Plasma Ouabain-Like Factor During Acute and Chronic Changes in Sodium Balance in Essential Hypertension

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Abstract—An ouabain-like factor has been implicated repeatedly in salt-sensitive hypertension as a natriuretic agent. However, the response of plasma ouabain-like factor to acute and chronic variation of body sodium is unclear. We studied 138 patients with essential hypertension who underwent an acute volume expansion/contraction maneuver (2 days) and 20 patients who entered a blind randomized crossover design involving chronically controlled sodium intake and depletion (170 to 70 mmol/d; 2 weeks each period). In both studies, plasma levels of ouabain-like factor were higher during sodium depletion (acute: 338.8±17.4 and 402.7±22.8 pmol/L for baseline and low sodium, respectively, \( P<0.01 \); chronic: 320.4±32.0 versus 481.0±48.1 pmol/L, \( P=0.01 \)). No significant change in plasma ouabain-like factor was observed after a 2-hour saline infusion (333.4±23.9 pmol/L) or controlled sodium (402.1±34.9 pmol/L). When patients were divided into salt-sensitive or salt-resistant groups, no differences in plasma ouabain-like factor were observed in the 2 groups at baseline or in response to the 2 protocols: salt resistant (n=69, 340.1±25.9 pmol/L) versus salt sensitive (n=69, 337.4±23.6 pmol/L) and chronic salt resistant (n=11, 336.0±53.2) versus salt sensitive (n=9, 301.1±331.4 pmol/L). However, circulating ouabain-like factor was increased by sodium depletion in both groups. These results demonstrate that circulating ouabain-like factor is raised specifically by maneuvers that promote the loss of body sodium. Acute expansion of body fluids with isotonic saline is not a stimulus to plasma ouabain-like factor. Moreover, basal levels of plasma ouabain-like factor do not differ among patients with salt-sensitive or salt-resistant hypertension. Taken together, these new results suggest that ouabain-like factor is involved in the adaptation of humans to sodium depletion and argue against the hypothesis that ouabain-like factor is a natriuretic hormone. (Hypertension. 2001;38:198-203.)

Key Words: sodium pump inhibitor ■ endogenous ■ salt sensitivity ■ high blood pressure

The involvement of a natriuretic hormone in the control of sodium homeostasis was hypothesized \( \sim \)40 years ago.\(^1,2\) Those studies demonstrated that an expansion of extracellular volume was associated with the appearance in the plasma of an endogenous substance that was suggested to augment sodium and water excretion by the kidney via its ability to inhibit the Na-K pump. This sodium pump inhibitor subsequently became associated with salt-sensitive (SS) forms of hypertension.\(^3\) Recently, an endogenous sodium pump inhibitor, also called ouabain-like factor (OLF), was isolated from the human circulation and found to be structurally similar to the cardiac glycoside ouabain.\(^4,5\) Subsequent cross-sectional studies showed that the plasma levels of OLF were elevated in \( \sim50\% \) of patients with essential hypertension.\(^6\) However, the response of circulating OLF to changes in body fluid volumes in humans is unclear. Several reports demonstrate that OLF increases in animal models of experimental hypertension,\(^7\) in sodium-loaded animals, and in humans,\(^8,9\) but others did not.\(^10\) Three main issues might explain these discrepancies. First, there may be methodological differences in the procedures used to detect OLF. Second, the intensity and duration of the protocols used to assess the effect of sodium load on circulating OLF may differ. Third, the response of OLF may have been obscured by prior or ongoing therapy.

The aim of this study was to determine the response of plasma OLF to acute volume expansion (2 hours) and acute sodium depletion (24 hours). In addition, we also investigated the behavior of circulating OLF during the chronic (14-day) variation of total body sodium. The studies were performed among a large group of patients with essential hypertension who had never received treatment. Plasma OLF was mea-
sured by use of a previously validated immunoassay in which the measured immunoreactivity has been shown to correlate with biological activity.11

Methods

Acute Sodium-Loading Protocol
One hundred forty-eight patients with essential hypertension, none of whom had received prior therapy, were recruited from the Outpatient Clinic for Hypertension of San Raffaele Hospital of Milan. The study was approved by the Ethical Committee of the San Raffaele Hospital, and informed consent was obtained from each individual.

