Abnormal Atrial Natriuretic Peptide and Renal Responses to Saline Infusion in Nonmodulating Essential Hypertensive Patients

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Background Nonmodulation seems to represent an inheritable trait characterized by abnormal angiotensin-mediated control of aldosterone release and renal blood supply and salt-sensitive hypertension. Recently, we demonstrated that atrial natriuretic peptide (ANP) response to angiotensin II also is altered in nonmodulators. Moreover, an abnormal ANP response to acute volume expansion has been shown by others in hypertensive patients displaying some features of nonmodulators. These data induced us to hypothesize that nonmodulation could be characterized by an abnormal ANP response to saline load.

Methods and Results Forty-three essential hypertensive men were subdivided into low-renin patients (n = 12), nonmodulators (n = 15), and modulators (n = 16) according to their renin profile and ability to modulate aldosterone and p-aminohippurate clearance responses to a graded angiotensin II infusion (1.0 ng·kg⁻¹·min⁻¹ and 3.0 ng·kg⁻¹·min⁻¹ for 30 minutes each) on both a low- (10 mmol Na⁺ per day) and a high- (210 mmol Na⁺ per day) Na⁺ intake. The intravenous saline load (0.25 mL·kg⁻¹·min⁻¹ for 2 hours) performed on a low-Na⁺ diet increased plasma ANP levels in low-renin (from 14.30±4.68 to 23.30±7.52 fmol/mL at 120 minutes, P<.05) and modulating patients (from 10.95±3.55 to 18.21±5.42 fmol/mL at 120 minutes, P<.05), whereas it did not change the hormone levels in nonmodulators (from 10.77±3.25 to 13.83±5.70 fmol/mL at 120 minutes, P=NS). When patients switched from a low- to a high-NaCl diet, plasma ANP levels increased significantly in all groups. However, when the saline load was repeated on a high-NaCl intake, ANP levels increased in both low-renin and modulating patients (P<.05), whereas it failed to increase in nonmodulators.

Conclusions Nonmodulating hypertensive patients showed a reduced ANP response to saline infusion in the presence of a normal increase of plasma ANP with dietary NaCl load. The impaired ANP response to saline infusion could be due to a different distribution of volume load and contribute to determining the reduced ability to excrete sodium that is commonly described in nonmodulators. (Circulation. 1994;90:2859-2869.)

Key Words • hypertension • kidney • atrial natriuretic factor • renin • hormones

Essential hypertension is a heterogeneous syndrome in which different mechanisms might identify patient subsets, such as salt sensitivity, renin status, and adrenergic activity.¹-³

In this context, the so-called “nonmodulation” seems to represent a strongly inheritable phenotype⁴,⁵ characterized by abnormal aldosterone and renal blood flow response to angiotensin II, abnormal renin response to saline load, and salt-sensitive hypertension.⁴-¹³

Although the most precise way to identify nonmodulating hypertensive patients is to assess the aldosterone increase after angiotensin II infusion on a low sodium intake and/or the p-aminohippurate (PAH) decrease after the same infusion on a high sodium intake,⁵ the nonmodulating phenotype is also characterized by elevated erythrocyte Na⁺/Li⁺ countertransport,¹⁴ increased plasma and urine dopamine levels,¹⁵ and fasting hyperinsulinemia.¹⁶ These findings suggest that the pathogenetic mechanisms involved in nonmodulation are related to an altered response of not only the zona glomerulosa but also the renal vasculature to the infused angiotensin.

In accordance with this hypothesis, we recently demonstrated¹⁶ that nonmodulating patients failed to increase plasma atrial natriuretic peptide (ANP) levels in response to an angiotensin II infusion, thus indicating that the cardiac myocyte response to angiotensin II also is altered in nonmodulation. Moreover, an impaired ANP response to saline load has been reported by Volpe et al¹⁷,¹⁸ in essential hypertensive patients displaying some characteristics of nonmodulation, such as reduced plasma renin activity (PRA) and aldosterone responses to saline infusion¹⁷,¹⁰,¹²,¹³ and decreased rate of sodium excretion.⁷,¹³ Taken together, these findings gave rise to the hypothesis that nonmodulators may be characterized by an abnormal ANP regulation involving the ANP response to both angiotensin II and saline infusions.

In the present study, we evaluated whether in human essential hypertension, the so-called nonmodulating phenotype⁵ was associated with an impaired ANP response to both salt and angiotensin II infusions. We also investigated whether changes in sodium intake were able to modify the ANP and the sodium excretory responses to both angiotensin II and saline infusions in the same patients. The dynamic modifications of ANP and of other salt-regulating hormones were also as-

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ness in nonmodulating hypertensive patients in comparison with age-matched modulating and low-renin hypertensive patients and in a group of normotensive subjects.

Methods

Patients

The study protocol was accepted by our Ethics Committee. An informed written consent to take part in this study was requested of all the eligible hypertensive patients, who were selected from a cohort of male outpatients who had never been recruited for previous studies performed by our Hypertension Unit.

