Plasma Endothelin in Chronic Heart Failure

John J. McMurray, MD; Simon G. Ray, MRCP; Ibrahim Abdullah, MRCP; Henry J. Dargie, FRCP; and James J. Morton, PhD

Background. Endothelins are recently characterized vasoconstrictor peptides. As chronic heart failure (CHF) is characterized by peripheral arteriolar and renal vasoconstriction, we have measured venous plasma endothelin-like immunoreactivity ("endothelin") in patients with this syndrome.

Methods and Results. Compared with age- and sex-matched healthy volunteers (mean±SEM plasma endothelin concentration 6.4±0.3 pmol/l, n=16), patients with severe CHF had elevated peripheral venous endothelin concentrations (12.4±0.6 pmol/l, n=47, p<0.01). Plasma endothelin did not increase with exercise in normal subjects or in patients. Plasma endothelin concentration (mean, 13.4±0.9 pmol/l) did not correlate with plasma atrial natriuretic factor concentration (mean, 88.9±11.9 pg/ml) in patients with CHF (n=21). There was also no correlation between plasma endothelin and serum urea or between endothelin and serum creatinine in patients with CHF (n=34). There was, however, significant renal extraction of endothelin (aorta, 11.1±0.8 pmol/l; renal vein, 8.8±0.6 pmol/l; p=0.02) in patients with CHF (n=13).

Conclusions. Evidence suggests that circulatory endothelin concentrations in the range 5–40 pmol/l are vasoactive. Consequently, the endothelin concentrations found in patients with CHF may be of pathophysiological significance. (Circulation 1992;85:1374–1379)

KEY WORDS • atrial natriuretic factor • heart failure, congestive • peptides • vasoconstriction

Chronic heart failure (CHF) is a syndrome characterized by reduced peripheral and renal perfusion.1 Impairment of blood flow is, in part, a result of altered vascular tone and responsiveness.1 Certain neurohumoral factors, such as angiotensin II, norepinephrine, and arginine vasopressin, are believed to contribute to hypoperfusion in CHF by causing vasoconstriction.2,3 Recently, a new family of vasoconstrictor peptides known as endothelins has been identified.4 Endothelins (hereafter referred to as "endothelin") are potent, long-acting arterial and venoconstrictors with a particularly pronounced effect on the renal vasculature.4,5 Endothelin mRNA is widely expressed in human tissues, and endothelin binding sites have been demonstrated in blood vessels, heart, and kidney.6 Sensitive assays for endothelin have been developed and show that it can be detected in the circulation (endothelin-I is believed to be the circulating form in humans).7 As endothelin could contribute to the pathophysiological disturbance of vascular tone in CHF, we have measured endothelin levels in patients with this syndrome. A twofold increase in circulating endothelin concentration has been reported in animal models of CHF.8,9

Methods

Healthy Subjects

Group A, 10 healthy subjects from the Department of Cardiology and the MRC Blood Pressure Unit, was studied at rest. Peripheral venous plasma endothelin was measured in these subjects. Subsequently, six healthy male subjects (group B) were studied before and after symptom-limited maximum exercise. Peripheral venous plasma endothelin was measured before and after exercise in these subjects.

CHF Patients

Group 1, consisting of 18 patients, was studied after a period of supine rest; peripheral venous plasma endothelin was measured in these patients. All had chronic (>3 months’ duration) heart failure. CHF was diagnosed on the basis of symptoms of breathlessness and fatigue associated with systolic left ventricular dysfunction. All were treated with diuretics, and the majority were also taking digoxin and vasodilators.

Group 2, consisting of 21 patients with CHF, was subsequently studied; in these patients, both endothelin and atrial natriuretic factor (ANF) were measured in peripheral venous blood.

A further eight patients with CHF were studied before and after a symptom-limited maximal treadmill exercise test (group 3). Peripheral venous plasma endothelin was measured before and immediately after exercise in these patients.