The following exclusion criteria were used: (1) history of myocardial infarction, congestive heart failure, stroke, creatinine clearance ≤50 mL/min, diabetes mellitus, or liver disease; (2) severe hypertension requiring immediate treatment; (3) oral contraceptive use; and (4) known drug or alcohol abuse. Inclusion criteria were office blood pressure (BP) >145/95 but <180/120 mm Hg in ≥2 consecutive visits at the Outpatient Clinic and a 24-hour ambulatory BP recording (Spacelabs 90207) with mean daytime BP >140/90 mm Hg.

Modified Weinberger-Grim Protocol
The patients were hospitalized on Saturday morning for 6 days, during which time they received a 150-mmol/d sodium diet (modified Weinberger-Grim protocol).1,11 They underwent clinical examination, routine biochemistry, and tests for exclusion of secondary forms of hypertension. Ten patients were excluded from the study.

On Wednesday, the patients were asked to void their bladders and assume the supine position between 7:00 and 8:00 AM in a quiet, comfortable room. A venous catheter was inserted into an antecubital vein. Patients remained in the supine position until the end of the sodium load. After the equilibration period and achievement of a steady state, a constant-rate intravenous infusion of 2 L of 0.9% NaCl was carried out in 2 hours. BP (mean of 3 measurements taken 3 minutes apart) was measured every 30 minutes during the 2 hours of loading and 3 times at 3-minute intervals at the end of the infusion. These last 3 BP values were averaged and used in the analysis. A subgroup of 81 patients continued collecting urine from the end of the infusion until 8:00 AM the following day (recovery period).

On Thursday, sodium and volume depletion were effected by giving a sodium-restricted (50 mmol) diet plus administration of 3 doses of 37.5 mg PO furosemide at 8:00 AM, 2:00 PM, and 6:00 PM. The patient was asked to drink ≤25 mL tap water per 1 kg BW. On Friday at 8:00 AM, BP was measured again, and the patient was discharged from the hospital. BP, hematocrit (Hct), plasma renin activity (PRA), aldosterone, OLF, sodium, potassium, and urinary aldosterone were measured at the end of the equilibration period after the sodium infusion (Na load), after the recovery period, and after sodium depletion.

Determination of Body Sodium Changes for Acute Studies
The body sodium balance after Na loading was obtained as the difference in the amount of NaCl infused in 2 hours (310 mmol) compared with that excreted during the infusion time (sodium load). During the recovery period, patients were allowed to have a light lunch and later a light dinner with a constant amount of sodium (100 mmol). The urine collected during the recovery period was used to calculate the sodium excretion rate. The body sodium balance at recovery was obtained by subtracting from the total amount of sodium intake (310 + 100 mmol) the amount of sodium excreted after sodium load and the urinary sodium excreted after recovery. The sodium depletion point was obtained by subtracting the amount of sodium excreted after sodium depletion (37.5 mg furosemide × 3/d PO) plus 50 mmol (sodium-restricted diet) to the point obtained at recovery.

Chronic Sodium Protocol
This study included patients recruited in collaboration with the Salt-Sensitivity Study Group of the Italian Society of Hypertension.13 Each patient gave informed consent to participate in the study. The patients’ high BP values were confirmed on 2 consecutive clinic visits after discontinuation of any previous antihypertensive treatment for ≥3 weeks. Exclusion and inclusion criteria were the same as described before for the acute protocol.

Because community intervention projects and multicenter prospective trials of salt restriction have agreed on the conclusion that continued high salt intake is not easily achieved,14,15 we studied hypertensive patients during both controlled sodium intake and moderate salt restriction for 2 weeks each period. On recruitment, BP was taken 3 times with a standard mercury sphygmomanometer at 2-minute intervals after patients were seated for ≥10 minutes. A questionnaire was administered for evaluation of the patient’s dietary habits and usual caloric intake. Patients were randomized to follow an isocaloric diet containing 50 to 80 mmol/d sodium for 14 days (low sodium) or 150 to 200 mmol/d sodium for 14 days (controlled sodium). At the end of each 2-week period, the patients collected 24-hour urine for analysis of compliance. Only those patients who achieved a difference in urinary sodium excretion of 100 mEq/d between the 2 diet periods were considered in the analysis. Clinic measurements of BW, BP, plasma OLF, PRA, and aldosterone were undertaken at baseline and at the end of each diet period.

Determination of Changes in Body Sodium for Chronic Studies
The urinary sodium excretion at baseline has been considered the reference point. Changes in body sodium were obtained by subtracting the urinary sodium excretion after both controlled sodium intake and low-sodium periods from the baseline urinary sodium excretion.

