Elevated Endothelin-1 in Heart Failure and Loss of Normal Response to Postural Change

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Background. The possible contribution of endothelin-1, a potent endothelium-derived vasoconstrictor peptide, to neurohumoral compensation for hemodynamic stress was examined in nine normal volunteers and six patients with severe congestive heart failure.

Methods and Results. Plasma levels of endothelin-1 were measured with a sensitive and specific radioimmunoassay. Venous blood samples were obtained after 90 minutes of supine rest and serially during 30 minutes of 60° upright tilt. Endothelin-1 levels were compared with those of known neurohumoral mediators of compensation. In normal subjects, the resting levels of endothelin-1 were low (0.74±0.11 pg/ml), and there was a rapid increase to 1.37±0.07 pg/ml at 5 minutes of upright tilting (p<0.05). This increase was not sustained at 10 and 15 minutes of tilt, but there was a trend toward a second rise at 30 minutes (1.14±0.17 pg/ml; p=0.06). This biphasic pattern of response was shared by dopamine and reflected the response of systemic blood pressure to postural change. In contrast, slower and more sustained increases in circulating levels were observed for norepinephrine, epinephrine, aldosterone, plasma renin activity, and vasopressin, whereas atrial natriuretic peptide tended to decrease progressively. Patients with congestive heart failure had markedly higher basal levels of circulating endothelin-1 than normal subjects (3.7±0.5 pg/ml; p<0.01), and there was no further increase on postural change. Similar patterns were observed for the other neurohumoral mediators measured, with the degree of blunting of the response to upright tilting in heart failure being inversely related to the magnitude of increase in basal levels.

Conclusions. Alterations in plasma levels of endothelin in congestive heart failure and in response to postural change were qualitatively and quantitatively similar to the alterations of known mediators of neurohumoral compensation. In addition, the increase in plasma endothelin-1 during upright tilting in normal subjects preceded the increases in circulating levels of the other vasoconstrictor mediators, consistent with a role of endothelin-1 in neurohumoral compensation for hemodynamic stress. (Circulation 1992;85:510–517)

The 21-residue vasoactive peptide endothelin-1, first isolated from the supernatant of cultured porcine endothelial cells,1 produces potent and protracted contraction of venous and arterial smooth muscle from a variety of animal species,1–3 including humans.4,5 Indeed, systemic infusion of synthetic endothelin-1 in humans has been shown to result in prolonged increases in blood pressure6; therefore, it has been suggested that endothelin-1 may contribute to the regulation of vascular resistance. The effect of endothelin-1 on systemic and regional vascular resistance has been studied extensively in a variety of animal models. The renal vascular bed appears to be particularly sensitive to the vasoconstrictor action of endothelin-1.7–9 The coronary bed is also susceptible to extreme endothelin-1–induced vasoconstriction,10–12 especially if the native coronary endothelium has been altered or damaged.13 Furthermore, on the basis of in vitro and in vivo animal experiments, it has been suggested that endothelin-1 may contribute indirectly to the regulation of regional and systemic vascular resistance by modifying the secretion of other neurohumoral mediators, including renin–angiotensin,14,15 aldosterone,16,17 and atrial natriuretic peptide.18–20 Endothelial cells in culture release large quantities of endothelin-1 in a constitutive manner.21 The expression and release of endothelin-1 can be further
enhanced by various vasoactive agents (norepinephrine, angiotensin II, thrombin)\textsuperscript{1,2} and cytokines (transforming growth factor-\(\beta\), interleukin-1).\textsuperscript{1,2,23-25} Stimulated release of endothelin-1 by endothelial cells in vitro appears to require de novo gene expression and protein synthesis.\textsuperscript{26} In humans, plasma endothelin-1 levels are very low\textsuperscript{27,28}; substantial increases in circulating endothelin-1 concentrations have been demonstrated in pathophysiological states,\textsuperscript{29-32} some of which are associated with decreased blood pressure or a compromise in cardiac output.\textsuperscript{28,33} In addition, increase in plasma endothelin-1 has been reported in response to physiological stimuli such as postural change\textsuperscript{34} and the cold pressor test.\textsuperscript{35,36} Congestive heart failure is a clinical condition characterized by marked activation of neurohumoral compensatory mechanisms that act to support the circulation in the face of reduced cardiac performance.\textsuperscript{37-40} Little is currently known about the possible contribution of endothelin-1 to hemodynamic alterations of congestive heart failure, including decreased renal plasma flow and reduced coronary reserve. In a preliminary report,\textsuperscript{28} we found a nonsignificant increase in endothelin levels in patients with stable heart failure. In animal models of cardiac insufficiency, however, substantial increases in plasma endothelin-1 have been reported.\textsuperscript{41,42} Therefore, the aim of the present study was to explore the possible contribution of endothelin-1 to hemodynamic compensation in humans, both in the short-term response to postural change and in the long-term adaptation to chronic cardiac insufficiency. Circulating levels of endothelin-1 and known neurohumoral mediators were determined at rest and during head-up tilt in normal subjects and patients with severe heart failure. We report a substantial elevation of circulating endothelin-1 levels in patients with severely compromised cardiac function in parallel with the increases in known neurohumoral compensatory mediators. However, the rapid increase in plasma endothelin-1 levels induced by postural change in normal subjects was lost in heart failure.

