Elevated Concentrations of Endogenous Ouabain in Patients With Congestive Heart Failure

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Background. An endogenous digitalis-like compound in mammals has long been postulated, but only recently has a substance indistinguishable from ouabain been identified in human plasma. Because of the potential significance of such a substance in patients with congestive heart failure, we sought to evaluate the pathophysiology of endogenous ouabain in these individuals.

Methods and Results. Using an immunoassay, we determined plasma ouabain concentrations in 51 patients with heart failure and in 19 control subjects. Plasma ouabain concentrations in control subjects ranged from 0.16 to 0.77 nM (mean, 0.44±0.20 nM). In 19 matched heart failure patients receiving digoxin, the mean ouabain was significantly elevated at 1.59±2.2 nM (range, 0.17–8.76 nM, p<0.05 versus control subjects). The ouabain concentration correlated inversely with both cardiac index (r=−0.62, p<0.005) and mean arterial pressure (r=−0.51, p<0.05). However, there was no correlation between ouabain and left ventricular filling (r=0.19, NS) or right atrial pressures (r=0.20, NS). In 16 heart failure patients not receiving digoxin, the mean ouabain was 1.52±2.58 nM. No relation between renal function and ouabain was detected.

Conclusions. The unanticipated lack of correlation of ouabain with atrial pressures indicates that volume is not the chief determinant of ouabain concentration in patients with congestive heart failure. However, the significant relations of plasma ouabain concentration with cardiac index and mean arterial pressure imply that endogenous ouabain may be an important homeostatic factor in humans. (Circulation 1992;86:420–425)

Key Words • ouabain • congestive heart failure • Na\(^+\),K\(^+\)-ATPase

The existence of an endogenous counterpart to the digitalis glycosides has been postulated for more than a century, but only recently has this factor been identified. We have reported that a substance indistinguishable from ouabain is present in human plasma. This endogenous substance inhibits the sodium-potassium pump (Na\(^+\),K\(^+\)-ATPase) and, in vitro, has cardiac and vascular actions similar to plant-derived ouabain. Although the physiological importance of endogenous ouabain has not previously been explored, the availability of a sensitive and specific immunoassay for endogenous ouabain now permits investigation of its actions in vivo.

The impact of an endogenous Na\(^+\),K\(^+\)-ATPase inhibitor in individuals with congestive heart failure could be considerable. First, the myocardial inotropic state is directly dependent on the function of the sodium-potassium pump. Second, inhibition of the Na\(^+\),K\(^+\)-ATPase in the renal tubule may lead to natriuresis. Third, inhibition of the pump in the vasculature might maintain or increase blood pressure by causing vasoconstriction—either directly or by effects on sympathetic innervation. These possible consequences of the actions of a Na\(^+\),K\(^+\)-ATPase inhibitor explain the efficacy of cardiac glycosides in some patients and suggest that deficiency of endogenous ouabain might exacerbate congestive heart failure. On the other hand, the decreased cardiac output, fluid overload, and hypotension associated with congestive heart failure might augment release of endogenous ouabain.

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To test the hypothesis that ouabain concentrations are abnormal in patients with congestive heart failure, we measured plasma ouabain in 19 patients with moderate to severe congestive heart failure who were receiving digoxin and 19 healthy, age-, sex-, and race-matched control subjects.

We further evaluated the physiology of endogenous ouabain by assessing its concentration in patients not receiving digoxin and by determining its relation with both renal function and hemodynamic parameters in patients with congestive heart failure. This is the first study to directly measure and evaluate endogenous ouabain in individual patients.

Methods

Patient Population

We studied 51 patients with moderate to severe left ventricular dysfunction who were referred for the treat-
ment of congestive heart failure. There were 39 men and 12 women aged 27–81 years (mean, 58±12 years). All patients had an ejection fraction <40% with a mean of 21±8% (range, 5–38%). Ejection fraction was assessed by radionuclide ventriculography in all except three patients. The etiology of heart failure was ischemic heart disease in 17 patients, primary dilated cardiomyopathy in 32 patients, and primary valvular disease in two patients. By New York Heart Association (NYHA) classification, four patients were class II, 39 patients were class III, and eight were class IV. Nineteen of the patients were receiving digoxin and were evaluated hemodynamically. Ouabain concentrations were also assessed in 16 patients who had never received digoxin. Renal function was evaluated in nine of these patients not receiving digoxin and in 16 additional patients receiving stable doses of digoxin.