The study was begun in 65 men affected by uncomplicated essential hypertension of a mild to moderate degree. In particular, after withdrawal from any antihypertensive medications, all the selected patients had an outpatient supine diastolic blood pressure between 95 and 114 mm Hg at six different visits performed at 1-week intervals. Further inclusion criteria were body mass index >19 and <25 kg/m², serum creatinine <100 μmol/L, normality of both the 99mTc-diethylenetriamine pentaacetic acid and the 123I-o-iodohippurate scintirenograms, absence of proteinuria, and microalbuminuria <20 μg/min on three different 24-hour collections. No patients had any cardiovascular alteration as evaluated on the basis of clinical evaluation, one- and two-dimensional echocardiogram, and 12-lead ECG. Hypertensive retinopathy was required to be less than grade II in all cases. Moreover, patients with a personal history of ischemic brain, cardiac, and/or leg disease, as well as those with either alcohol or smoking behavior, were excluded from the study. The secondary forms of hypertension were screened out by clinical and laboratory assessments, including the measurement of supine plasma norepinephrine, epinephrine, and dopamine levels. Since ANP regulation can be influenced by a severe autonomic neuropathy, this condition was excluded by Valsalva ratio, deep breathing, lying to standing, sustained handgrip, and orthostatic hypotension, according to the well-known criteria of Ewing et al.21

A normol glucose tolerance was proved by the presence of fasting plasma glucose levels <6.0 mmol/L, absence of glycosuria, and plasma glucose levels 2 hours after oral glucose load (75 g) <7.7 mmol/L. All patients had normal serum cholesterol and triglyceride levels (ie, serum cholesterol <5.20 mmol/L and >3.80 mmol/L and serum triglycerides <1.1 mmol/L and >1.1 mmol/L).

During this part of the study, all patients were on a normocaloric diet with constant sodium intake (120 mmol NaCl daily) and took no medications for 6 weeks. The above sodium intake was achieved by a daily supplement of four capsules (each capsule containing 27.5 mmol NaCl) added to a diet containing about 1 g/kg protein, 2 g/kg carbohydrates, 0.5 g/kg fat, 10 mmol Na⁺, and 60 mmol K⁺ per day. Participants were carefully instructed by us and experienced nutritionists on how to avoid any kind of high-sodium food, to appreciate low-sodium food, and to absolutely eliminate use of added salt.

Dietary written advice was also given to all patients, and our medical staff was at their disposal from 8 AM to 8 PM for any possible inquiry about diet. To simulate as closely as possible the Italian way of eating, the capsules were administered twice daily, during lunch and dinner. Patients were advised to drink 1.5 L water per day.

Adherence to the diet was assessed by measuring the 24-hour urinary sodium and chloride excretion on the 4 days of each week. Patients were considered compliant when sodium and chloride excretions were >80 and <130 mmol/d. After 6 weeks of constant sodium intake, 9 patients were considered not compliant and were screened out. The remaining 56 patients continued the study, and a family history of hypertension and myocardial infarction was evaluated by a questionnaire and consultation with each primary care physician according to a previously described methodology.22 In brief, at the first visit, patients were required to answer standard questions. During the following visits, patients' wives were asked to confirm previous information. The primary care physician was asked to give at least a final confirmation of the resulting family history. A positive history of hypertension or myocardial infarction was defined as the presence of at least one first-degree relative who had suffered from one or both of these conditions before the age of 55 years.1,18 In the case of hypertension, prescribed antihypertensive medication was sufficient to consider the patient to be hypertensive. Family history was obtained in each case by researchers who were unaware of the study purpose, conditions, and results.

A group of 13 normotensive men (blood pressure levels <140/90 mm Hg) without any of the above-cited family backgrounds served as control subjects. Both the inclusion criteria and the study conditions were identical to those used for hypertensive subjects.

The erythrocyte Na⁺/Li⁺ countertransport activity was also evaluated after at least 6 weeks of pharmacological washout.

Protocol

An overview of the study design is given in Fig 1. As is shown, after the definitive enrollment in the study, both hypertensive patients (n=56) and control subjects (n=13) were hospitalized and continued the previous diet containing 120 mmol NaCl per day. During week 1 of hospitalization, 5 patients showed a supine diastolic blood pressure <95 mm Hg and were screened out. All the remaining patients (n=51) were assigned first to a low- (10 mmol Na⁺ per day for 2 weeks) and then to a high- (210 mmol Na⁺ per day for 2 weeks) sodium diet in a double-blind fashion. Both the low- and the high-sodium intakes were achieved by continuing the previous diet but substituting in a double-blind fashion the daily supplement of four capsules containing 27.5 mmol NaCl each with four identical capsules containing either placebo (meal) or 50 mmol NaCl each. Compliance to the diet was verified by measuring both sodium and chloride excretions on the last three days of each week. Three patients with a urinary excretion of Na⁺ >20 mmol/d during the low-sodium intake as well as 5 patients and 2 normotensive control subjects with a sodium excretion <180 mmol NaCl during the high-sodium

![Fig 1. Schematic represents the study protocol. After recruitment by our outpatient unit, patients followed a constant-NaCl diet (120 mmom/d for 6 weeks). During this period, clinical and biochemical evaluations were performed to enroll patients according to the inclusion criteria. Then, the enrolled patients were hospitalized, and the infusions of angiotensin II and saline were performed, at 1-week intervals, during both a low-salt (10 mmol NaCl per day) and a high-salt (210 mmol NaCl per day) diet. The different NaCl intakes were obtained by means of capsules (containing NaCl or placebo) added to a standard diet containing 10 mmol NaCl per day. Compliance to the diet was assessed measuring the 24-hour urinary Na⁺ excretion on the last 3 days of each week.](http://circ.ahajournals.org/DownloadedFrom)
intake were considered not compliant and were excluded from the study. Thus, the evaluation of the nonmodulating phenotype was performed in 43 hypertensive patients, and 11 normotensive subjects were enrolled in the control group. To avoid bias, all procedures regarding changes in sodium intake, adherence to the diet, and blood pressure measurements were made by a staff of researchers who were unaware of the conditions, the purpose, and the results of the study.

