Left Ventricular Mass, Stroke Volume, and Ouabain-Like Factor in Essential Hypertension

Paolo Manunta, Paola Stella, Rodolfo Rivera, Daniele Ciurlino, Daniele Cusi, Mara Ferrandi, John M. Hamlyn, Giuseppe Bianchi

Abstract—Many patients with essential hypertension (EH) exhibit increased left ventricular mass. Similarly, elevated circulating levels of an endogenous ouabain-like factor (OLF) have been described in some but not all patients with EH. Moreover, ouabain has a hypertrophic influence on isolated cardiac myocytes. Accordingly, we investigated relationships among plasma OLF, left ventricular mass, and cardiac function in patients with EH. Plasma OLF was determined in 110 normotensive subjects and 128 patients with EH. Echocardiographic parameters and humoral determinants were measured in EH. Plasma OLF levels were increased (P<0.0001) in patients with EH (377±19 pmol/L) versus normotensive (253±53 pmol/L) subjects. The distribution of plasma OLF was unimodal in normotensives, whereas it was bimodal in EH. Twenty-four–hour diastolic ambulatory blood pressure was slighter higher in EH with high OLF compared with EH with normal OLF (93.2±1.14 versus 89.4±1.33 mm Hg, P=0.03). Left ventricular mass index and stroke volume in EH with high OLF were greater than in EH with normal OLF (101.9±3.3 versus 86.1±2.5 g/m², P=0.0003, and 57.10±1.48 versus 52.30±1.14 mL/m², P=0.02, respectively), although heart rate was slower (74.2±1.3 versus 80.5±1.3 bpm, P=0.005). Multiple regression analysis that tested the influence of body mass index, age, gender, 24-hour blood pressure, and OLF on left ventricular mass revealed independent contributions of systolic (13.2%) and diastolic (12.4%) blood pressure and plasma OLF (11.6%) to left ventricular mass. We conclude that ≈50% of patients with uncomplicated EH have elevated-high circulating OLF levels, higher diastolic blood pressure, greater left ventricular mass and stroke volume, and reduced heart rate. We propose that the OLF affects cardiovascular function and structure and should be considered as a factor that contributes to the risk of morbidity events.

Key Words: sodium ■ sodium-potassium pump ■ cardiac glycosides ■ digoxin ■ human ■ hypertension, essential ■ heart

Arterial hypertension is a risk factor for sudden cardiac death. Moreover, among hypertensives, this risk is increased further in those with left ventricular hypertrophy. The molecular background of cardiac hypertrophy has been associated with changes in myocardial gene expression as well as activation of the tissue renin-angiotensin system and the sympathetic nervous system. The sodium pump is of major importance for active ion transport across the sarcolemma and contributes to the electrical and contractile function of the myocardium. Low concentrations of ouabain, via partial inhibition of the Na pump, cause a small decrease in intracellular Na, affect sarcoplasmatic Na-Ca exchange, and lead to an increase in intracellular Ca and contractility. The rise in cell Ca²⁺ stimulates the signal transduction pathways that regulate the expression of growth-related genes in heart. It has recently been shown that the incubation of cultured rat neonatal cardiac myocytes with nontoxic concentrations of ouabain induces the transcription of some cardiac early-response proto-oncogenes and late-response fetal genes, which have been implicated as markers of myocyte hypertrophic growth. In contrast with these findings, other investigators have demonstrated that the acute inotropic effect of ouabain is associated with the inhibition of protein synthesis in papillary muscle of adult rats. Evaluation of the controversy between these 2 types of findings should take into account the differences between experimental conditions that may, per se, affect the type of response and the variables measured. There is no doubt, however, that the long-term effect triggered by increased load is toward hypertrophy and not toward inhibition of protein synthesis. It was recently shown that the human circulation contains an isomer of ouabain or a closely related isomer. This endogenous ouabain (ouabain-like factor [OLF]) has been quantified both in pure form and in mammalian tissues and

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small volumes of plasma via independent assays. Functionally, OLF is a high-affinity, reversible, and specific inhibitor of the Na pump, with inotropic and vasopressor activity. Elevated plasma OLF levels have been described in ~45% of patients who were initially diagnosed with essential hypertension (EH) and referred for possible secondary hypertension.

