Hormones Regulating Cardiovascular Function in Patients With Severe Congestive Heart Failure and Their Relation to Mortality

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There is a varying hormonal activation in heart failure. To be able to evaluate this activation and relate it to prognosis, we took blood samples at baseline and after 6 weeks from 239 patients with severe heart failure (all in New York Heart Association class IV) randomized to additional treatment with enalapril or placebo. In this study (CONSENSUS), which has previously been reported, there was a significant reduction in mortality among patients treated with enalapril. The present data show in the placebo group a significant positive relation between mortality and levels of angiotensin II \((p<0.05)\), aldosterone \((p=0.003)\), noradrenaline \((p<0.001)\), adrenaline \((p=0.001)\), and atrial natriuretic factor \((p=0.003)\). A similar relation was not observed among the patients treated with enalapril. Significant reductions in mortality in the groups of patients treated with enalapril were consistently found among patients with baseline hormone levels above median values. There were significant reductions in hormone levels from baseline to 6 weeks in the group of patients treated with enalapril for all hormones except adrenaline. There were no correlations between these changes in hormone levels. Summarily, there is a pronounced but variable neurohormonal activation in heart failure even in patients with similar clinical findings. This activation is reduced by enalapril therapy. The results suggest that the effect of enalapril on mortality is related to hormonal activation in general and the renin-angiotensin system in particular. \((\text{Circulation} \ 1990;82:1730–1736)\)

The long-term prognosis for patients with severe congestive heart failure has been very poor.\(^1\) In severe congestive heart failure, compensatory activation of neurohormonal mechanisms,\(^2\) the importance of which is unclear, takes place. The increased sympathetic activity may even induce myocardial deterioration in certain types of congestive heart failure.\(^3\) The neurohormonal activation may increase myocardial oxygen consumption by elevating heart rate and afterload and may also induce coronary vasoconstriction. A direct cardiotoxic effect of angiotensin II has also been recently reported.\(^4\) Diuretic therapy may enhance this activation and also induce electrolyte disturbances that might lead to arrhythmias.

It has been recently demonstrated by our group that the addition of the angiotensin converting enzyme (ACE) inhibitor enalapril to conventional therapy markedly reduced mortality in severe congestive heart failure.\(^5\) This trial was a multicenter Scandinavian trial called CONSENSUS. A secondary objective in CONSENSUS was to study the activation of hormones related to cardiovascular function and the effect of the overall hormone response to ACE inhibition. This report presents the hormonal data from the CONSENSUS trial and relates the hormone profiles to mortality.

Methods

Patients

The study design as well as inclusion and exclusion criteria have been reported previously.\(^5\) Two hundred fifty-three patients with severe congestive heart failure in New York Heart Association class IV were randomized from 35 Scandinavian centers in a double-blind fashion to placebo \((n=126)\) or enalapril
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The
diuretics (mean

heart

failure

48%

(n=127), in addition to their conventional therapy. The mean age was 70 years (range, 36–91 years). Coronary artery disease was the cause of congestive heart failure in 73% of the patients. All patients were on diuretics (mean furosemide dose, 210 mg), 94% were on digitalis, and 50% were on vasodilators, usually isosorbide dinitrate. ACE inhibitors were not permitted. The treatment dose of enalapril/placebo was 2.5–40 mg daily. This dose was titrated during the initial 3 weeks. The 6-month mortality (primary objective) in the group of patients receiving placebo was 48% as calculated from life-table analysis, and the corresponding figure in the group of patients receiving enalapril was 29%.

Blood Samples

Blood samples for assessment of hormone levels were taken at randomization and at 6 weeks. The protocol stated that samples should be drawn after 30 minutes’ rest in the supine position and should be frozen at −70°C if possible, otherwise at −20°C. Samples were collected regularly, transferred every month to the central laboratory, and stored there at −70°C. Two hundred thirty-nine samples were received at the central laboratory, however, for 14 patients, blood samples were missing for various reasons.

At 6 weeks, there were 28 deaths in the group of patients receiving placebo and 12 deaths among the patients receiving enalapril. Additionally, 17 survivors (nine receiving placebo and eight receiving enalapril) had less than 6 weeks of follow-up. Thus, 196 patients (89 receiving placebo and 107 receiving enalapril) were alive at the 6-week follow-up and potentially available for hormone analysis.