Identification of the Nonmodulating Phenotype

According to the criteria of Williams et al., low-renin patients were first identified as those having PRA levels after 1 hour of active orthostatism on a low-sodium intake (10 mmol Na+/day for 1 week) that were <0.30 ng of angiotensin I per liter per second. In hypertensive patients with a normal to high renin activity, the nonmodulating phenotype was defined as the simultaneous presence of the following two characteristics: (1) an aldosterone increase <420 pmol/L in response to angiotensin II infusion during a low-sodium diet (10 mmol Na+/day for 7 days) and (2) a PAH clearance decrement <120 mL/min per 1.73 m² in response to angiotensin II infusion during a high-sodium diet (210 mmol Na+/day for 7 days).

In particular, after 1 week on low-Na+/intake, blood samples for PRA evaluation were taken in both hypertensive and normotensive individuals after 1 hour of lying in the supine position and then again after 1 hour of upright posture. After blood collections, renal plasma flow was assessed in both hypertensive patients and control subjects according to the method described by Rystedt et al. Briefly, at 9:30 AM, after 1 hour with the patient in the supine position, an intravenous catheter was installed in the right arm. A control blood sample was obtained, and PAH (bouls injection of 8 mg/kg) was infused. A constant infusion of PAH (12 mg/min) was then started, with the infusion rate controlled by a peristaltic pump (Abbot/Shaw Life Care Pump). PAH clearance was calculated from the plasma concentrations and the infusion rate and corrected for body surface area. After basal PAH clearance was measured, angiotensin II amide (Hypertensin, CIBA-GEIGY Corp, Pharmaceutical Division) was infused at successive doses of 1.0 and 3.0 ng·kg⁻¹·min⁻¹ for 30 minutes each, using the above-mentioned peristaltic pump, without discontinuing the contemporaneous PAH infusion.

During the angiotensin II infusion, blood samples for PRA, aldosterone, ANP, sodium, and potassium were drawn at time 0, 30, and 60 minutes by use of a heparin lock catheter system installed in an antecubital vein of the left forearm. Blood pressure was constantly measured every 5 minutes by a standard Riva-Rocci sphygmomanometer with the cuff position over the brachial artery of the left arm.

Before the angiotensin II infusion was begun, patients were asked to void at 8:30 AM to complete the previous 24-hour collection and to make a 1-hour collection before each infusion was begun. At the end of this procedure, another urine collection was made.

Saline Infusion

After the infusion of PAH and angiotensin II, both patients and control subjects continued the low-sodium intake for another week. At the end of this period, after an overnight fast, at 8 AM with the patient in the supine position, a catheter was inserted in an antecubital vein of the right forearm. After 1 hour, blood samples for PRA, aldosterone, ANP, sodium, and potassium evaluations were taken by a heparin lock catheter system inserted in an antecubital vein of the left forearm. Baseline urine collections for urine volume and sodium and potassium excretions were also obtained, and a 0.9% NaCl infusion was started and maintained at the constant rate of 0.25 mL·kg⁻¹·min⁻¹ (by use of the previously mentioned peristaltic pump) for 2 hours. Blood samplings were repeated at 30, 60, and 120 minutes for the above-noted determinations. Urine samples for sodium and potassium excretions were obtained at 30-minute intervals. Blood pressure was controlled every 10 minutes throughout the experiment by a standard mercury sphygmomanometer and a stethoscope. All measurements were taken again 60 minutes after the saline infusion was stopped (ie, at 180 minutes from baseline).

High-Sodium Diet

Both the PAH and angiotensin II infusions as well as the intravenous saline load were repeated in each patient after 1 and 2 weeks on a high-sodium intake (210 mmol Na+/day) (Fig 1).

To maintain the complete double blindness, all the infusions were made by a separate staff of researchers who did not have any information about the study design, results, and conclusions.

Blood and Urine Samplings, Laboratory Procedures, and Blood Pressure Measurements

Plasma renin activity and aldosterone were assayed by radioimmunoassay (RIA) (Sorin Biomedica). Plasma ANP was evaluated as previously described. Briefly, ANP was extracted from plasma by Amprep c-18 columns (Amersham International) that were first activated with 5 mL methanol and 10 mL distilled water. Each column was loaded with 2.5 mL acidified plasma. The eluate was collected in 2 mL ethanol, dried under vacuum, reconstituted in 1 mL phosphate buffer solution (pH 7.4, Sigma Chemical Co), and measured by RIA with a commercially available human-ANP (99-126) RIA kit (Peninsula Laboratories Inc). Synthetic human ANP (99-126) (Bissendorf GmbH Peptide) was used as standard. Mean recovery was 85% (range, 79% to 91%); interassay and intra-assay variations were <10%. The RIA sensitivity was 1 fmol per tube. Plasma norepinephrine, epinephrine, and dopamine were assayed by reverse-phase high-performance liquid chromatography (LKB-Pharmacia) with electrochemical detection (ESA) after the extraction and concentration by absorption onto activated alumina.

All the assays were performed no later than 1 week after blood sampling (mean, 3 days). In all cases, blood samples were collected on ice and immediately centrifuged. The plasma was then separated and frozen at −80°C until assayed.

The Na+/Li+ countertransport activity was evaluated according to the method proposed by Canessa et al. Briefly, peripheral blood was collected in heparin-containing vacuum tubes (Becton-Dickinson Vacutainer Systems Europe). Red blood cells were separated within 3 hours by centrifugation for 10 minutes at 300g. The plasma and Buffy coat were separated by suction. Erythrocytes were then washed three times in a Na+-containing washing solution (in mmol/L: magnesium chloride 75, sucrose 85, glucose 10, Tris-MOPS 10; pH 7.4) at 4°C, and the maximal velocity (Vmax) of the Na+/Li+ countertransport was then assayed from the external sodium-stimulated lithium efflux after lithium loading.

Sodium, potassium, and the other routine laboratory measurements were made by standard laboratory methods on fresh samples of blood or urine. HDL cholesterol was assayed by an enzymatic method after precipitation of apoprotein B-containing lipoproteins by phosphotungstate and magnesium (Boehringer Mannheim). LDL cholesterol levels were assayed by the Friedewald method. Plasma insulin levels were assayed by a commercially available RIA kit (Ares Serono).