The induction of hypertrophic characteristics in vitro by ouabain and the frequency of increased circulating OLF and left ventricular mass among patients with EH led us to consider the possibility of a link between these phenomena. Accordingly, this study reports results of the first large-scale investigation of circulating OLF in patients with EH in whom parallel measurements of left ventricular mass and function have been performed.

Methods

Patient Selection and Blood Pressure Measurements

Normotensive healthy subjects (n=110, 23 to 50 years of age) who attended the San Raffaele Hospital in Milan gave informed consent for blood sampling. Patients with EH were recruited at random from our outpatient clinics by 2 of us (P.M. and D.C.). Secondary hypertension was excluded by routine blood chemistry, urinalysis, and renal echography. Subjects were excluded if they had a medical history that included myocardial infarction, congestive heart failure, stroke, diabetes mellitus, liver disease, use of oral contraceptives, or the abuse of drugs or alcohol. A large portion of the hypertensive patients (n=128) had developed high blood pressure within the previous 2 years and had not previously received antihypertensive drugs. Treated patients discontinued medication for ≥3 months before entering the study. During this period, blood pressure was monitored every 2 weeks and therapy was started; the patient was excluded from the study if diastolic blood pressure (DBP) increased >15 mm Hg or reached 105 mm Hg. Office blood pressures were used during the recruitment of the study subjects. The hypertensive patients. However, plasma levels of OLF did not differ between normotensive men (286 ± 21 versus 310 ± 45 pmol/L, respectively).

Statistical Analyses

Values are expressed as mean±SEM. Comparison of the different groups of patients was performed with 1-way ANOVA followed by a posteriori multiple comparison with Fisher's least significant difference test. Tests for bimodality were performed with the method of Day13 by a program written ad hoc for an Apple Macintosh personal computer. The program assesses the presence of multiple normal distributions in the data and computes the means, standard deviations, and proportion of each subpopulation to the total population. To investigate the relationship between plasma OLF and the other known variables that may affect left ventricular mass, multiple regression analysis was performed with a probability of inclusion criterion of 0.05.

Results

Table 1 presents the clinical characteristics of the 110 normotensives and 128 hypertensives studied. Office blood pressures were used during the recruitment of the study subjects. The hypertensives were similar to the normotensives but included a higher proportion of men than women. Circulating OLF values were significantly higher (P<0.0001) in the hypertensive patients. However, plasma levels of OLF did not differ between normotensive men (286±17 pmol/L) and women (259±17 pmol/L) or between their hypertensive counterparts (389±21 versus 310±45 pmol/L, respectively). Thus, the difference in circulating OLF between the normotensive and hypertensive patients did not appear to be gender related. Plasma OLF in hypertensive men and women was higher than in their normotensive controls, but only the latter reached statistical significance (P=0.014). Urinary Na and K excretion were slightly greater in EH, but the difference was not significant.

The distribution of plasma OLF was unimodal in normotensives (Figure 1, top), with a mean of 253±53 pmol/L; the plasma concentration ranged between 0 (the threshold concentration in the RIA was ≈30 pmol/L) and 600 pmol/L. Plasma OLF levels ranged from 53 to 930 pmol/L in hypertensives (Figure 1, bottom). The distribu-
The computed plasma OLF for the first mode was 207 ± 74 pmol/L, a value similar to that observed in the normotensives (253 ± 53 pmol/L). The plasma OLF of the higher mode was 540 ± 197 pmol/L. Accordingly, the hypertensives were split into 2 subgroups by drawing 2 normal distributions. We defined hypertensive patients with plasma OLF levels of <281 pmol/L as “normal” on the basis of the similarity of their circulating levels to those observed among the normotensive group. The cutoff value used was calculated from the mean of the normal mode plus 1 SD (207 ± 74 pmol/L). Similarly, we apportioned hypertensive patients into the “high” subgroup when the OLF was >343 pmol/L. This value was calculated from the mean of the higher mode minus 1 SD (540 ± 197 pmol/L). Among EH subjects, the normal- and high-OLF subgroups contained 60 (49 men and 11 women) and 62 (54 men and 8 women) individuals, respectively. The 6 patients whose plasma OLF levels ranged between 281 and 343 pmol/L were excluded from further subgroup analyses. In the combined group of normals and all patients, plasma OLF correlated positively with systolic blood pressure (SBP) ($R^2 = 5.6\%, P = 0.0004$) and DBP ($R^2 = 4.5\%, P = 0.0015$), although only half of the patients exhibited elevated plasma OLF.

