but not in salt-sensitive patients. Thus, during this phase cardiopulmonary baroreceptor reflex sensitivity was significantly higher in salt-resistant than in salt-sensitive patients (Figure 4).

When graded positive pressure levels were applied on neck tissues during both low and high salt diet significant increases of mean arterial pressure and FVR and reductions of heart period were observed in both groups. The sensitivity of carotid baroreceptor reflexes assessed by the slope of the regression line obtained by plotting the changes in FVR (mm Hg/ml/min/100 g) or in heart period (msec) against the corresponding levels of positive pressure (mm Hg) applied to neck tissues was similar in the two groups while on low sodium diet (FVR slope: salt-sensitive, 0.46±0.1; salt-resistant, 0.37±0.1; NS; heart period slope: salt-sensitive, -1.56±0.4; salt-resistant, -1.64±0.2; NS) and remained unchanged during high salt intake in both salt-resistant and salt-sensitive patients (FVR slope: salt-sensitive, 0.52±0.1; salt-resistant, 0.37±0.1; NS; heart period slope: salt-sensitive, -1.61±0.2; salt-resistant, -1.63±0.2; NS).

**Discussion**

The patients with essential hypertension enrolled for our study, in keeping with previous reports, fall into two subgroups: about 70% are resistant to high sodium intake and do not display a rise in mean blood pressure, and the remaining 30% are sensitive.
to high sodium intake and display significant increments in arterial pressure. The favored pathogenetic model of hypertension resulting from salt intake is based on the cybernetic framework of Guyton. According to this model in early hypertension, cardiac output increases in response to renal sodium retention. Thereafter, because of general total body autoregulation, the peripheral resistance increases, blood pressure increases further, diuresis ensues, and cardiac output decreases to normal.

Indeed, we found that during salt loading, sodium retention was greater in salt-sensitive than in salt-resistant hypertensive patients thus suggesting a possible greater increase in cardiac output in these patients. Analysis of the hemodynamic data, however, did not show any significant difference in the response of forearm blood flow between the two groups. The greater increase in right atrial pressure observed in salt-sensitive patients could be accounted for by a reduction of venous capacitance, as described in sodium-sensitive subsets of the normotensive and borderline hypertensive groups.

The increase in blood pressure observed in salt-sensitive patients could be accounted for by the lack of an appropriate fall in vascular resistance. This finding is consistent with previous data showing that during salt loading, cardiac index increases equally in salt-sensitive and salt-resistant hypertensive patients, but the fall in peripheral resistance is twofold greater in the latter group. This phenomenon could be ascribed to structural impairment of the vascular bed in salt-sensitive hypertensive patients since Takeshita et al observed that reactive hyperemic forearm blood flow was significantly smaller in salt-sensitive than in salt-resistant hypertensive patients. However, Sullivan and his coworkers reported that sodium-sensitive subjects are able to decrease resistance significantly after isotonic exercise, to levels near those of sodium-resistant subjects, indicating that the lack of a significant fall of vascular resistance during salt loading in salt-sensitive hypertensive patients could not be entirely ascribed to permanent structural changes but mostly to impaired vascular adjustments to volume expansion.

The results of our study may contribute to elucidate the mechanisms underlying this phenomenon by showing that long-term high salt diet potentiates the response to LBNP in salt-resistant but not in salt-sensitive patients. Mark and Kerber suggested that the reflex response induced by LBNP largely results from the reduction of the tonic inhibitory influence of cardiopulmonary and arterial baroreceptors on vaso-motor centers. In particular, low levels of LBNP decrease central venous pressure and PCWP but have no significant effect on mean arterial pressure, whereas higher levels of LBNP also decrease arterial pressure. Cardiopulmonary receptors with sympathetic afferents mediate mainly excitatory influences. LBNP should reduce the discharge of these latter receptors, thereby promoting an inhibitory response rather than the excitatory response observed in our study. Cardiopulmonary receptors with vagal afferents appear to exert an inhibitory influence on vasomotor discharge. These receptors are sensitive to changes in cardiac filling pressure. LBNP would be expected to decrease the inhibitory input from these receptors thus increasing sympathetic activity. Although the precise type and location of cardiopulmonary receptors that mediate the reflex response to LBNP cannot be determined from this type of study, we believe that the vasomotor response elicited by LBNP results from the selective unloading of cardiopulmonary receptors with vagal afferents. Therefore, the finding that in salt-resistant hypertensive patients, a comparable reduction in right atrial and PCWPs caused a greater increase in plasma norepinephrine and forearm vascular resistance during salt loading suggests the existence of a salt-induced plasticity of cardiopulmonary baroreceptor reflexes in humans, as already described in Dahl salt-resistant rats. Further, the finding that high sodium diet failed to augment the response to cardiopulmonary receptor unloading in salt-sensitive patients suggests the lack of salt-induced plasticity in cardiopulmonary baroreceptor reflexes in these patients. Alternatively, data reported by Ferrario et al suggest that sodium may affect the baroreceptor reflex loop also by a central mechanism or by acting directly on the sympathetic efferent limb.