Throughout the hospitalization period, blood pressure was measured at 8 AM, after 15 minutes with the patient in the supine position, by a standard mercury sphygmomanometer and a stethoscope. The first measurement was not considered, and the average of the last three pressures, taken at 3-minute intervals, was considered. Blood pressure was taken by re-
Table 1. General Characteristics of the Study Population

<table>
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<tr>
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<th>Hypertensives</th>
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<td></td>
<td>Low Renin</td>
<td>Nonmodulators</td>
<td>Modulators</td>
<td>Normotensives</td>
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<tr>
<td></td>
<td>(n=12)</td>
<td>(n=15)</td>
<td>(n=16)</td>
<td>(n=11)</td>
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<tr>
<td>Age, y</td>
<td>40±5</td>
<td>41±3</td>
<td>42±3</td>
<td>40±4</td>
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<td>Duration of hypertension, mo</td>
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<td>42±5</td>
<td>34±10</td>
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<td>Body mass index, kg/m²</td>
<td>21.9±1.1</td>
<td>22.6±1.2</td>
<td>20.8±2.5</td>
<td>21.5±0.9</td>
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<td>Family history, yes/no</td>
<td>Hypertension</td>
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<td>11/4</td>
<td>4/12</td>
<td>0/9</td>
<td></td>
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<td></td>
<td>Myocardial infarction</td>
<td>1/11</td>
<td>9/6†</td>
<td>3/13</td>
<td>0/9</td>
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<td>SBP, mm Hg</td>
<td>169.2±3.2‡</td>
<td>170.5±3.2‡</td>
<td>170.4±3.5‡</td>
<td>124±2.5</td>
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<td>DBP, mm Hg</td>
<td>103.2±2.1‡</td>
<td>104.5±2.5‡</td>
<td>104.0±2.9‡</td>
<td>83.5±3.5</td>
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<td>Heart rate, bpm</td>
<td>73.4±5.0</td>
<td>70.2±4.5</td>
<td>75.1±4.6</td>
<td>71.5±4.8</td>
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<td>Total cholesterol, mmol/L</td>
<td>4.52±0.10</td>
<td>4.69±0.11</td>
<td>4.53±0.09</td>
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<td>HDL cholesterol</td>
<td>1.02±0.20</td>
<td>0.95±0.19</td>
<td>1.09±0.16</td>
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<td>LDL cholesterol</td>
<td>3.20±1.02</td>
<td>3.42±1.32</td>
<td>3.15±1.16</td>
<td>3.33±1.05</td>
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<td>Serum triglycerides, mmol/L</td>
<td>1.49±0.06</td>
<td>1.58±0.05j</td>
<td>1.46±0.07</td>
<td>1.55±0.05</td>
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<td>Fasting insulin, pmol/L</td>
<td>49.2±21.0</td>
<td>87.4±30.1*</td>
<td>53.6±27.0</td>
<td>55.2±13.8</td>
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<td>Fasting glucose, mmol/L</td>
<td>4.9±0.2</td>
<td>5.0±0.2</td>
<td>5.0±0.3</td>
<td>4.9±0.2</td>
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<td>BUN, mmol/L</td>
<td>10.7±3.9</td>
<td>11.1±1.8</td>
<td>10.8±1.6</td>
<td>10.2±1.8</td>
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<td>Serum creatinine, mmol/L</td>
<td>79.5±8.8</td>
<td>79.5±17.6</td>
<td>91.2±8.8</td>
<td>88.4±8.9</td>
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<td>Na⁺/Li⁺ countertransport, mmol/L - cell⁻¹ h⁻¹</td>
<td>0.310±0.02</td>
<td>0.490±0.1§</td>
<td>0.332±0.03</td>
<td>0.301±0.02</td>
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<td>Plasma norepinephrine, pmol/mL</td>
<td>1012.6±121.5</td>
<td>1131.1±101.5</td>
<td>1023.5±213.3</td>
<td>1101.5±98.2</td>
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<td>Plasma epinephrine, pmol/mL</td>
<td>234.1±26.2</td>
<td>258.3±75.2</td>
<td>288.9±43.2</td>
<td>235.5±50.2</td>
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<tr>
<td>Plasma dopamine, pmol/mL</td>
<td>212.1±40.4</td>
<td>268.3±76.2</td>
<td>245.0±32.4</td>
<td>220.6±56.1</td>
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</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; bpm, beats per minute; and BUN, blood urea nitrogen. Values are mean±SEM.

*P<.05, nonmodulating hypertensives vs modulating and low-renin hypertensives and normotensives; †P<.01, nonmodulating hypertensives vs low-renin and modulating hypertensives; ‡P<.0001, low-renin, nonmodulating, and modulating hypertensives vs normotensives; §P<.0001, nonmodulating vs modulating and low-renin hypertensives, and normotensives; ||P<.0001, nonmodulating hypertensives vs low-renin and modulating hypertensives.

searchers who did not participate in the study and were not informed about its purpose, conditions, and results.

Statistical Analysis

All data were collected by use of the database Supercalc-3 (Computer Associates Inc). Statistical evaluations were made with software for biomedical statistics (Primer of Biostatistics, New York, NY: McGraw-Hill Book Co; 1992) and a PC Olivetti M380.

Data are presented as mean±SD. Statistical significances are considered as a value of P<.05. Differences among the groups examined were tested for significance by one-way ANOVA followed by the Newman-Keuls test for pairwise comparisons. For multiple comparisons, ANOVA was used, followed by the modified Student's t test and Bonferroni's test for adjusting the significance level. Differences in the same group after the different diet periods were evaluated by the paired Student's t test. χ² analysis was used for comparison of descriptive parameters. Pearson’s correlation coefficients were used to examine relations among variables.