Hormone Analysis

All hormone concentrations were determined at the same laboratory, and all samples from the same patient were analyzed in one assay. The coefficient of variation for each method was calculated from analysis of frozen aliquots (−20°C except for catecholamine samples, which were stored at −70°C) of pooled human plasma or serum. Twenty aliquots were measured for determining the coefficient of variation within the assay, and 3–5 aliquots were analyzed on not less than 15 different occasions to determine the interassay coefficient of variation. All hormones could not be analyzed for every patient due to the lack of sufficient amounts of blood. The number of tested patients for each hormone is shown in the tables and the figures.

Noradrenaline, adrenaline, and dopamine were determined in EDTA-plasma by a radioenzymatic method6 by using kits from Amersham (UK). The intra-assay and interassay coefficients of variation were below 19% and 31%, respectively. Reference values in the laboratory are as follows (mean±SD): noradrenaline, 484±318 pg/ml; adrenaline, 132±112 pg/ml; dopamine, 81±79 pg/ml; with the following respective median values: 462, 126, and 42 pg/ml.

Aldosterone was determined by radioimmunoassay according to the procedure described by Walsh et al7 using kits from Diagnostic Products (Los Angeles, California). Cross-reactions with related steroids (e.g., 18-hydroxycorticosterone <0.05%, cortisol <0.0005%, and spironolactone <0.08%) were negligible. Intra-assay and interassay coefficients of variation were 7% and 10%, respectively. (Reference values in our laboratory [1 ng/dl=27.8 pmol/l], 404±208 pmol/l; median, 363 pmol/l.)

Angiotensin II in EDTA-plasma was measured by radioimmunoassay as described by Nussberger et al8 with kits from Buhlman Laboratories AG (Basel, Switzerland). The antiserum cross-reacts with angiotensin III to 100% and with angiotensin I to 0.16%. Due to potential cross-reactions in the angiotensin II radioimmunoassay,9,10 the levels we report should be considered as immunoreactive angiotensin II. The intra-assay and interassay coefficients of variation were 9% and 14%, respectively. (Reference values in our laboratory, 20±7 pg/ml; median level, 16 pg/ml.)

ACE activity was determined in serum by a radioenzymatic method11,12 using kits from Buhlman Laboratories.13 The low molecular weight substrate used probably means that angiotensin II generation by Tonin-macroglobulin complex14 will be measured as ACE activity. The intra-assay and interassay coefficients of variation were 6% and 8%, respectively. (Reference values in our laboratory, 25±12 U/min/l; median activity, 28 U/min/l. Regarding variations between different laboratories in terms of reference intervals, see Evans.15)

Atrial natriuretic factor was measured in EDTA-plasma by using radioimmunoassay kits from Immunotechnology Service (Wyckon, Holland) as recently described.16 Intra-assay and interassay coefficients of variation were 8% and 13%, respectively. (Reference values in our laboratory, 57±21 pg/ml; median level, 47 pg/ml.)

Statistical Methods

The protocol stated that baseline and 6-week hormone levels should be related to 6-month mortality. Other comparisons were not prespecified. The treatment groups were compared with respect to mean hormone values, as well as mean changes in values, by using Wilcoxon’s rank sum test. The signed rank test was used for within-group changes from baseline. Differences between survivors and deaths were also tested by using the rank sum test. The relations between hormone levels were evaluated by using the Spearman correlation coefficient.

The relation between hormone levels and mortality was tested with logistic regression models by using continuous hormone values. The log rank test was used to compare the mortality experiences of the two treatment groups of patients and for the subgroup of patients determined by baseline hormone levels. All comparisons were made according to “intention to treat.” A p value of less than 0.05 was considered significant.
Table 1. Baseline Hormone Levels