**Methods**

**Study Subjects**

The study population consisted of 15 subjects, nine normal volunteers and six patients with New York Heart Association Class III congestive heart failure. The characteristics of the heart failure patients are shown in Table 1. This group displayed evidence of severe compromise of circulatory function, with decreased left ventricular ejection fraction and serum sodium and increased serum creatinine. All patients were clinically stable at the time of study and were being managed as outpatients on multiple drug therapy for heart failure, including digoxin and angiotensin converting enzyme inhibitors. No medications were given for at least 12 hours before study, and all subjects signed informed consent.

**Experimental Protocol**

Normal volunteers and heart failure patients were kept fasting overnight. An intravenous line was introduced into the left antecubital vein for blood sampling. An automated blood pressure measuring device (Dynamap, Critikon, Tampa, Fla.) was attached to the right arm for periodic blood pressure and heart rate determinations. Subjects were placed on a tilt table and were allowed to rest in the horizontal position for 90 minutes in a darkened, quiet room before the initial baseline venous samples were obtained. The table was then rapidly tilted to 60°, with the head upright and feet supported by a base platform, and the subjects were left in this position for 30 minutes. Blood was sampled after 5, 10, 15, and 30 minutes of upright tilt. Subjects were then returned to the horizontal position and allowed to rest for an additional 30 minutes before final blood samples were obtained. Blood pressure and heart rate were recorded at each sampling interval.

**Sample Collection and Processing**

Blood was drawn through the indwelling venous catheter and transferred to specially prepared tubes stored on ice. Immediately after completion of the protocol, samples were centrifuged at 4°C, and the plasma was stored at -70°C until the time of assay. Norepinephrine, epinephrine, and dopamine were measured using the radioenzymatic technique of Peuler and Johnson.\textsuperscript{43} Arginine vasopressin, plasma renin activity, and aldosterone were determined according to methods previously reported.\textsuperscript{38} Atrial natriuretic
peptide was measured by radioimmunoassay according to the technique of Wilson et al. Serum sodium and creatinine were measured by standard clinical laboratory techniques.

**Measurement of Plasma Endothelin-1**

Venous blood was collected into EDTA-containing tubes and centrifuged at 1,800g for 20 minutes at 4°C. Plasma endothelin was determined by a modification of the previously described radioimmunoassay. Samples were extracted with SepPak C18 cartridges (Waters, Mississauga, Ontario, Canada) activated with methanol, 8 mol/l urea, and water, and eluted with methanol (recovery, 75±3%). Samples and standards (endothelin-1, Peninsula Laboratories, Belmont, Calif.) were reconstituted in assay buffer and incubated for 24 hours with anti-endothelin-1 antibody (Peninsula Laboratories) at 4°C. The addition of approximately 4,000 cpm of [125I]endothelin-1 (Peninsula Laboratories) was followed by a second 24-hour incubation. Bound radioactivity was separated from free radioactivity by the second antibody method and evaluated after logit/log transformation. Immunoreactive endothelin-1 (irET-1) is presented after correction for recovery.