Analytical Methods
Urinary and plasma sodium and potassium were determined by flame photometry. Creatinine was determined by an autoanalyzer. Plasma OLF was determined by radioimmunoassay on C-18 extracted samples with a specific antiserum as previously described.11 PRA and aldosterone were measured by commercial radioimmunoassay (Sorin Laboratories).

Statistical Analyses
Statistical analysis was performed by the StatView 5.0.1 package (SAS Institute Inc) on a Macintosh Performa 5260. All data are expressed as mean±SEM. Statistical comparisons among groups were performed by Student’s t test. One-way ANOVA with Tukey’s test for repeated measures was used to analyze the effect of the different maneuvers during the acute or chronic protocols on the parameters considered. Regression analysis was used to assess the relative influence of different variables on the variation of OLF during acute or chronic protocols. A value of P<0.05 was considered significant.

Results

Acute Protocol
The main clinical characteristics of the 138 hypertensive patients who underwent the acute test are shown in Table 1. Figure 1 illustrates the responses of mean BP (MBP), plasma OLF, PRA, and changes in body sodium during the acute protocol. The time course of MBP and changes in body sodium (Figure 1) were similar; there was a significant
A significant increase in both parameters from baseline after sodium load (116.3±0.9 mm Hg and 255.4±2.9 mEq/24 h, respectively) and a significant decrease relative to baseline after sodium depletion (107.1±0.8 mm Hg, and −186.1±9.4 mEq/24 h). PRA (Figure 1) was suppressed during sodium load (0.44±0.06 ng·mL⁻¹·h⁻¹) compared with the basal condition (1.17±0.10 ng·mL⁻¹·h⁻¹). A significant increase in PRA was observed after sodium depletion (2.26±0.23 ng·mL⁻¹·h⁻¹). Similarly, plasma aldosterone was suppressed after volume expansion (baseline, 39.41±1.98 pmol/L; sodium load, 9.90±0.72 pmol/L) and raised after furosemide (86.32±0.04 pmol/L). Plasma OLF levels (Figure 1) did not show any significant change from baseline (338.8±26.6 pmol/L). However, circulating OLF levels were significantly higher (421.7±37.1 pmol/L) in a subgroup of 81 hypertensive patients, a small increase in circulating OLF (390±23.9 pmol/L) after 2 hours of saline infusion (333.4±17.4 pmol/L). In a subgroup of 81 hypertensive patients, a small increase in circulating OLF (390±23.9 pmol/L) after 2 hours of saline infusion (333.4±17.4 pmol/L). In a subgroup of 81 hypertensive patients, a small increase in circulating OLF (390±23.9 pmol/L) after 2 hours of saline infusion (333.4±17.4 pmol/L).

**Figure 1.** Acute protocol: Time course of MBP, plasma OLF, PRA, and change in body sodium with acute sodium loading and depletion. Na load indicates 0.9% NaCl for 2 hours; Na Depl, sodium depletion with furosemide (37.5 mg PO 3 times over 24 hours). *P<0.05, **P<0.01 vs baseline (ANOVA repeated measurement).**
SS patients had higher MBP than SR patients at baseline and after sodium load (Table 2), whereas after sodium depletion, SBP was significantly lower in SS than SR. PRA was lower in SS compared with SR at baseline, suppressed similarly by sodium loading, but was significantly lower in SS compared with SR after salt depletion. OLF levels were not statistically different between SS and SR patients in the 2 conditions studied (Table 2). Body sodium changes increased after sodium load and decreased after sodium depletion similarly in SS and SR.

SBP increased after sodium loading and decreased after sodium depletion compared with baseline in SS patients, whereas no significant change was observed in SR. As expected, plasma renin decreased after the sodium load and increased after sodium depletion in both groups compared with baseline. OLF levels did not change after salt loading but increased significantly in SS and SR after salt depletion compared with baseline (Table 2). After the sodium load, urinary sodium excretion was higher in the SS than SR patients.