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Treatment group</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE (U/min/l)</td>
<td>Enalapril</td>
<td>109</td>
<td>30.8</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>114</td>
<td>32.2</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>223</td>
<td>31.5</td>
<td>14.2</td>
</tr>
<tr>
<td>Angiotensin II (pg/ml)</td>
<td>Enalapril</td>
<td>120</td>
<td>68.4</td>
<td>71.0</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>119</td>
<td>76.0</td>
<td>74.8</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>239</td>
<td>72.2</td>
<td>72.9</td>
</tr>
<tr>
<td>Aldosterone (pmol/l)</td>
<td>Enalapril</td>
<td>119</td>
<td>1,435</td>
<td>895</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>119</td>
<td>1,331</td>
<td>879</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>238</td>
<td>1,383</td>
<td>887</td>
</tr>
<tr>
<td>Noradrenaline (pg/ml)</td>
<td>Enalapril</td>
<td>114</td>
<td>909</td>
<td>612</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>116</td>
<td>935</td>
<td>597</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>230</td>
<td>922</td>
<td>603</td>
</tr>
<tr>
<td>Adrenaline (pg/ml)</td>
<td>Enalapril</td>
<td>114</td>
<td>147</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>116</td>
<td>170</td>
<td>212</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>230</td>
<td>158</td>
<td>191</td>
</tr>
<tr>
<td>Dopamine (pg/ml)</td>
<td>Enalapril</td>
<td>114</td>
<td>102</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>116</td>
<td>86</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>230</td>
<td>94</td>
<td>81</td>
</tr>
<tr>
<td>ANF (pg/ml)</td>
<td>Enalapril</td>
<td>114</td>
<td>521</td>
<td>412</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>112</td>
<td>405</td>
<td>322</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>226</td>
<td>463</td>
<td>374</td>
</tr>
</tbody>
</table>

Note: Mean ANF at baseline was significantly greater in the group of patients receiving enalapril than in the group of patients receiving placebo (p=0.02).
ACE, angiotensin converting enzyme; ANF, atrial natriuretic factor.

Results

The mean ACE activities and hormone levels at baseline are depicted in Table 1. All hormones were markedly elevated above normal. Hormone levels in relation to sex or diabetes were also analyzed. The only important difference between sexes was the plasma level of adrenaline, which was higher in males (166±203 vs. 139±159 pg/ml; p<0.05). Diabetic patients had lower adrenaline levels than nondiabetic patients (108±153 vs. 173±199 pg/ml; p<0.05).
The aldosterone values were related to the use of spironolactone at baseline. The 90 patients with previous spironolactone therapy had 1,603±882 pmol/l vs. 1,249±665 pmol/l for the nonusers, possibly reflecting cross-reactions between the exogenous steroid and its metabolites in the aldosterone assay.

The correlations between the analyte concentrations are shown in Table 2. There was a slight but significant correlation between noradrenaline and angiotensin II, adrenaline, dopamine, and atrial natriuretic factor. The correlation was greater between angiotensin II and aldosterone.
The mean baseline hormone levels among survivors and nonsurvivors in the placebo group are shown in Table 3. The survivors had significantly lower baseline concentrations of angiotensin II (p<0.001), aldosterone (p<0.001), atrial natriuretic factor (p<0.01), noradrenaline (p=0.001), and adrenaline (p<0.05) than the nonsurvivors.

Hormone Levels and Mortality

The cause of death was cardiovascular in all patients but one (bleeding ulcer), with death due to

Table 2. Correlations Between Baseline Hormone Levels

<table>
<thead>
<tr>
<th></th>
<th>ACE</th>
<th>Angiotensin II</th>
<th>Aldosterone</th>
<th>Noradrenaline</th>
<th>Adrenaline</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II</td>
<td>r = -0.06</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>223</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>r = -0.01</td>
<td>0.37†</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>222</td>
<td>238</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>r = 0.01</td>
<td>0.27†</td>
<td>0.26†</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>215</td>
<td>229</td>
<td>230</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>r = -0.03</td>
<td>0.09</td>
<td>0.11</td>
<td>0.23†</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>212</td>
<td>229</td>
<td>230</td>
<td>230</td>
<td>...</td>
</tr>
<tr>
<td>Dopamine</td>
<td>r = -0.05</td>
<td>0.06</td>
<td>0.00</td>
<td>0.18†</td>
<td>0.25†</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>215</td>
<td>229</td>
<td>230</td>
<td>230</td>
<td>230</td>
</tr>
<tr>
<td>ANF</td>
<td>r = 0.08</td>
<td>0.07</td>
<td>0.06</td>
<td>0.16*</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>213</td>
<td>226</td>
<td>226</td>
<td>217</td>
<td>217</td>
</tr>
</tbody>
</table>

Note: This table presents the Spearman correlation coefficient and sample size for all pairs of hormones. ANF, atrial natriuretic factor; ACE, angiotensin converting enzyme.
* p<0.05; † p<0.01.
progression of heart failure predominating. The baseline hormone levels in this particular group of patients were calculated and compared with those from patients who died of other causes. There were no significant differences between these groups of patients.