On the contrary, in hypertensive patients carotid baroreceptor response was not affected by salt loading in either study group. This finding may appear in contrast with the observation of Ferrari et al, who demonstrated that high salt diet potentiates also afferent aortic baroreceptor function in Dahl salt-resistant rats. However, there are some relevant methodological differences between our study and that of Ferrari et al that could account for the different findings. For instance, in the animal studies cardiopulmonary and arterial afferent influences on the medullary cardiovascular centers could be isolated and afferent activity from sensory receptors directly recorded. Our interpretation of the results, on the contrary, is based on the measurement of the hemodynamic determinants of activity of the cardiopulmonary (i.e., cardiac filling pressure) and carotid (i.e., carotid transmural pressure) baroreceptor reflexes and on the measurement of efferent responses (i.e., plasma norepinephrine and forearm blood flow). Another substantial difference is that we studied only patients with high blood pressure who may have an impairment of arterial baroreceptor reflex responsiveness, whereas Ferrari et al demonstrated salt-induced arterial baroreceptor reflex sensitization only in rats with normal blood pressure. It may be speculated that in our study, an impairment of carotid baroreceptor responsiveness may have obscured or prevented a salt-induced potentiation of the reflex response to carotid baroreceptor unloading.

Theoretically, the lack of salt-induced plasticity in baroreceptor reflex function demonstrated in salt-sensitive hypertensive patients in our study might
play a pivotal role in the genesis of their salt sensitivity. In fact, it may account for the blunted suppression of the renin-angiotensin-aldosterone axis and the abnormal response of the adrenergic system to volume expansion, which have been proposed as potential mechanisms underlying salt-sensitive hypertension.\(^1\) The assessment of the hemodynamic effects of chronic salt loading performed in our study seems to corroborate this hypothesis. In fact, consistently with the results of the LBNP study, we showed that during volume expansion salt-sensitive hypertensive patients are characterized by a blunted reduction of vascular resistance probably linked to a defective suppression of plasma norepinephrine as compared with salt-resistant patients.

Furthermore, salt-sensitive as compared with salt-resistant hypertensive patients showed a reduced ability to modify ANF release in response to changes in atrial pressure. Also, this phenomenon may contribute to salt sensitivity. In fact, although a pathophysiological role of ANF in the development and maintenance of systemic hypertension is not demonstrated, it has been reported that long-term blockade of endogenous ANF with monoclonal antibody to ANF accelerates the development and exacerbates the severity of hypertension in stroke-prone spontaneously hypertensive rats and deoxycorticosterone acetate–salt hypertensive rats.\(^2\) Furthermore, Jin and coworkers\(^3\) also demonstrated that chronic ANF infusion prevents the increase in arterial pressure in response to a high sodium chloride diet in salt-sensitive spontaneously hypertensive rats but had no effect in low sodium–fed rats. These authors concluded that a deficiency of circulating endogenous ANF may play a role in salt-sensitive hypertension in these rats. Our results did not demonstrate any difference in plasma ANF levels between salt-sensitive and salt-resistant hypertensive patients given both low and high sodium diet. However, the LBNP study performed during high salt diet showed a reduced ability to modify ANF levels in response to changes in PCWP in salt-sensitive hypertensive patients. Also, in this case the effects of chronic salt loading demonstrated the clinical relevance of this observation since the slope of the linear regression between changes in plasma ANF and the corresponding changes in PCWP induced by chronic salt loading was significantly smaller in salt-sensitive as compared with salt-resistant patients. This finding suggests that salt-sensitive patients are characterized by a reduced ability to increase ANF levels in response to volume expansion, as compared with salt-resistant patients.

Our study does not permit the determination of the mechanisms involved in the salt-induced changes in cardiac baroreceptor reflex function in salt-resistant patients. However, the observation that high salt diet altered the ratio between changes in PCWP and sympathetic nervous activity as expressed by plasma norepinephrine concentration, rules out the possibility of a receptor resetting. More likely, it reflects sensitization of cardiac vagal afferents.

Although the increase in ANF release in salt-sensitive hypertensive patients appeared to be inadequate to volume expansion as compared with salt-resistant patients, the two groups had comparable levels of plasma ANF. For this reason it is not likely that ANF is involved in the sensitization of cardiopulmonary baroreceptor reflexes during high salt diet in salt-sensitive hypertension as hypothesized by Victor et al.\(^7\) The potentiation of the ANF response to the fall in atrial pressure may be the consequence rather than the cause of cardiopulmonary baroreceptor reflexes sensitization induced by salt loading in salt-resistant patients. In fact, we reported\(^36\) that changes in sympathetic discharge are inversely related to ANF release in humans.

In conclusion, our study demonstrates that high salt diet sensitizes cardiopulmonary baroreceptor reflexes in salt-resistant but not in salt-sensitive hypertensive patients. To the extent that the observation performed during cardiopulmonary baroreceptor unloading can be generalized to the complete baroreceptor reflex function, the salt-induced plasticity of cardiac baroreceptor reflex in salt-resistant patients may represent a protective mechanism against the development of salt-induced hypertension. In fact, it could potentiate the inhibitory effect of cardiopulmonary baroreceptor reflexes on vasomotor centers. The lack of the compensatory augmentation in cardiopulmonary baroreceptor reflex function in salt-sensitive hypertension may contribute to salt sensitivity.

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**References**


**By guest on March 31, 2017**


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