In addition to the 51 congestive heart failure patients, ouabain concentrations were assessed in 19 individuals who were age, sex, and race matched for the 19 hemodynamically evaluated patients. These normal control subjects did not have hypertension, diabetes mellitus, or any known cardiac disease. They were not receiving cardioactive medications.

Fifteen of the hemodynamically evaluated patients were men, and four were women. They ranged in age from 40 to 68 years (mean, 56±8 years). Ejection fraction by radionuclide ventriculography ranged from 5% to 34% (mean, 19±9%). The cause of heart failure was ischemic heart disease in seven patients, valvular disease in one patient, and primary dilated cardiomyopathy in 11 patients. By NYHA classification, three patients were categorized as class II, 13 as class III, and three as class IV. All of these patients were receiving constant doses of digoxin and diuretics, and all medication was withheld for at least 8 hours before hemodynamic and ouabain assessment.

To ensure that the findings were not merely secondary to digoxin administration, endogenous ouabain concentrations were measured in 11 men and five women who had never received digoxin. In these 16 patients, the mean ejection fraction was 27±7% (range, 13–38%). There were two NYHA class IV patients, 13 class III patients, and one class II patient (mean age, 63±11 years). In these patients, the etiology of heart failure was evenly divided between primary dilated cardiomyopathy and ischemic heart disease.

Glossomeric filtration rate was measured in 25 patients with congestive heart failure to assess whether ouabain concentrations reflect renal excretion. The ejection fraction in these patients ranged from 9% to 38% (mean, 22±8%). There were 17 men and eight women (mean age, 60±13 years). By NYHA classification, 20 patients were categorized as class III and five as class IV. Congestive heart failure was due to ischemic cardiac disease in seven patients, valvular disease in one patient, and primary dilated cardiomyopathy in 17 patients.

Hemodynamic Measurements

After written informed consent was obtained, right heart catheterization and arterial cannulation were performed in 19 patients who were then permitted to rest overnight to allow dissipation of hemodynamic changes related to intravascular instrumentation. On the following morning, after all cardioactive medications were withheld for at least 8 hours, the following hemodynamic variables were measured repeatedly in the fasting state until hemodynamic stability was achieved: mean arterial pressure, heart rate, left ventricular filling pressure, mean right atrial pressure, and cardiac output, using procedures that have been described previously. Mean systemic pressures were determined by electronic analysis. Cardiac index was calculated as cardiac output divided by body surface area (l/min/m²).

Determination of Glomerular Filtration Rate

Glossomeric filtration rate (GFR) was determined in 25 patients by the urinary accumulation of 99mTc DTPA (99mTc-diethylenetriamine pentaacetic acid), as described by LaFrance and colleagues. Urine was collected hourly between 1 and 4 hours after injection of 200 µCi of 99mTc DTPA, and serum samples were drawn 1, 2, 3, and 4 hours after injection. Three 1-hour measurements of GFR were computed, corrected for body surface area, and averaged.

Measurement of Osouabain

Venous blood was collected for assessment of ouabain in cold EGTA tubes containing reduced glutathione to minimize oxidation. The plasma was immediately frozen at −70°C. Ouabain was then measured in Bond Elut C-18 extracts (AnalyticChem International, Harbor City, Calif.) by immunoassay as previously described. The intra-assay and interassay coefficients of variation were 4.8% and 9.2%, respectively. The use of digoxin in patients with congestive heart failure should not directly influence the measurement of ouabain concentrations. Not only is the cross-reactivity of the antiserum with digoxin only 0.5%, but the extraction procedure is known to eliminate digoxin from the sample used for the assay.

Statistics

Data were compared by using ANOVA. For normally distributed data, significance was tested with the Student’s t test for unpaired data, with a value of p<0.05 considered to be significant. For data not normally distributed, the Mann-Whitney U test was used. All correlations were determined by simple linear regression. Group data are expressed as mean±SD. All statistics were computed using the STATVIEW II statistics package on an Apple Macintosh II computer.