Results

Baseline Comparison

Twelve hypertensive patients had a low renin activity and 31 a normal to high activity (ie, a PRA >0.30 ng angiotensin I per liter per second after 1 hour of upright posture on a 10 mmol sodium intake). The latter group was further divided into nonmodulators (n=15) and modulators (n=16) according to the capability to modulate (1) the plasma aldosterone response to a graded infusion of angiotensin II on a low Na⁺ intake and (2) the PAH clearance response to a graded infusion of angiotensin II on a high Na⁺ intake. In agreement with several previous reports by Williams and colleagues,5,7,12,13 in our study too a few patients (n=2) had a mild positivity for the aldosterone criterion while showing a normal PAH clearance response to infused angiotensin II. As a consequence, these patients were classified in the modulator subgroup. In baseline conditions (Table 1), the three patient subgroups were comparable with respect to age, weight, blood pressure, duration of hypertension, serum creatinine, fasting glucose, HDL and LDL cholesterol, plasma norepinephrine, epinephrine, and dopamine levels. On the contrary, nonmodulating hypertensive patients showed significantly higher fasting plasma insulin (P<.05) than low-renin and modulating hypertensive and normotensive individuals. As already demonstrated,14 red blood
cell Na⁺/Li⁺ countertransport was significantly higher (P<.0001) in nonmodulating hypertensive than in modulating and low-renin hypertensive and in normotensive individuals, while no differences were found among low-renin hypertensive, modulating hypertensive, and normotensive subjects (Table 1). A family history of hypertension and myocardial infarction was more frequent (P<.01) in nonmodulators than in low-renin and modulating hypertensive patients (Table 1).

**Renal and Hormonal Response to Saline Infusion and ANP Response to Dietary NaCl Supplementation**

Before the saline infusion was begun on a low-Na⁺ diet, plasma ANP levels were higher (P<.05) in low-renin patients (14.30±4.68 fmol/mL) than in modulators (10.95±3.55 fmol/mL), nonmodulators (10.77±3.25 fmol/mL), and normotensive subjects (10.27±3.01 fmol/mL). Plasma ANP levels increased with saline infusion in both low-renin (21.26±7.38 fmol/mL at 60 minutes, 23.30±7.52 fmol/mL at 120 minutes, P<.05 versus baseline) and modulating (16.08±5.77 fmol/mL at 60 minutes, 18.21±5.42 fmol/mL at 120 minutes, P<.05 versus baseline) patients, while remaining unchanged in nonmodulators (12.05±3.05 fmol/mL at 60 minutes, 13.83±5.70 fmol/mL at 120 minutes, P=NS) (Fig 3, third panel). Compared with nonmodulators, significantly higher ANP levels were observed in low-renin patients (P<.0001 at 60 and 120 minutes, P<.002 at 180 minutes), modulators (P<.03 at 60, P<.04 at 120, and P<.03 at 180 minutes), and normotensive subjects (P<.03 at 60, P<.04 at 120, and P<.05 at 180 minutes).

As expected, PRA levels were incompletely suppressed by saline infusion in nonmodulators compared with the other hypertensive (P<.0001 at 120 minutes) and normotensive (P<.02 at 120 minutes) control subjects (Fig 3, second panel). The plasma aldosterone response also was delayed in nonmodulators compared with normotensive subjects and the other hypertensive subgroups (Fig 3, top). In regard to renal sodium handling, baseline urinary sodium excretion was similar in all hypertensive subgroups and in normotensive control subjects, ranging from 9.1±2.5 µEq/min in low-renin hypertensive patients to 6.9±3.3 µEq/min in normotensive control subjects (Fig 3, bottom). During the saline infusion, all subjects showed a marked increase in urinary Na⁺ excretion, but nonmodulating

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**Table 2. Response of p-Aminohippurate Clearance to Angiotensin II Infusion During a Low Sodium Intake (10 mmol Na⁺/d for 7 days) and a High Sodium Intake (210 mmol Na⁺/d for 7 days) in Low-Renin (n=12), Nonmodulating (n=15), and Modulating (n=16) Essential Hypertensive Males and in Normotensive Control Subjects (n=11)**

|                      | Hypertensives |          |          |          |          |
|----------------------|--------------|----------|----------|----------|
|                      | Low Renin    | Nonmodulators | Modulators | Normotensives |
| PAH clearance, mL·min⁻¹·1.73 m⁻² | (n=12) | (n=15) | (n=16) | (n=11) |
| **Low-sodium diet**  |            |          |          |          |
| Baseline             | 570±36      | 574±45   | 560±83   | 539±44   |
| 30 min               | 533±31      | 561±40   | 520±75   | 506±54   |
| 60 min               | 529±78      | 546±50   | 549±65   | 503±28   |
| **High-sodium diet** |            |          |          |          |
| Baseline             | 628±32      | 582±39   | 629±65   | 643±44   |
| 30 min               | 580±32      | 525±50   | 600±87   | 523±40   |
| 60 min               | 486±35†     | 514±43*  | 440±35‡  | 429±62§  |

PAH indicates p-aminohippurate. Values are mean±SD. 
*P<.05, †P<.001, ‡P<.0001 versus baseline.
hypertensive patients showed their typical delayed natriuretic response to saline infusion (Fig 3, bottom). In fact, although the urinary excretion of sodium increased significantly in nonmodulators as well ($P<.05$ at 60, 120, and 180 minutes), their natriuretic response to saline infusion was significantly lower ($P<.0001$) than in modulating and low-renin hypertensive patients and in normal control subjects at 60 and 120 minutes (Fig 3, bottom).