Table 2 presents the major clinical characteristics of the 2 OLF subgroups of EH patients. The ambulatory values for 24-hour SBP and DBP were lower than the office pressures recorded during patient recruitment (Table 1). Both ambulatory SBP and DBP were higher in the subgroup with elevated OLF, but only the latter reached statistical significance. All other variables (creatinine clearance, sodium excretion, PRA, and plasma aldosterone) were similar in the 2 OLF subgroups between normal- and high-OLF EH.

Figure 2 shows the echocardiographic parameters in the 2 subgroups of EH patients. The left ventricular mass index was substantially greater in EH with high OLF (102 ± 3.3 g/m²) than in the subgroup with normal OLF (86 ± 2.5 g/m²), $P < 0.001$. With gender-specific cutoffs to define left ventricular hypertrophy (LVH) (women 106 g/m² and men 114 g/m²), 11 12 (10 men and 2 women) (80%) of the 15 (13 men and 2 women) patients with LVH were in the high-OLF subgroup. Thus, the relative risk of LVH was 4.6-fold higher in the high-OLF subgroup. As shown in Figure 2B and 2C, stroke volume was higher and heart rate was lower in the subgroup of patients with high plasma OLF. Table 3 presents...
other echocardiographic findings for cardiac dimensions and function in the 2 subgroups. In the subgroup with high plasma OLF, the mean septum thickness, end-diastolic diameter, end-diastolic volume (EDV), and cardiac output were greater than in the subgroup with normal OLF. Although not measured simultaneously, both the higher blood pressure and higher cardiac output in the high-OLF subgroup suggest that total peripheral resistance also should have been greater. Ejection fraction did not differ between the subgroups.

Plasma OLF and left ventricular mass index (LVMI) correlated positively ($R^2=0.14; P<0.0001$) in the entire group with hypertension (Figure 3), whereas heart rate and plasma OLF correlated inversely ($R^2=0.17, P=0.0001$). A positive but weak correlation was present between plasma OLF and left ventricular EDV ($R^2=3.4\%$, $P=0.038$, not shown). To estimate the independent contribution of the measured variables to the variance of LVMI, a multiple regression analysis was performed with gender, age, body mass index, 24-hour ambulatory SBP, and plasma OLF as independent variables (Table 4). Among these variables, only 24-hour ambulatory SBP, OLF, age, and body mass index, 24-hour ambulatory SBP, and plasma OLF as independent variables (Table 4). Among these variables, only 24-hour ambulatory SBP, OLF, age, and body mass index contributed significantly to the variance of LVMI, whereas gender did not. As for the obvious reason that SBP and DBP are correlated, only 1 pressure at a time was entered into the model. We chose SBP because it appears to be the major determinant of LVMI, but DBP was also tested. The LVMI variance explained independently by 24-hour ambulatory DBP was 12.4\%, which was similar to that obtained for 24-hour ambulatory SBP.

**Discussion**

The major new findings of the present studies are as follows:

1. Patients with uncomplicated essential hypertension of

**TABLE 3. Echocardiography Parameters in Hypertensive Patients With Normal and High Levels of Circulating OLF**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal OLF</th>
<th>SEM</th>
<th>High OLF</th>
<th>SEM</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septum, cm</td>
<td>1.01</td>
<td>0.02</td>
<td>1.09</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Posterior left ventricular wall thickness, cm</td>
<td>0.95</td>
<td>0.03</td>
<td>1.00</td>
<td>0.02</td>
<td>NS</td>
</tr>
<tr>
<td>End-diastolic diameter, cm</td>
<td>4.70</td>
<td>0.06</td>
<td>4.89</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>End-systolic diameter, cm</td>
<td>2.76</td>
<td>0.07</td>
<td>2.87</td>
<td>0.06</td>
<td>NS</td>
</tr>
<tr>
<td>End-diastolic volume, mL/m$^2$</td>
<td>79.6</td>
<td>1.93</td>
<td>86.4</td>
<td>1.92</td>
<td>0.02</td>
</tr>
<tr>
<td>End-systolic volume, mL/m$^2$</td>
<td>27.2</td>
<td>1.23</td>
<td>29.3</td>
<td>1.23</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiac output, L/m/m$^2$</td>
<td>3.53</td>
<td>0.10</td>
<td>3.87</td>
<td>0.11</td>
<td>0.02</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>0.660</td>
<td>0.01</td>
<td>0.663</td>
<td>0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS indicates not significant.
short duration have, on the average, elevated circulating OLF levels that are positively correlated with both blood pressure and left ventricular mass. (2) The frequency distribution of plasma OLF is bimodal in EH, whereas it is unimodal in normotensives. The mean of the primary mode was similar in both normotensives and EH patients, but \( \approx 50\% \) of the EH patients had high circulating levels of OLF that were described by an additional mode. The observation that elevated plasma OLF is not found in all EH patients suggests that the raised levels are not simply secondary to hypertension but are consistent with the present view that EH is multi-etiologic.\(^{14} \)

The proportion of EH patients with high plasma OLF in this study is similar to that found in a previous study (\( \approx 45\% \))\(^{11} \) in which the patients had EH that was more severe, more complicated, and of longer duration. The frequency with which elevated plasma OLF is observed among unselected patients with EH is notable and suggests that this sodium pump inhibitor either is a marker for a large, previously unrecognized, intermediate phenotype or that it is related to the pathogenesis of elevated blood pressure in the afflicted individuals. (3) Multiple regression analyses revealed that 13.2% and 11.6% of the variance of left ventricular mass is explained by 24-hour SBP and OLF, respectively. The importance of the former relationship is widely recognized and was verified in this study with patients whose hypertension was of short duration and free from complications. Under these conditions, the specific linkage of OLF with cardiac mass independent of blood pressure now reinforces the possibility that sodium pump inhibitors may be linked with cardiac structure in vivo in addition to its known effects on cardiac function.

# Variance Explained

<table>
<thead>
<tr>
<th>Variables</th>
<th>( B )</th>
<th>( T )</th>
<th>Significance</th>
<th>( R^2, % )</th>
<th>Significance of ( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hr SBP</td>
<td>0.142</td>
<td>3.602</td>
<td>(&lt;0.001)</td>
<td>13.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>OLF</td>
<td>0.008</td>
<td>3.625</td>
<td>(&lt;0.001)</td>
<td>11.6</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>Age</td>
<td>0.212</td>
<td>3.256</td>
<td>0.002</td>
<td>6.7</td>
<td>0.0018</td>
</tr>
<tr>
<td>BMI</td>
<td>0.721</td>
<td>2.381</td>
<td>0.019</td>
<td>4.0</td>
<td>0.019</td>
</tr>
<tr>
<td>Gender</td>
<td>5.455</td>
<td>1.03</td>
<td>0.304</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( B \) indicates partial regression coefficient; \( T \) the \( T \) value of \( B \). Dependent variable was LVMI. Multiple regression analysis: results and individual variance explained by each independent variable entered into a forward regression model. Gender did not contribute significantly to the variance. The total LVMI variance explained by the above variables in the model was 35.6%. BMI indicates body mass index.

Human OLF is a specific, high-affinity, and reversible inhibitor of the sodium pump that is an isomer of ouabain.\(^{7,8} \)

We have shown that our method measures human OLF specifically and quantitatively\(^7 \) and that a direct relationship between immunoreactivity and biological activity exists under extraction and assay conditions identical to those used in this study.\(^9 \)

Similar radioimmunoassay methods have been used to determine serum digoxin concentrations. In the elderly with heart failure, serum digoxin levels that ranged between 0.4 and 1 ng/mL (ie, 0.52 to 1.3 nmol/L) were related to stroke volume.\(^{15,16} \) The therapeutic concentrations of digoxin overlap those of the high mode of distribution for plasma OLF in the EH patients. Thus, the relationships between OLF and heart rate and stroke volume that we observed are comparable to those obtained with therapeutic concentrations of digitalis glycosides.\(^{15,16} \)