Results

Ouabain Concentrations

Plasma ouabain concentrations in normal controls ranged from 0.16 to 0.77 nM, with a mean of 0.44±0.20 nM (Figure 1). In 19 matched patients with congestive heart failure who were receiving digoxin, the mean ouabain concentration was significantly elevated at 1.59±2.2 nM, p<0.05. However, the concentration ranged from 0.17 to 8.76 nM, with nine patients having a concentration below 0.77 nM, the upper limit seen in the normal control subjects.

Sixteen additional heart failure patients who had never received digoxin demonstrated ouabain concentrations similar to the 19 patients who had received digoxin. In the patients not receiving digoxin, the mean
ouabain concentration was 1.52±2.58 nM (range, 0.02–8.27 nM), with values <0.77 nM in 10 patients.

Ouabain concentrations in various subgroups of patients are shown in Table 1. These data reflect analysis of all patients with congestive heart failure studied. There were no differences in ouabain concentration related to etiology, sex, or age. The patients with the lowest ejection fractions demonstrated the highest concentrations of ouabain. Similarly, class III patients by NYHA classification had (statistically insignificant) increased concentrations compared with the less severely ill class II patients. However, the ouabain concentrations in class III patients were also (insignificantly) greater than those noted in class IV patients.

Relation Between Hemodynamic Variables and Ouabain

Hemodynamic comparisons between congestive heart failure patients with normal (ouabain <0.77 nM) and elevated ouabain concentrations are detailed in Table 2. Patients with elevated ouabain concentrations demonstrated more advanced heart failure, with a lower cardiac output and mean arterial pressure and elevated right atrial and left ventricular filling pressures. However, only the cardiac output was statistically significantly different (p<0.005) between the two groups.

Endogenous ouabain concentrations were then compared with hemodynamic parameters in 19 patients with congestive heart failure. The log of the ouabain concentration correlated negatively with the cardiac index (r = −0.62, p<0.005; Figure 2). There was also a negative correlation between the log of the ouabain concentration and the mean arterial pressure (r = −0.51, p<0.05; Figure 2). However, there was no correlation between ouabain concentration and left ventricular filling (r=0.19, p=NS) or right atrial pressures (r=0.20, p=NS; Figure 3).

Relation Between Renal Function and Ouabain

The differences in ouabain concentration observed among hemodynamically evaluated patients could not be explained by differences in renal function. The mean serum creatinine was the same in patients with elevated and normal ouabain concentrations (1.3±0.2 and 1.3±0.4 mg/dl, respectively). Similarly, the blood urea nitrogen did not differ between groups (26±9 and 25±13 mg/dl, respectively).

In more accurate assessments of renal function obtained in 25 patients with heart failure, there was no relation between GFR and plasma ouabain concentration (Figure 4). In these patients, ouabain ranged from 0.01 to 8.27 nM (mean, 1.2±1.9 nM). GFR ranged from 9 to 113 ml/min/1.73 m² (mean, 42±25 ml/min/1.73 m²).

Discussion

Plasma concentrations of endogenous ouabain are elevated in patients with congestive heart failure and correlate with hemodynamic abnormalities. This is the first demonstration of abnormal ouabain concentrations in a pathological condition, and these findings suggest that endogenous ouabain might contribute to cardiovascular homeostasis in humans.

Regulation of Ouabain

Endogenous ouabain has the same elemental composition as plant-derived ouabain, and mass spectrometry and high performance liquid chromatography do not demonstrate any differences between endogenous ouabain and plant-derived ouabain.10 Because plant-derived ouabain is excreted primarily via the kidneys11—and endogenous ouabain is probably similarly cleared—the lack of relation between renal function and ouabain concentration indicates that the concentrations noted in the present study are not the result of variations in renal

TABLE 1. Ouabain Concentrations in Various Subgroups of Patients With Congestive Heart Failure