When the data obtained on a high-Na$^+$ diet were analyzed, no differences in PRA response to sodium load were observed among the different subgroups. In particular, PRA levels were suppressed after the 14-day period on a high-Na$^+$ intake in all patients, and slight further decrements were observed during saline infusion in all groups (Fig 4, second panel). Plasma aldosterone levels also were suppressed by the high sodium intake in all subjects, and similar decreases were induced by saline infusion in all subgroups (Fig 4, top).

It was of particular interest to note that the high Na$^+$ intake increased plasma ANP levels in modulators (from $10.95\pm3.55$ to $18.91\pm4.56$ fmol/mL, $P<.0005$), nonmodulators (from $10.77\pm3.25$ to $18.84\pm2.74$ fmol/mL, $P<.0005$), low-renin patients (from $14.30\pm4.68$ to $24.2\pm5.61$ fmol/mL, $P<.0001$), and normotensive subjects (from $10.27\pm3.01$ to $17.81\pm4.10$ fmol/mL, $P<.002$). The increase in plasma ANP levels due to dietary sodium supplementation was more pronounced in low-renin patients than in other hypertensive patients. Accordingly, as observed during the low-NaCl diet, plasma peptide levels after dietary NaCl supplementation were still higher ($P<.005$) in low-renin patients than in the other two hypertensive groups and normotensive subjects (Fig 4, third panel).

Despite the normality of the plasma ANP response to dietary NaCl load, the cardiac hormone showed a delayed increase during saline infusion in nonmodulators (from $18.84\pm2.74$ to $21.15\pm3.84$ fmol/mL at 120 minutes, $P=NS$) but not in the other patients. In particular, a significant ($P<.05$ versus baseline) and rapid increment in plasma ANP was observed during saline infusion in both the remaining hypertensive patients and the control group (Fig 4, third panel).

As already shown by other reports,7,10,12 saline infusion induced nonsignificant changes in renal plasma flow or blood pressure in all patients. In contrast, urinary sodium excretion increased significantly with saline infusion in all patient subgroups (Fig 4, bottom). Also in this case, the natriuretic response to saline load was delayed in nonmodulators, being lower ($P<.0001$) than in low-renin and modulating hypertensive patients and in normotensive subjects at 60 and 120 minutes (Fig 4, bottom). During recovery (ie, at 180 minutes), the urinary sodium excretion was slightly but insignificantly reduced in nonmodulating hypertensive patients compared with the other subgroups (Fig 4, bottom).
Salt Sensitivity

As far as the blood pressure sensitivity to changes in sodium intake is concerned, our data confirmed the well-known salt sensitivity of low-renin and nonmodulating hypertensive patients. In fact, at the end of the 2 weeks on a high sodium intake, both the systolic and the diastolic blood pressures were significantly higher than at the end of the normal sodium intake in low-renin patients (systolic, from 168.4±4.5 to 175.9±5.4 mm Hg, P<.001; diastolic, from 99.3±2.1 to 108.5±4.3 mm Hg, P<.0001) and in nonmodulators (systolic, from 165.4±4.0 to 169.7±3.2 mm Hg, P<.005; diastolic, from 98.5±2.7 to 105.4±3.2 mm Hg, P<.0001). As expected, no significant salt-related changes were observed in modulators and normotensive control subjects. In a similar way, compared with the normal sodium intake, the low-sodium intake significantly reduced both systolic and diastolic blood pressures in low-renin (systolic, P<.01; diastolic, P<.001) and nonmodulating (systolic, P<.05; diastolic, P<.005) hypertensive patients, while it was ineffective in modulators and control subjects.
Discussion

We demonstrate for the first time in this study that nonmodulating hypertensive patients showed an impaired plasma ANP response to saline infusion compared with low-renin and modulating hypertensive patients. Chronic dietary NaCl supplementation increased normal plasma ANP levels in all patients. However, when saline infusion was repeated on a high-salt diet, circulating ANP levels failed to increase further in nonmodulators.

As is known, a target-tissue refractoriness to angiotensin II has been suggested as the primary cause of nonmodulating essential hypertension.\textsuperscript{12,13} The facts that angiotensin II did not increase ANP levels in nonmodulators,\textsuperscript{16} whereas it promoted ANP release in unselected subjects\textsuperscript{16,25,26} and dogs,\textsuperscript{27} and that angiotensin II itself may raise ANP secretion from isolated rat\textsuperscript{28} and rabbit heart\textsuperscript{29} gave rise to our hypothesis\textsuperscript{16} that angiotensin II sensitivity could be primarily impaired at the myocyte level in nonmodulators. Nevertheless, circulating ANP levels failed to increase even after saline infusion in these patients. In this context, the role for an impaired angiotensin II sensitivity as well as for a primary abnormality in the cellular mechanisms of stretch-secretion coupling\textsuperscript{30} is questionable, since oral chronic NaCl supplementation was followed by a normal ANP increase.

As a consequence, it seems possible that the abnormal ANP response to saline infusion is also related to a different fluid compartmentalization into the vascular bed. In accordance with this hypothesis, several studies carried out in hypertensive patients have demonstrated that saline infusion may result in variable natriuretic, hormonal, and hemodynamic responses in hypertensive patients matched for age, race, sex, duration of hypertension, degree of target organ damage, and baseline blood pressure levels.\textsuperscript{10,17,18,31-35} With regard to plasma ANP, an impairment of its release after saline load has already been demonstrated in patients showing several hormonal and renal characteristics of nonmodulators.\textsuperscript{17,18} Moreover, the possibility that differences in intravascular distribution of volume load may influence the response to acute saline load has been suggested by some studies, indicating that borderline hypertensive patients unable to suppress PRA and increase ANP in response to saline infusion (ie, two hormonal characteristics of nonmodulators) had an abnormal venous distensibility during the same infusion.\textsuperscript{35,38} In keeping with this hypothesis, the same patients showed normal ANP and venous distensibility responses to dietary NaCl supplementation.\textsuperscript{39} Furthermore, the latter revealed in these patients another characteristic feature of nonmodulation, ie, salt sensitivity.\textsuperscript{39} Therefore, it could be hypothesized that the abnormal ANP response to intravenous saline load observed in nonmodulators may reflect a different intravascular distribution of volume load. However, measurements of body fluid distribution were not performed in this study, and we can only speculate on the matter.