**Chronic Protocol**

Twenty patients achieved differences in urinary sodium excretion of \( \geq 100 \) mEq/24 h between the 2 weeks of controlled normal- and low-sodium diets. Only these patients were included in the analysis. These patients (Table 3) had a greater body mass index and a higher PRA than those studied with the acute protocol. The latter differences are probably related to the fact that plasma samples were collected in a different manner in the 2 protocols (sitting versus supine position). Figure 2 illustrates the changes in MBP, OLF, PRA, and body sodium after the 2 dietary periods. During the controlled-sodium diet, MBP, OLF, and body sodium were slightly higher compared with baseline without reaching statistical significance. After the low-sodium diet, each of the 6 parameters measured showed significant changes compared with baseline values. For example, body sodium and MBP decreased, whereas PRA and OLF increased. A similar behavior was observed for plasma aldosterone (controlled sodium, 33.55±0.03 pmol/L; low sodium, 48.02±0.03 pmol/L; \( P<0.01 \)). BW fell significantly during the low-salt diet (ΔBW, \(-0.66\pm0.3 \) kg; \( P<0.05 \)). A positive relationship was observed between the increase in plasma OLF and PRA after the low-sodium diet (\( r=0.45, P<0.05 \)).

When patients were separated according to their salt sensitivity, 11 individuals were SR and 9 were SS. Table 4 presents the parameters studied in these 2 groups. After the normal-salt diet, the SS group showed a significantly larger increase in MBP compared with SR. OLF did not differ between SS and SR after 2 weeks of normal diet. The SS group also showed a trend toward decreased MBP after the low-sodium diet. PRA was increased significantly in both groups after the low-sodium period. Plasma OLF levels did not change after the controlled-sodium diet in both in SR and SS patients compared with their respective basal conditions (335±53.20 and 301±29 pmol/L, respectively), whereas plasma OLF was increased similarly in SR and in SS after the low-sodium diet.

**Discussion**

The major new findings of the present studies are as follows. First, acute diuretic-induced sodium depletion and chronic dietary restriction of salt intake raise the circulating levels of OLF among patients with mild to moderate essential hypertension who had never received antihypertensive therapy. Second, and in contrast to long-held hypotheses, acute salt loading is not an immediate stimulus to plasma OLF. Third, basal OLF levels do not differ among patients with SS and SR essential hypertension. When taken together, these new re-
TABLE 4. Clinical Parameters Before and Following 2-Week Periods With Diets Containing Controlled Sodium Intake or Low Sodium Among Hypertensive Patients Divided According to Salt Sensitivity

<table>
<thead>
<tr>
<th></th>
<th>Controlled Sodium</th>
<th>Low Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP, mm Hg</td>
<td>105.4±2.1</td>
<td>103.6±1.6</td>
</tr>
<tr>
<td>OLF, pmol/L</td>
<td>405.4±48.3</td>
<td>485.9±75.5</td>
</tr>
<tr>
<td>PRA, ng · mL⁻¹ · h⁻¹</td>
<td>3.6±0.78</td>
<td>4.57±0.98</td>
</tr>
<tr>
<td>Urinary sodium excretion, mEq/24 h</td>
<td>197.6±18.5</td>
<td>80.4±8.3</td>
</tr>
<tr>
<td>SS (n=9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>111.5±2.4*</td>
<td>97.9±3.5‡</td>
</tr>
<tr>
<td>OLF, pmol/L</td>
<td>398.1±53.5</td>
<td>475.0±59.3†</td>
</tr>
<tr>
<td>PRA, ng · mL⁻¹ · h⁻¹</td>
<td>2.06±0.39</td>
<td>3.3±0.68†</td>
</tr>
<tr>
<td>Urinary sodium excretion, mEq/24 h</td>
<td>180.4±14.7</td>
<td>78.0±13.3</td>
</tr>
</tbody>
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*P<0.05, SS vs SR; †P<0.05, low sodium vs controlled sodium; ‡P<0.05, low sodium vs controlled sodium.

sults indicate that increases in circulating OLF are triggered by reductions in sodium balance. Moreover, the behavior of circulating OLF is inconsistent with its hypothesized role as a natriuretic factor.

Previous studies have shown that chronic expansion of extracellular fluid volume in rats and several clinical conditions in humans that are accompanied by chronic retention of salt and water, such as hypertension, primary aldosteronism, renal failure, and congestive heart failure, are often associated with elevated circulating levels of ouabain-like activity. At first glance, those data taken together seem to support the proposal of de Wardener et al that the expansion of extracellular fluid volume per se provides a stimulus sufficient for the release of an endogenous Na pump inhibitor and that such an inhibitor may promote natriuresis and diuresis.

Recently, circulating and urinary compounds other than OLF, including marinobufagenin-like factor, and substances structurally unrelated to steroids, such as those proposed by Kramer and coworkers and Garay et al, were shown to be increased under conditions of volume expansion. The effects of these compounds on renal function are not clear. Nevertheless, it appears that a variety of distinct factors could, in principle, contribute to the regulation of sodium balance after increases in body fluid volume.