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>Ouabain concentration (nM)</th>
<th>p = NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic</td>
<td>17</td>
<td>1.43±2.34</td>
<td>p = NS</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>32</td>
<td>1.20±1.82</td>
<td></td>
</tr>
<tr>
<td>Valvular disease</td>
<td>2</td>
<td>1.47±1.16</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>1.26±2.01</td>
<td>p = NS</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>1.38±1.88</td>
<td></td>
</tr>
<tr>
<td>NYHA classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>0.61±0.19</td>
<td>p = NS</td>
</tr>
<tr>
<td>III</td>
<td>39</td>
<td>1.46±2.20</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>0.78±0.71</td>
<td></td>
</tr>
<tr>
<td>Age*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤61 Years</td>
<td>26</td>
<td>1.48±2.23</td>
<td>p = NS</td>
</tr>
<tr>
<td>&gt;61 Years</td>
<td>25</td>
<td>1.08±1.66</td>
<td></td>
</tr>
<tr>
<td>Ejection fraction†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤21%</td>
<td>25</td>
<td>2.04±2.54</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>&gt;21%</td>
<td>23</td>
<td>0.52±0.63</td>
<td></td>
</tr>
</tbody>
</table>

NYHA, New York Heart Association.
*Patients divided by the median age of 61 years.
†Ejection fraction determined by radionuclide ventriculography in 48 patients; patients divided by the median value of 21%.
excretion. It is more likely that production of ouabain is increased in patients with heart failure.

Analysis of the differences between heart failure patients with elevated and normal plasma ouabain concentrations may help to elucidate the factors that contribute to increased ouabain production. The most severely ill patients generally demonstrate the greatest neurohormonal activation and hemodynamic abnormalities,12,13 and we similarly observed that ouabain concentrations were greatest in the patients with the lowest ejection fractions. However, ouabain concentrations do not appear to merely reflect the severity of heart failure. NYHA class IV patients did not have the highest concentrations, and left ventricular filling pressure (a sensitive indicator of the extent of heart failure) did not correlate with the ouabain concentration. Indeed, we noted that the only hemodynamic parameters that correlated with ouabain concentration were cardiac index and mean arterial pressure, which suggests that these factors may directly influence ouabain production.

The relation of endogenous ouabain with both cardiac index and mean arterial pressure suggests that these hemodynamic parameters may be determinants of ouabain production. Ouabain concentrations could conceivably depend on cardiac function and the consequent effects on perfusion of the organ(s) where ouabain is made. For example, changes in Na+,K+-ATPase inhibitor activity and concentration have been associated with hypothalamic and adrenal function,11,14,15 and it is possible that decreased perfusion of one or both of these organs stimulates ouabain production. On the other hand, hypotension secondary to left ventricular dysfunction might stimulate ouabain release in a physiological response to the relatively low blood pressure. Of course, the observation that heart failure patients with the highest mean arterial pressures have the lowest concentrations of ouabain does not rule out the possibility that hypertension might be caused by pathological elevations of plasma ouabain concentration. Endogenous ouabain is likely to be a vasoconstrictor,16 and

| Table 2. Comparison of Hemodynamic Variables of Patients With Normal and Elevated Ouabain Concentrations |
|-----------------|---------|--------|--------|--------|--------|
| Ouabain (nM) | n | CI (l/min/m²) | MAP (mm Hg) | LVFP (mm Hg) | RAP (mm Hg) | HR (min⁻¹) |
| >0.77 nM | 10 | 2.66±2.64 | 1.38±0.24 | 86±8 | 24±7 | 15±7 | 88±14 |
| <0.77 nM | 9  | 0.40±0.21* | 1.80±0.21† | 93±16 | 20±7 | 10±5 | 87±16 |

CI, cardiac index; MAP, mean arterial pressure; LVFP, left ventricular filling pressure; RAP, right atrial pressure; HR, heart rate.

*p<0.05; †p<0.005.

**Figure 2.** Plot shows relation between plasma ouabain and both cardiac index and mean arterial pressure in 19 patients with congestive heart failure. Highest ouabain concentrations are associated with lowest cardiac indexes and lowest arterial pressures.

**Figure 3.** Plot shows relation between plasma ouabain and both left ventricular filling and right atrial pressures in 19 patients with congestive heart failure. There was no correlation between plasma ouabain and either of the two pressures.
several previous studies using nonspecific assays have reported elevated circulating concentrations of unidentified Na\textsuperscript{+},K\textsuperscript{+}-ATPase inhibitors in patients with essential hypertension.\textsuperscript{17-21}