Baseline hormone levels were analyzed in relation to 6-month mortality in the two treatment groups of patients. In Figures 1–5, these are shown for the groups of patients above and below the median level of the various hormones. Between the two treatment groups of patients, there were no significant differences at baseline in age, weight, height, systolic or diastolic blood pressure, heart rate, serum sodium, potassium, or creatinine. There was a significant positive relation in the group of patients receiving placebo between mortality and angiotensin II (p<0.05), aldosterone (p=0.002), atrial natriuretic factor (p=0.003), noradrenaline (p<0.001), and adrenaline (p=0.001). Such relations were not observed among the patients treated with enalapril. The reduction in 6-month mortality was pronounced in particular for patients with baseline concentrations of angiotensin II (from 55% to 25%, p<0.001) or aldosterone (from 55% to 20%, p<0.01) that were above median. There was no significant effect among patients with hormone levels below median. Accordingly, the significant reduction in mortality was confined to patients with baseline hormone levels above median for these five hormones. For noradrenaline and adrenaline levels below median, however, there was a nonsignificant reduction in mortality of 29% (from 31% to 22%) and 23% (from 35% to 27%), respectively.

Changes in Hormone Levels

Changes in analyte concentrations from baseline to 6 weeks are presented in Figure 6. There were significant reductions in the group of patients receiving enalapril for angiotensin II, aldosterone, atrial natriuretic factor, and noradrenaline. The decrease in ACE activity was pronounced. In 63 patients, the ACE activity at 6 weeks was less than 2 U/min/l; in six patients, it was 2–10 U/min/l; and in a further six patients, it was above 10 U/min/l. None of the latter six patients had been withdrawn from blinded therapy.

The correlation between these hormone changes in the group of patients receiving enalapril was analyzed, and it was found that there was no relation between these changes at all. Changes in furosemide dosages correlated significantly to changes in aldosterone concentrations (r=0.29; p<0.01) but not to any other hormonal alteration.

The 6-week changes were compared with the subsequent 6-month mortality to determine whether the outcome could be predicted from the effects on hormone levels. In both treatment groups, there were no significant relations between hormonal changes from baseline to 6 weeks and subsequent mortality. When angiotensin II decreased with more than 16 pg/ml (median change), however, the mortality in the group of patients receiving enalapril was 7% (3 of 44), compared with 21% (7 of 37) when the decrease was less. The corresponding values for aldosterone were 11% for an aldosterone reduction exceeding 232 pg/ml versus 20% when the reduction was less.

Discussion

Increased neurohormonal concentrations have previously been reported in congestive heart failure.17,18
The present study confirms these observations in a large group of patients with severe heart failure. The high hormonal levels in our heart failure patients demonstrated the pronounced activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system as well as the release of atrial natriuretic factor. Even among patients with similar symptoms, all in New York Heart Association class IV, however, there was a large variation in hormone levels. It thus seems that neurohormonal compensatory mechanisms are activated to a very variable extent in severe congestive heart failure.

All patients were on medication, and the importance of this therapy to the neuroendocrine activation is obviously unknown. In this context, it is interesting to consider the observation by Bayliss et al.19 In untreated patients with congestive heart failure, they observed elevations of plasma noradrenaline but not of plasma renin. There was a significant increase of plasma renin after the administration of diuretics.

**Correlations Between Hormone Levels**

The correlations between levels of noradrenaline, angiotensin II, aldosterone, and atrial natriuretic factor were significant although slight, with $r$ values from 0.16 to 0.37. This may in part be due to the wide standard deviations in our methodology. A positive but weak correlation, however, is not changed into a negative by a reduced variance. The systems are activated simultaneously but apparently to a variable extent in each case.

The poor correlations between changes in hormone levels from baseline to 6 weeks were somewhat surprising. Particularly, the lack of any correlation between changes in angiotensin II and changes in aldosterone demonstrates the complexity of this hormonal activation. The changes in plasma ACE activity were not correlated to changes in angiotensin II or aldosterone. This may imply that plasma ACE does not reflect tissue ACE activity adequately. The importance of the tissue ACE has been emphasized by Dzau.20 Plasma ACE is simply the most easily inhibited component of the net ACE activity in the body. Other ACE activity may be more important for the functional status of the cardiovascular system. The clinical importance is that although the smallest dose of enalapril inhibits plasma ACE effectiveness, maximal reduction of angiotensin II may require larger doses of enalapril. Plasma ACE activity reflects compliance in the study as well as bioavailability of enalapril. A
plasma ACE level of more than 10 U/min/1 at the time of follow-up therefore probably represents evidence of noncompliance with enalapril treatment.