Finally, 13 patients with CHF were studied at the time of cardiac catheterization (group 4). Aortic and renal venous plasma endothelin concentrations were measured in these patients.
Sample Collection and Handling

In healthy controls (groups A and B) and in patient groups 1–3, 10 ml of blood was drawn from an antecubital vein after at least 15 minutes of rest. Patients in groups 1 and 2 were studied after supine rest, and those in group 3 and healthy controls (groups A and B) were studied after seated rest. In patient group 3 and control group B, a second sample was taken 3–5 minutes after maximum exercise. Patients in group 4 were studied after diagnostic right heart catheterization but before contrast left ventriculography. Simultaneous 10-ml blood samples were taken from either the right or left renal vein (identified by hand injection of a few milliliters of contrast) and the aorta. All blood samples were collected into chilled K+-EDTA tubes and immediately placed on ice. Samples were then centrifuged at 4°C, and the plasma was separated and stored at −20°C until assay.

Endothelin Assay

All samples were assayed within 2 months of collection. Paired samples from any one individual were always run within the same batch of samples. Plasma endothelin-1 and -2-like immunoreactivity was measured by the method of Davenport et al. Briefly, 3 ml of plasma was acidified with 2 M hydrochloric acid and applied to preactivated cartridges (Bond-Elut, LRC bonded phase PH, Jones Chromatography). The columns were washed with 0.1% trifluoroacetic acid, and immunoreactive endothelin was eluted with 80% methanol/0.1% trifluoroacetic acid. The extracts were evaporated to dryness under a stream of compressed air. The dried extracts were reconstituted in borate buffer. Antiserum cross-reactivity was endothelin-1, 100%; endothelin-2, 204%; and endothelin-3, 0.024%. Mean recovery of added “cold” endothelin (100 pmol/l) from plasma was 61±1.6% (n=16). The lower limit of detection of the assay is 0.5 pmol/l.

ANF Assay

ANF was measured with a sensitive and specific radioimmunoassay, as previously described. The normal concentration range for plasma ANF with this assay is <30 pg/ml.

Statistical Analysis

Normal and patient endothelin concentrations were compared by independent t test. Renal vein and aortic concentrations and concentrations before and after exercise were compared by a paired t test.

Results

Healthy Volunteers

Group A consisted of seven men and three women aged 42–64 years (mean, 52 years). All subjects in group B were men aged 38–54 years (mean, 48 years).

Resting plasma endothelin concentration in group A was 5.9±0.2 pmol/l (mean±SEM) (Figure 1). The mean resting plasma endothelin concentration before exercise in group B was 7.2±0.4 pmol/l, and the concentration after exercise was 7.2±0.4 pmol/l (Figures 1 and 2).

CHF Patients

The characteristics of the patients in each group are given in Table 1. Groups 1–3 were similar. Group 4 contained more women, was younger, and had less severe heart failure.

Resting endothelin concentrations. See Figure 1. The mean peripheral venous plasma endothelin concentration in resting CHF patients was 11.5±1.1 pmol/l in group 1 and 13.4±1.0 pmol/l in group 2. Endothelin

FIGURE 1. Plots of resting venous plasma endothelin concentrations in healthy volunteers (normal) and patients with chronic heart failure (CHF). ○, Group A; △, group B (healthy volunteers). ●, Group 1; ●, group 2; and ▲, group 3 (before exercise; no sample was obtained after exercise in one subject) (CHF patients). ET-IR, endothelin-like immunoreactivity.