The antibody exhibited a cross-reactivity of 10% with human “big” endothelin-1 and 5% with endothelin-3 but no cross-reactivity with unrelated peptides (i.e., ANF1-28, brain natriuretic peptide, vasoressin, angiotensin I and II). The standard curve was very stable, with a midpoint (IC50) of 7.23±0.58 pg/tube. The limit of detection, defined as the least amount of irET-1 distinguishable from zero at a 95% confidence level, was 0.12 pg/tube. The intra- and interassay coefficients of variation were 9% and 12%, respectively. Serial dilutions of the plasma extract inhibited binding of radioligand in parallel with the standard curve, and high-performance liquid chromatography analysis of plasma extract demonstrated a dominant peak of irET-1 coeluting with synthetic endothelin-1. To establish the intraindividual variation in plasma endothelin-1 over the time course of the tilt protocol, blood samples were drawn from five normal volunteers at 0, 5, 15, and 30 minutes of supine rest. The mean plasma endothelin-1 concentration was 0.63±0.09 for the group, and there were no significant changes over the study period. The intraindividual coefficient of variation was 21% (range, 11–34%).

**Statistical Analysis**

Statistical analysis was performed with SYSTAT statistical software (SYSTAT Inc., Evanston, Ill.). Differences between heart failure and normal groups were determined with a two-tailed independent-samples t test. The significance of differences within groups during the study protocol was determined with a two-tailed Wilcoxon signed ranks test. All values are presented as mean±SEM.

**Results**

**Hemodynamic Response to Tilting**

One subject, a normal volunteer, experienced syncope at 15 minutes of tilt and therefore could not complete the protocol and was excluded from further analysis. The remaining eight normal subjects demonstrated a biphasic change in systemic blood pressure (Figure 1A). Diastolic pressures rose significantly at 5 and 10 minutes of tilt and again at 30 minutes, returning to baseline after the termination of upright tilting. There was a sustained increase in heart rate throughout the tilt period (Figure 1B). Patients with congestive heart failure (Figure 2A) had lower baseline systolic blood pressure (102±6 versus 113±14 mm Hg, respectively; p<0.05) and higher heart rates (86±9 versus 64±4 beats per minute, respectively; p<0.05) than the normal subjects. They showed a biphasic pattern of blood pressure response to upright tilting similar to that of the normal group; the heart rate response, however, was more variable and less marked than in the normal group (Figure 2B).

**Neurohumoral Response to Postural Change in Normal Subjects**

As shown in Figure 3, four patterns of response to postural change were observed in normal subjects for endothelin-1 and the neurohumoral mediators studied. Endothelin-1 and dopamine both exhibited a biphasic response to postural change (Figure 3A). The initial increase in plasma endo-
theulin-1 from 0.74±0.11 to 1.14±0.17 pg/ml at 5 minutes (p<0.02) was not sustained and returned to baseline by 10–15 minutes of tilt. There was, however, a trend toward a second rise for both agents by 30 minutes, returning to baseline after termination of the upright tilt. Norepinephrine and epinephrine levels increased slightly more gradually during postural change (Figure 3B), with peak responses occurring at 30 and 15 minutes, respectively. These increases were sustained throughout the tilt protocol and returned to baseline only when the horizontal position was reassumed. A more delayed pattern of response was displayed by vasopressin, plasma renin activity, and aldosterone (Figure 3C), whereas the vasodilator atrial natriuretic factor tended to decline progressively when the upright position was assumed (Figure 3D).