On the other hand, our findings could offer alternative explanations for some of the renal and hormonal abnormalities shown by nonmodulators. First, the blunted ANP response to saline infusion could be a contributor to the reduced urinary excretion of sodium observed in these patients. As in previous reports,\textsuperscript{17,18} we did not find any significant correlation between plasma ANP levels and urinary sodium excretion. Nonetheless, it does not seem arbitrary to hypothesize that the reduced response of a natriuretic substance, ie, ANP, might contribute to the delayed sodium excretory response to saline loading. In contrast with this hypothesis, captopril has been reported to normalize sodium excretion\textsuperscript{7-38} but not ANP increase\textsuperscript{38} after saline infusion in nonmodulators\textsuperscript{7} and in essential hypertensive patients with several characteristics reminiscent of those observed in nonmodulators.\textsuperscript{39} Therefore, the impaired renal response to saline load should be due to more complicated mechanisms, probably involving the blunted PRA response to sodium load.

In this context, our results confirmed that PRA suppression due to both angiotensin II\textsuperscript{11,16} and saline infusions\textsuperscript{7,10,16} is impaired in nonmodulators. Several studies have reported that increased levels of plasma ANP, such as those obtained during exogenous infusion,\textsuperscript{40-45} water immersion,\textsuperscript{45} or volume expansion,\textsuperscript{46} are combined with a reduction in PRA. In agreement with the possible role of ANP in determining the lack of renin suppression, Volpe et al\textsuperscript{17,18} reported that increments in ANP concentration and decrements in PRA induced by a saline load were directly correlated. Interestingly, this result was obtained in patients showing several characteristics of nonmodulators, such as reduced PRA, aldosterone, and natriuretic responses to saline infusion,\textsuperscript{18} supporting the hypothesis that a reduced ANP release during volume loading could contribute to the abnormalities of PRA behavior and sodium excretory capability in these patients. In agreement with this hypothesis, Singer et al\textsuperscript{45,46} demonstrated that a sustained suppression of the angiotensin II levels is fundamental in determining the early sodium excretion after a saline load. Therefore, an ANP-related lack of renin suppression could represent a main contributor to the reduced ability to excrete a sodium load shown by nonmodulators.

Alternative explanations for our findings seem to be unlikely. In particular, in agreement with previous reports,\textsuperscript{7,12} we found that renal plasma flow and blood pressure response to saline load were not significantly different among patient subgroups. As a consequence, the delay in sodium excretion shown by nonmodulators cannot be related to different blood pressure levels achieved during saline load. In accordance with this interpretation, Rystedt et al\textsuperscript{7} asserted that the exaggerated natriuresis that follows a saline infusion in low-renin patients is not due to parallel pressor response. Furthermore, compared with nonmodulators, modulators showed a normal natriuretic response to saline, even though blood pressure levels did not change with acute volume expansion in both groups.\textsuperscript{7} In this context, a different renal plasma flow response to saline load cannot have determined the impaired sodium excretory response to saline shown by nonmodulators. Indeed, as already demonstrated,\textsuperscript{7,12,13} the time required for renal blood flow to increase after a sodium load involves many more hours than required for the fall in PRA and the increase in plasma ANP levels and sodium excretion.

In the present study, we confirmed that nonmodulators show a marked salt sensitivity of blood pressure.\textsuperscript{5,6,12,13,16} Williams and Hollenberg\textsuperscript{2,13,47,48} sug-
gested that the lack of renal blood flow changes in response to chronic dietary NaCl supplementation could lead to an inappropriate state of sodium retention and, as a consequence, determine the increase in blood pressure.\textsuperscript{48} This hypothesis could be supported by our data, confirming the presence of an altered renal blood flow response to dietary NaCl supplementation in nonmodulators (Table 2). In light of these findings, the normality of the ANP response to chronic sodium loading suggests that the peptide does not play a significant role in determining the abnormal responses of blood pressure and renal blood flow to dietary NaCl supplementation. In agreement with this hypothesis, normal and even exaggerated ANP response to dietary NaCl supplementation have been demonstrated in salt-sensitive normotensive\textsuperscript{49} and hypertensive patients.\textsuperscript{50}

With regard to family history of hypertension, our data totally agree with previous findings, demonstrating that the nonmodulating phenotype can be observed in the normotensive offspring of essential hypertensive subjects\textsuperscript{51} and that 84\% of nonmodulators have a positive family history of hypertension compared with 25\% to 30\% in the other hypertensive patients.\textsuperscript{4,13,52,53} On the other hand, impaired PRA, aldosterone, ANP, and natriuretic responses to saline infusion have been described recently in hypertensive patients having a parental history of cardiovascular events.\textsuperscript{17,18} In this report, a positive family history of myocardial infarction seemed more frequent in nonmodulators, but the small number of subjects studied does not allow any speculation on this matter. Indeed, family histories were often positive for both hypertension and cardiovascular events in nonmodulators, suggesting that hypertension is responsible for this finding.