**Mortality Versus Hormone Concentrations**

A relation between plasma noradrenaline levels and mortality has previously been shown by Cohn et al. Our observations are in accordance with their findings. Circulating noradrenaline is probably of limited physiological importance because it amounts to less than 1% of what is available in the synaptic cleft. Increased plasma norepinephrine is the result of increased spillover from this cleft. At best, plasma norepinephrine reflects sympathetic activity.

We found an even greater correlation between mortality and angiotensin II or aldosterone, which has not been reported earlier. Packer and colleagues have described a relation between hyponatraemia and mortality in a retrospective follow-up. They did not demonstrate a significant relation, however, between renin levels and survival.

The beneficial effect of enalapril therapy on survival in the present study was related to the activity of the renin-angiotensin-aldosterone system. (This could be seen in patients with high levels of angiotensin II or aldosterone.) The relation between baseline concentrations and mortality seen in the group of patients receiving placebo was absent in the patients receiving enalapril. Furthermore, the entire effect on mortality was observed among those patients who had concentrations above median shown experimentally by Rieger et al. Recently, it has been demonstrated by Tan et al that angiotensin II may induce direct myocardial toxicity.

It cannot be concluded from our data that patients with low hormone concentrations will not benefit from enalapril. Because these patients have a lower mortality risk, it will be necessary to monitor larger numbers for a longer time to be able to address this issue. There may be patients with severe heart failure who have a low hormonal activation, and enalapril would therefore have no effect on short-term mortality. Their long-term mortality, however, is relatively high and they may nevertheless benefit symptomatically from ACE inhibition. It should also be pointed out that long-term (>6 month) mortality was not studied.

The effect of enalapril might also be due to differing hemodynamic states in our patients, in which those patients with greater reduction in myocardial function benefit from a vasodilator effect. All our patients, however, had advanced heart failure in functional class IV and we did not measure hemodynamics.

**Atrial Natriuretic Factor**

Atrial natriuretic factor increases diuresis, causes venodilation, and inhibits renin release in normal patients. Elevated levels of this peptide have been reported in heart failure. Hara et al found plasma atrial natriuretic factor concentrations to be related to the ejection fraction and the severity of heart failure. A reduction in atrial natriuretic factor may reflect hemodynamic improvement, expressed as a lowering of the left ventricular filling pressure.

Atrial natriuretic factor may modulate the effects of the renin-angiotensin-aldosterone system in heart failure. A relation between atrial natriuretic factor levels and mortality has been reported recently by Gottlieb et al. They found in a retrospective analysis of 103 patients that patients with a blood level of atrial natriuretic factor above 125 pg/ml (median) had a significantly lower rate of survival than those with a lower level. We also found in the group of patients receiving placebo that survivors had lower levels of atrial natriuretic factor than nonsurvivors. The mortality was significantly reduced by enalapril in patients with above-median but not below-median baseline levels. Whether atrial natriuretic factor has direct harmful effects on the myocardium cannot be determined from this study.

**Cause of Death**

The baseline hormone levels were not related to subsequent cause of death. As 65% of the patients in the group receiving placebo died of congestive heart failure and this cause of death was significantly reduced among patients on enalapril, the importance of renin-angiotensin-aldosterone system activation for the progression of heart failure is demonstrated in this study. The mechanisms of this deleterious influence, however, remain to be clarified. It is not possible to make any conclusion as to whether the results from this study may be translated directly for patients with less severe heart disease in New York Heart Association class II–III and who have high hormone levels. If so, ACE inhibition may prevent deterioration of myocardial function.

**Conclusion**

In summary, there is a pronounced but variable neurohormonal activation in severe heart failure. This activation is reduced by enalapril. There is a significant relation between neurohormonal activation and mortality in the patients treated with placebo. The high mortality was observed to be reduced by enalapril, primarily in those patients with the most pronounced activation. This study suggests that the reduction in mortality is related to the inhibition of neurohormonal activation and of the renin-angiotensin-system in particular.

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