The stimulus for the elevated circulating OLF levels in a subgroup of patients is unknown. Increased secretion and diminished renal or metabolic clearance are possibilities not addressed by the design of our study and will require future investigation. Increased salt intake, renal failure, and raised extracellular fluid volumes have been suggested as a stimulus to increase OLF.\(^{17} \) However, neither creatinine clearance nor sodium excretion differed in EH with normal and high OLF. Thus, overt impairment of renal function and variation of sodium intake within the ranges measured can be excluded. Moreover, acute expansion of extracellular fluid volume in dogs and in humans did not raise plasma OLF.\(^{18,19} \) Nevertheless, increased circulating levels of OLF were observed in patients with volume-sensitive hypertension and congestive heart failure, which raises the possibility that a chronic as opposed to an acute tendency for expansion of fluid volume may be relevant.\(^8,16 \) EDV was greater in EH patients with high OLF, which suggests increased central blood volume. Multiple regression analysis suggested that OLF accounted for a small portion (3.4%) of EDV variance in patients with EH.

Among the EH patients, the high-OLF subgroup exhibited increased 24-hour DBP, left ventricular mass index, and stroke volume, although their heart rates were lower. All other variables, including PRA and aldosterone levels, did not differ between the 2 groups. Multiple regression analysis demonstrated that OLF contributed to left ventricular mass independent of its relationship with blood pressure. Therefore, the nature of the observed relationship between cardiac parameters, blood pressure, and circulating OLF is of interest. The experimental design used suggests a possible cause-and-effect relationship. However, long-term clinical studies similar to those that demonstrated the role of elevated blood pressure in raising cardiac mass will be required to address causality. Nevertheless, the observation that the patients with elevated OLF...
have higher 24-hour DBP is noteworthy. In both normotensives and hypertensives, the long-term DBP is correlated with the risk of cardiovascular and cerebrovascular disease without apparent threshold. Differences of as little as 4 to 6 mm Hg in DBP have been linked with significant risk outcomes. Moreover, in addition to higher 24-hour DBP, the EH patients with high OLF had larger left ventricular mass, which itself has been shown to worsen outcome. Thus, irrespective of whether the links of OLF with blood pressure and cardiac structure are causal, high OLF is a potential marker for heightened overall risk of premature morbidity and mortality.

A number of observations do, however, demonstrate cause-and-effect relationships for ouabain, blood pressure, and cardiac mass in other settings. As one example, prolonged infusion of low doses of plant ouabain induced sustained hypertension in normal rats. The steady-state plasma levels of ouabain were dose-dependently related to blood pressure and embrace the circulating levels of OLF in the high-OLF patient subgroup studied here. Second, a novel class of antihypertensive agents that are antagonists of ouabain has been described recently. These agents blocked the chronic hypertensinogenic and renal effects of prolonged ouabain administration. Third, inhibition of the majority of sodium pumps by ouabain leads to marked increases in cell Na and inhibition of protein synthesis, the latter associated with K loss and eventual cell death; whereas studies conducted in cultured neonatal rat cardiac myocytes demonstrate that concentrations of ouabain that inhibit a small fraction of the available sodium pumps activate the expression of growth-related genes and induces cellular hypertrophy. These results suggest that ouabain affects the signal transduction pathways through the interaction with gene regulatory mechanisms specifically localized in cardiac myocytes. Similarly, cellular hypertrophy can be induced in cardiac and arterial myocytes by raising the sodium concentration in their cell culture media. These reports emphasize the role of small sustained increases in cell sodium in accelerating cell growth. In apparent contrast with these findings, others demonstrated that the acute inotropic effect of ouabain in papillary muscle of adult ferret already stimulated to contract demonstrates that the acute inotropic effect of ouabain in papillary muscle of adult ferret already stimulated to contract. Moreover, the long-term effect that is trigged by an increased load is hypertrophy and not inhibition of protein synthesis.

In summary, among patients with uncomplicated EH, ≈50% had elevated circulating levels of an OLF. This subgroup of patients was characterized by greater DBP, left ventricular mass, and stroke volume and lower heart rate. Elevated circulating OLF appears to be a new factor that contributes to increased risk among patients with EH. Additional cross-sectional as well as longitudinal investigations are indicated.

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References


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