FIGURE 2. Plots of venous plasma endothelin concentrations in healthy volunteers (△—△, normal; group B) and patients with chronic heart failure (▲—▲, CHF; group 3) sitting before exercise (pre ex) and immediately after upright exercise on a treadmill (post ex). ET-IR, endothelin-like immunoreactivity.
TABLE 1. Characteristics of CHF Patients

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=18)</th>
<th>Group 2 (n=21)</th>
<th>Group 3 (n=8)</th>
<th>Group 4 (n=13)</th>
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<tr>
<td>Age (mean±SEM)</td>
<td>61±4</td>
<td>68±5</td>
<td>67±4</td>
<td>52±4</td>
</tr>
<tr>
<td>Sex (men:women)</td>
<td>18:0</td>
<td>15:6</td>
<td>6:2</td>
<td>4:9</td>
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<td>Primary cause of CHF</td>
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<td></td>
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<tr>
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<td>14</td>
<td>16</td>
<td>5</td>
<td>2</td>
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<tr>
<td>Valvular</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>6*</td>
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<td>0</td>
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<td>NYHA class</td>
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</tr>
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<td>II</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
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<td>III</td>
<td>12</td>
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<tr>
<td>IV</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>FS (%)</td>
<td>16.0±4.1</td>
<td>18.4±4.8</td>
<td>19.0±3.2</td>
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<td>LVEDD (cm)</td>
<td>6.3±0.6</td>
<td>6.4±0.5</td>
<td>6.0±0.4</td>
<td>5.6±0.7</td>
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<tr>
<td>Creatinine†† (μmol/l)</td>
<td>171±25</td>
<td>135±11</td>
<td>158±32</td>
<td>107±22</td>
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<tr>
<td>Urea†† (mmol/l)</td>
<td>16.9±4.3</td>
<td>10.7±1.0</td>
<td>15.0±5.2</td>
<td>9.6±3.3</td>
</tr>
</tbody>
</table>

Group 1, 18 patients with CHF studied after supine rest; group 2, 21 other patients with CHF studied as for group 1; group 3, 8 patients with CHF studied before and after a symptom-limited treadmill exercise test; and group 4, 13 patients with CHF studied at the time of cardiac catheterization.

CHF, chronic heart failure; IHHD, ischemic heart disease; DCM, dilated cardiomyopathy; NYHA, New York Heart Association; FS, echocardiographic fractional shortening of the left ventricle; LVEDD, left ventricular end-diastolic diameter.

*One patient also had significant coronary artery disease.
†n=13, Group 1; n=15, group 2; n=6, group 3 in Figure 5.
‡Creatinine, 1 mg/dl=88.4 μmol/l; blood urea nitrogen, 10 mg/dl=3.6 mmol/l.

concentration did not appear to relate to the level of left ventricular dysfunction, New York Heart Association class, or cause of heart failure; it should be noted, however, that the majority of patients had moderately severe or severe heart failure caused by coronary artery disease.

Effect of exercise. See Figure 2. Peak Vo2 was measured in five of this group of patients and was 15.4±2.0 ml/kg/min. Plasma endothelin concentrations before and after exercise in these patients (group 3) were 11.0±1.5 and 10.8±1.4 pmol/l, respectively (p=NS).

Aortic and renal vein plasma concentrations. See Figure 3. The mean aortic plasma level of endothelin was 11.1±0.8 pmol/l, and the mean renal vein plasma concentration was 8.8±0.6 pmol/l (p=0.02). One patient was studied at rest, before exercise, and at cardiac catheterization. His endothelin levels in these circumstances were 17.9, 18.4 (before exercise; no sample was obtained after exercise), and 15.4 pmol/l.

Relation With ANF

See Figure 4. Mean plasma ANF in the patients with heart failure (group 2) was 88.9±11.9 pg/ml, compared with 13.6±4.4 pg/ml in healthy subjects (group B; p<0.001). There was no correlation between plasma ANF and endothelin.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Plots of aortic plasma (aorta) and renal venous plasma (renal vein) plasma endothelin concentration in patients with chronic heart failure (group 4). ET-IR, endothelin-like immunoreactivity.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Plots of venous plasma atrial natriuretic factor (ANP) and endothelin concentrations in individual patients (n=21) with chronic heart failure (group 2). There was no correlation between the concentrations of the two peptides in plasma.
Endothelin concentrations did not correlate with plasma ANF concentrations. This finding is of interest for two reasons. First, plasma endothelin concentration has been reported by Margulies et al.\(^9\) to correlate with right atrial pressure and pulmonary artery occlusion pressure in an animal model of heart failure. Atrial distension is believed to be the stimulus for ANF release, and right and left atrial pressures have been found in previous studies to correlate with ANF concentrations. Consequently, a relation between ANF and endothelin levels might have been expected in the present study. We did not find this (Margulies et al did not report whether they found a correlation between ANF and endothelin). Furthermore, in a study of patients with primary and secondary pulmonary hypertension, Stewart et al.\(^{11}\) found no relation between pulmonary artery pressure and plasma endothelin concentration.