In the present study, we addressed the specific question of the relationship between plasma OLF and acute and chronic changes in sodium balance among patients with essential hypertension. First, we used a reliable method for a precise and selective quantification of OLF. As demonstrated in our previous work, the sensitive radioimmunoassay we used incorporates a highly selective anti-ouabain antibody that allows the specific and quantitative measurement of OLF in extracted plasma. We have previously demonstrated a direct relationship between immunoreactive OLF and the biological potency of this compound on the sodium pump. Our results suggest little or no association between SS essential hypertension and OLF, even though the SS patients clearly had higher MBP and lower PRA than SR patients under basal conditions and SS patients responded to acute salt loads with raised MBP and a decrease in PRA (Table 2). Given that the hemodynamic and hormonal alterations are consistent with a state of expanded body fluid volumes as confirmed by the increase in total body sodium, the absence of a significant change in plasma OLF under these conditions is noteworthy.

Our findings raise doubt concerning the hypothesis that OLF functions as a natriuretic factor in vivo. Second, the frequent association of raised circulating OLF with several pathological states characterized by fluid excess and mentioned previously will require reinterpretation. Third, the increase in circulating OLF evoked by a reduction in total body sodium (Tables 2 and 4) now raises the possibility that OLF participates with the conservation of sodium. The positive correlation between PRA and OLF during salt depletion in both protocols is also of interest given that many earlier studies linked elevated humoral levels of a sodium pump inhibitor with low PRA.

Our results do not establish directly whether the stimulus to plasma OLF is due primarily to sodium restriction per se or whether it arises secondarily as a consequence of the increase in PRA or some other factor. The finding during the recovery phase (20 hours after acute saline loading) of an increased OLF while plasma renin is still suppressed suggests a dissociation of these 2 hormonal systems. The direct relationships among Hct, PRA, aldosterone, and OLF indicate that the major portion of the increase in the circulating levels of these 3 hormones is stimulated by volume depletion. Thus, the aforementioned increases are considerably greater than the physical concentration of these factors affected by reduced plasma volume alone.

Thus far, it is likely that the increases in circulating OLF observed in this study may be attributed primarily to raised secretion. Ouabain has a slow urinary clearance, and if OLF is handled similarly, large changes in excretion would appear to be required to explain the circulating changes, especially in the acute maneuvers. This possibility is unlikely because no change in the index of glomerular filtration rate (creatinine) was detected (data not shown).

Regardless of the nature of the mechanism that raises circulating OLF, the observations presented in this study warrant revision of the role of the renal sodium pump in the pathophysiological action of OLF. For example, the chronic infusion of low doses of ouabain in normal rats induces sustained hypertension and increases the maximal activity of renal Na⁺,K⁺-ATPase activity. Moreover, incubation of rat kidney cells for prolonged periods with 10⁻⁷ mol/L ouabain, a concentration similar to those achieved during salt depletion, also increased the activity and expression of Na-K pumps. The mechanism of this effect of low nanomolar concentrations of ouabain on the renal Na⁺,K⁺-ATPase is not understood but clearly would be expected to promote rather than enhance sodium excretion. Under conditions of low-sodium intake and high PRA in vivo, elevated OLF may enhance the ability of the kidney to reabsorb sodium. These new observations and conclusions are paradoxical given that prior hypotheses proposed a reduction in renal Na-K pump activity and natriuresis in response to increased levels of ouabain or OLF.
The results presented in our study also contrast with previously published articles reporting that endogenous sodium pump inhibitors increase during salt loading.3,9 Because other sodium pump inhibitor(s) are present in the circulation,1,20–22 it is possible that less specific assay systems could account for the difference in our results. Our findings are in agreement with other recent studies in which either an acute or prolonged salt loading did not modify plasma levels of OLF in normal men10 and patients with primary aldosteronism.26 Moreover, studies in awake sodium-replete dogs27,28 have shown that the adrenal secretion of OLF was not affected by acute hypervolemia.

In conclusion, circulating levels of OLF are not elevated by acute volume expansion among patients with essential hypertension regardless of the salt sensitivity of their BP. The unanticipated observation that circulating OLF rises specifically during both acute sodium depletion and chronic salt restriction now suggests that this steroid is involved in the physiological adaptation to sodium retention. Further studies are needed to explore the control of and role of OLF in electrolyte homeostasis in humans.

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