It has been postulated that increased intravascular volume causes release of endogenous inhibitors of Na\textsuperscript{+},K\textsuperscript{+}-ATPase.\textsuperscript{22-24} Indeed, a high sodium intake increases the plasma concentration of a Na\textsuperscript{+},K\textsuperscript{+}-ATPase inhibitor with properties similar to ouabain.\textsuperscript{25} Our data demonstrate, though, that right and left atrial pressures do not correlate with plasma concentrations of ouabain and suggest that intravascular volume alone does not regulate ouabain production. Other recent studies also argue against the hypothesis that increased extracellular fluid volume directly leads to increased circulating concentrations of ouabain. Chronic volume expansion in normal humans with 9-α-fluorohydrocortisone does not increase plasma concentrations of endogenous ouabain,\textsuperscript{26} and ouabain concentrations do not correlate with weight gains in patients on dialysis.\textsuperscript{27} These results contrast with observations concerning atrial natriuretic peptide\textsuperscript{28} and indicate distinct control mechanisms for the two hormonal substances with natriuretic properties. Nevertheless, the possibility remains that sodium loading may influence ouabain production by mechanisms other than intravascular volume. For example, there are sodium-sensing elements in the gastrointestinal system that may link dietary sodium and circulating Na\textsuperscript{+},K\textsuperscript{+}-ATPase inhibitors.\textsuperscript{29}

Ouabain and Heart Failure

The observation that the patients with the highest ouabain concentrations had the lowest cardiac indexes argues against the hypothesis that lack of ouabain contributes to the cardiac dysfunction of most patients with heart failure.\textsuperscript{30} Rather, it seems likely that elevated ouabain concentrations result from the abnormal physiology of congestive heart failure.

The mean plasma ouabain concentration of the patients with heart failure (1.59 nM) is similar to the generally accepted therapeutic concentration of digoxin (1.28–2.56 nM).\textsuperscript{31} This suggests that the magnitude of the physiological effect of endogenous ouabain is similar to that of the pharmacological effect of digoxin. The physiological importance of endogenous ouabain is underscored by the finding that ouabain has a greater affinity for the human cardiac Na\textsuperscript{+},K\textsuperscript{+}-ATPase than does digoxin.\textsuperscript{32} The effect of high concentrations of endogenous ouabain on the inotropic state, however, may well depend on the concentration of sodium-potassium pump sites,\textsuperscript{33} which is reportedly altered in patients with heart failure.\textsuperscript{34} Furthermore, it is not clear whether chronic inotropic stimulation is desirable; arrhythmias, progression of heart failure, and ischemia could possibly result from chronic Na\textsuperscript{+},K\textsuperscript{+}-ATPase inhibition.\textsuperscript{35}

Because both digoxin and endogenous ouabain may compete for the same receptor, the response to digoxin could be influenced by the plasma concentration of ouabain. Thus, patients with high ouabain concentrations may respond less well to digoxin or be at increased risk of toxic complications. Conversely, patients with severe heart failure and low plasma ouabain may benefit most from therapy with digoxin or other cardiac glycosides. The use of cardiac glycosides needs to be reevaluated in light of the high concentrations of endogenous ouabain noted in most patients with congestive heart failure. Digoxin assays also need to be reassessed in order to detect the extent of cross-reactivity with endogenous ouabain.

All patients evaluated hemodynamically in this study were receiving digoxin, which could conceivably influence endogenous ouabain concentrations. Ouabain and digoxin may compete for receptor binding, and administration of digoxin might therefore increase circulating concentrations of ouabain. It is also possible that digoxin directly influences ouabain production. However, ouabain concentrations were similar in the cohort of hemodynamically evaluated patients receiving digoxin and the cohort of heart failure patients not receiving digoxin, demonstrating that ouabain concentrations are not elevated in heart failure patients merely because of the administration of digoxin. Because the patients in the two groups are not directly comparable, this finding cannot indicate the nature of any interaction between exogenous digoxin and endogenous ouabain. We also cannot exclude the possibility that digoxin administration influenced the observed relation between ouabain and the hemodynamic variables. However, the ability to detect a relation between ouabain concentration and both mean arterial pressure and cardiac index suggests that digoxin administration cannot explain the lack of relation between ouabain and atrial filling pressures.

Conclusions

The presence of highly conserved receptor sites for the cardiotonic steroids has long implied the existence of an endogenous digitalis-like substance. The recent discovery of endogenous ouabain in mammals and the correlation of this compound with hemodynamic parameters in patients suggests that this system may be involved with homeostasis in humans.

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