The second reason why the present observation is of interest is that a physiological antagonism between ANF and endothelin has been postulated.\(^{12}\) It has been suggested that endothelin stimulates ANF release and that ANF counters the actions of the former; for example, it antagonizes endothelin-induced vasoconstriction. Our findings do not support this hypothesis, at least in heart failure. Our findings are also in keeping with those of Shichiri et al,\(^{13}\) who found no correlation between ANF and endothelin in patients with chronic renal failure.

We also failed to find any relation between plasma endothelin concentration and renal function as assessed by serum urea and creatinine. Plasma endothelin concentration has not been found to correlate with blood urea nitrogen or creatinine in most studies of patients with renal failure and/or hypertension.\(^{13-17}\) One study by Kohn et al.\(^{15}\) did, however, find a positive correlation between plasma endothelin and serum creatinine and a negative one between endothelin and glomerular filtration rate in untreated hypertensives; it is not clear why this study is the exception. Margulies et al\(^9\) were also unable to find a correlation between plasma endothelin concentration and a number of indexes of renal function (glomerular filtration rate, renal vascular resistance, and fractional sodium excretion) in their experimental model of heart failure. The same group did, however, find that exogenous endothelin infusion resulted in higher plasma concentrations in heart failure dogs than in normal dogs.\(^8\) There was no difference in glomerular filtration rate between the two animal groups in this experimental setting. These findings suggest that endothelin clearance is decreased in heart failure but that this decrease is not a result of reduced glomerular filtration. In another study, however, Kohn et al.\(^{16}\) showed that bilateral nephrectomy reduced the clearance of exogenous endothelin in rats, suggesting a role for the kidneys. Anggard et al.\(^{17}\) found that in the rat, the kidneys were second only to the lung in their capacity to bind and clear exogenous (injected) radiolabeled endothelin. In the present study, we also found that the kidneys extract endothelin, in that there was a 20% decrease in plasma endothelin concentration across the kidney (i.e., aortic compared with renal vein) in patients with heart failure. One possible explanation for all of these observations is that the kidney does remove endothelin from the circulation but not by glomerular

Relation With Serum Urea and Creatinine

See Figure 5. Neither the concentration of serum urea nor that of creatinine showed any relation to plasma endothelin concentration (there was also no correlation between renal extractions of endothelin and either serum urea or creatinine in patients in group 4). No relation was found between plasma ANF concentration and serum urea or creatinine concentration.

Discussion

In this study, patients with CHF were found to have a mean peripheral venous plasma endothelin concentration twice that of age- and sex-matched control subjects (our normal levels were in close agreement with those of Davenport et al\(^7\)). Our findings are in keeping with recent reports of a twofold to threefold increase in plasma endothelin concentration in animal models of CHF.\(^8,9\)

We also found that upright treadmill exercise did not cause a rapid increase in circulating endothelin concentrations either in healthy volunteers or in patients, in marked contrast to other neurohormones.\(^2\) It is possible that endothelin secretion does not respond as quickly as these other hormones or that an increase in local production has to be sustained for an increment in systemic concentration to be detected.
filtration. Endothelin-1 binding sites have been identified in the human kidney (though not in the glomeruli), and it is possible that these account for renal removal of the peptide.\textsuperscript{30} Whether renal removal of endothelin is increased or decreased from normal in these patients, or whether it even occurs normally, cannot be determined from the present study. Extrarenal clearance (e.g., pulmonary) of endothelin may also occur, as has been described for other peptides.\textsuperscript{21,22} In this context, it is noteworthy that our findings contrast with other reports that peripheral venous plasma endothelin concentration is significantly higher than the arterial concentration in healthy volunteers and patients with coronary artery disease (this finding has been used to infer pulmonary clearance of endothelin).\textsuperscript{11,22,23}