In the present study, we selected only nonobese hypertensive patients with normal carbohydrate and lipid metabolisms. Nevertheless, fasting insulin levels were significantly higher in nonmodulators than in other hypertensive subgroups. The same patients also showed the highest triglyceride and total cholesterol and the lowest HDL cholesterol levels. The reasons leading to these findings are unclear. Fasting hyperinsulinemia is often observed in insulin-resistant conditions.\textsuperscript{54} Moreover, a reduced insulin sensitivity has been observed recently in salt-sensitive normotensive individuals,\textsuperscript{55} while mild fasting hyperinsulinemia is more frequent in salt-sensitive than in salt-resistant essential hypertensive patients.\textsuperscript{16,56} Taken together, these findings could suggest a possible link among insulin resistance, hyperdyslipidemia, sodium sensitivity, and nonmodulation. In accordance with this hypothesis, we confirmed that erythrocytes from nonmodulating hypertensive patients show increased Na$^+$/Li$^+$ countertransport activity compared with those from low-renin and modulating hypertensive patients\textsuperscript{14} (Table 1). As is known, erythrocyte Na$^+$/Li$^+$ countertransport activity is accelerated in hypertensive patients with hyperdyslipidemia\textsuperscript{57,58} and insulin resistance\textsuperscript{59} and in hypertensive and normotensive subjects with a positive family history of essential hypertension.\textsuperscript{15} These data lead to the fascinating theory that an increased Na$^+$/Li$^+$ countertransport activity in vitro may reflect an increased Na$^+$/H$^+$ antiport activity in vivo.\textsuperscript{60} The aforementioned should influence both vascular tone and insulin-mediated glucose uptake in insulin-sensitive tissues via different mechanisms, involving enhanced response to vasoconstrictors,\textsuperscript{63} impaired insulin-stimulated vasodilation in skeletal muscles,\textsuperscript{64} intracellular alkalization,\textsuperscript{64} and increased intracellular free calcium.\textsuperscript{63} Nevertheless, the hypothesis that an increased activity of the Na$^+$/Li$^+$ countertransport may influence both blood pressure and insulin sensitivity in nonmodulators is speculative, since insulin-mediated glucose uptake\textsuperscript{65} was not evaluated in this study.

Unlike previous reports,\textsuperscript{12} we were unable to find elevated plasma dopamine levels in nonmodulators. In our opinion, this discrepancy could be due to the different study conditions. Indeed, plasma dopamine was evaluated by Gordon et al\textsuperscript{5} during a very-low-sodium diet, while we collected blood samples for dopamine assay on a diet containing 120 mmol NaCl per day. Therefore, since salt-sensitive patients in general\textsuperscript{56} and nonmodulators in particular\textsuperscript{15} are unable to increase their dopamine levels with high salt intake, it seems likely that the lack of any significant difference in plasma dopamine levels between nonmodulators and the other hypertensive patients may simply reflect the different sodium intake that we used in this study. We also failed to show significant differences in the aldosterone response to angiotensin II between low-renin and modulating hypertensive patients. In this context, the more likely explanation seems to be simply related to the different PRA values we chose to define low-renin hypertension.\textsuperscript{16,22}

With regard to possible study limitations, our data should not be related to incorrect definition of nonmodulation. The most convenient way to identify nonmodulators is to measure the aldosterone and the PAH clearance responses to the infusion of angiotensin II on different sodium diets.\textsuperscript{3} Although the concordance between renal and adrenal responses is excellent,\textsuperscript{5,13} we prevented misclassification by considering as nonmodulators only patients showing positivity for both criteria. On the other hand, the level of sodium intake and the accurate control of sodium balance are fundamental for the correct identification of nonmodulating hypertension.\textsuperscript{5,13} However, adherence to a very-low-NaCl diet is often extremely problematic,\textsuperscript{65} since most adults in western society consume 6 to 12 g NaCl per day, ie, 100 to 200 mmol per day,\textsuperscript{66} and salty foods are commonly defined as more pleasant than low-salt foods.\textsuperscript{66} Nevertheless, different studies have demonstrated that careful instruction of each patient and the help of a nutritional staff may result in an elevated number of subjects adherent to a very-low-NaCl diet (5 to 10 mmol Na$^+$ per day).\textsuperscript{66,70} Correspondingly, in the present study several hypertensive patients and normotensive subjects were adherent to the assigned diet. Therefore, our data should not have been altered by uncontrolled sodium intake, lack of sodium balance achievement, or incorrect identification of the nonmodulating phenotype.

Similarly, the differences we found between nonmodulators and the other subgroups were not due to age, stage of hypertension, or the degree of involvement of target organs. Indeed, we studied only relatively young subjects with negative microalbuminuria, absence of myocardial hypertrophy, normal glucose tolerance, and hypertensive retinopathy less than grade II. Moreover, since renin behavior\textsuperscript{71} and salt sensitivity\textsuperscript{72} are both influenced by sex, unlike previous studies\textsuperscript{4,5,17,18} we
decided to assess the nonmodulating phenotype only in male hypertensive patients.

In conclusion, the present study demonstrates that ANP release during saline infusion is impaired in a patient subset already identified by Hollenberg, Williams, and colleagues as nonmodulating hypertensive patients.5-15 On the contrary, the ANP response to a 14-day period of high NaCl intake was normal in the same patients. The delayed ANP response to saline infusion could participate in reducing both the renin and sodium excretory responses to saline loading. Conversely, the normality of the ANP response to dietary NaCl load suggests that the peptide has a poor role in determining the marked blood pressure sensitivity to NaCl intake that represents a common feature of the nonmodulating phenotype. In this context, the impairment of ANP regulation as well as the presence of some characteristics reminiscent of those observed in insulin-resistant conditions,52,65,73 such as elevated Na+/Li+ countertransport and fasting hyperinsulinemia, further supports the hypothesis that nonmodulation is a multifaceted syndrome, involving both renal and extrarenal abnormalities.

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