Before the potential pathophysiological significance of these findings can be gauged, it is important to consider how specific the assay used to measure endothelin is. Endothelin-1 is the biologically active peptide that circulates in humans. The antibody used in this study does not cross-react with degraded endothelin; i.e., it will not measure metabolites of endothelin (this is because it reacts with the N-terminal variant region and does not recognize ring opened forms).\textsuperscript{7,24} The antibody we used does significantly cross-react with endothelin-2. Endothelin-2, however, has not been found to circulate in humans.\textsuperscript{4,7,15,25,26} The antibody we used also cross-reacts with proendothelin-1 ("big endothelin"). Consequently, the "endothelin" immunoreactivity found in our patients probably reflects both circulating endothelin-1 and proendothelin-1. Proendothelin-1 is normally converted to the active peptide endothelin-1. This assay, therefore, will usually give an estimate of both potential and actual endothelin biological activity. It is possible, however, that conversion of proendothelin-1 to endothelin-1 is abnormal in heart failure. Consequently, the high plasma levels we found could, for example, reflect an increase mainly in proendothelin-1 rather than endothelin-1 because of a decreased conversion rate (though there would have to be a very large increase in proendothelin-1 to account for a doubling or trebling of plasma concentrations).

Assuming, however, that the raised levels of endothelin in our patients do reflect biologically active peptide, why are circulating concentrations increased and what might the significance of this increase be?

The increase in circulating endothelin concentration could be a result of increased secretion. Endothelial function as assessed by endothelium-mediated vasodilation is abnormal in heart failure.\textsuperscript{27} Hypoxia, catecholamines, angiotensin II, and arginine vasopressin increase endothelin production experimentally.\textsuperscript{4,26,29} It is also possible that endothelin clearance is diminished in CHF, as already alluded to.

What of the possible significance of the increase in circulating endothelin concentration? Though it is widely held that the threshold concentration for a vasoconstrictor effect of endothelin is $10^{-10}$ to $10^{-11}$ M, infusion of endothelin at a dose between 1 and 2.5 mg/kg/min, resulting in circulating levels of approximately 3–10 pmol/l, causes adverse systemic and renal hemodynamic effects in both humans and experimental animals.\textsuperscript{30–32} Similarly, Cockroft et al\textsuperscript{33} reported that brachial arterial infusion of endothelin at 1 pmol/l/min, giving an estimated local concentration of 20 pmol/l, reduced forearm blood flow by 15%. The threshold concentration for contraction of human pulmonary artery in vitro by endothelin is 10 pmol/l.\textsuperscript{34} Firth et al\textsuperscript{35} have shown that endothelin at 40 pmol/l reduces renal blood flow by 10% in the isolated rat kidney.

Furthermore, there is some evidence that subthreshold doses of endothelin can augment the vasoconstrictor response to norepinephrine.\textsuperscript{36} Abnormal endothelium, as is found in heart failure, may also be more sensitive to the effects of endothelin.\textsuperscript{27,37} These recent findings suggest the concentrations of endothelin measured in some of our patients may be vasoactive. It is also possible that, as systemic concentrations are elevated, local concentrations may be even higher. Weighed against these considerations, however, are data from Caverio et al\textsuperscript{38} that show that the systemic vascular (but not other) effects of exogenous endothelin are reduced in an experimental model of heart failure. It is possible that sustained high endothelin concentrations lead to the development of tolerance to some of the effects of endothelin.

The recent development of endothelin-1 antagonists/synthesis inhibitors should help address the role of endothelin in heart failure and may ultimately be of therapeutic benefit in this syndrome.\textsuperscript{38}

Acknowledgments

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