Changes in Circulating Levels of Endothelin-1 and Other Vasoactive Factors in Heart Failure

Baseline levels of vasoactive factors were measured after 90 minutes of supine rest in normal subjects and in patients with congestive heart failure. Plasma levels of endothelin-1 (3.7±0.47 pg/ml) were more than fivefold higher in heart failure patients than in normal subjects (p<0.01). The degree of elevation of baseline endothelin-1 levels in patients with heart failure (compared with normal subjects) was similar to the elevations in mean levels observed for neurohumoral mediators of hemodynamic compensation in vasopressin (3.6±1.1 versus 0.6±0.1

**FIGURE 2.** Graphs show systolic and diastolic blood pressure (panel A) and heart rate (panel B) for six patients with heart failure during supine rest (time 0), during 30 minutes of 60° upright tilt (filled bar), and on recovery (time 60 minutes). Values are mean±SEM. Symbols indicate significance of differences compared with resting value: +, 0.1>p>0.05; *, p<0.05; **, p<0.02.

**FIGURE 3.** Graphs show changes in circulating levels of vasoactive mediators during postural change in normal subjects. Panel A: Endothelin-1 (ET-1) and dopamine (DA); panel B: norepinephrine (NE) and epinephrine (EPI); panel C: arginine vasopressin (AVP), plasma renin activity (PRA), and aldosterone (ALDO); panel D: atrial natriuretic factor. Horizontal bar indicates period of upright tilting. Symbols indicate significance of differences compared with resting value: +, 0.1>p>0.05; *, p<0.05; **, p<0.02.
pg/ml, fivefold, $p<0.05$), norepinephrine (396±63 versus 140±20 pg/ml, threefold, $p<0.02$), plasma renin activity (8.1±2 versus 1.3±0.2 ng/ml/hr, sixfold, $p<0.05$), aldosterone (83±14 versus 14±2 ng/dl, $p<0.02$), and atrial natriuretic factor (230±59 versus 32±5 pg/ml, sevenfold, $p<0.05$). In contrast, the levels of epinephrine (33±6 versus 31±8 pg/ml) and dopamine (14±2 versus 9±3 pg/ml) were not significantly increased in heart failure.

Neurohumoral Response to Postural Change in Heart Failure

During tilting, there was no further increase in the already elevated basal plasma endothelin-1 levels in the heart failure group (Figure 4A). Likewise, there was a marked blunting of the response of vasopressin, renin, aldosterone, and atrial natriuretic factor to postural change (Figures 4C and 4D). Epinephrine and, to a lesser degree, norepinephrine levels still increased during tilt (Figure 4B), whereas the late increase in circulating concentration of dopamine was exaggerated in heart failure (Figure 4A). In Figure 5, the relative increase in concentration of the vasoactive factors in response to tilt is plotted against the magnitude of mean baseline elevation in heart failure compared with control. There was an inverse relation such that the blunting of the response to postural change was most marked for those factors (including endothelin-1) that were greatly increased in the failure state. Conversely, dopamine and epinephrine, which were little changed in heart failure, showed the greatest response to tilting.

Discussion

In the present study, plasma levels of endothelin-1 were fivefold higher in patients with New York Heart Association Class III congestive heart failure than in the normal volunteers. In an earlier study, only a
trend toward increases in endothelin-1 was observed, which did not reach the level of significance. It is likely, therefore, that there exists a spectrum of elevation in plasma endothelin-1 in heart failure depending on the hemodynamic severity in a manner analogous to other circulating vasoconstrictor factors.\textsuperscript{37,38} Indeed, the increases in circulating endothelin-1 levels paralleled those of several important neurohumoral mediators in the heart failure group, consistent with a possible contribution to circulatory compensation in the face of important limitation in cardiac performance.

Before a role for endothelin in hemodynamic compensation can be entertained, several important considerations must be addressed. The functional importance of circulating endothelin-1 remains uncertain. The highest plasma concentrations measured in the present study were still below the threshold for inducing contractile responses in vitro.\textsuperscript{1} However, the sensitivity of isolated vessels in vitro may not accurately reflect in vivo vascular responsiveness. A recent report on endothelin infusion in humans\textsuperscript{6} demonstrated a significant vasopressor action at plasma concentrations only fivefold to 10-fold higher than those observed in heart failure. In an animal model, infusions of endothelin-1 that resulted in elevations of plasma levels similar to those found in the present report produced significant hemodynamic effects.\textsuperscript{41} Therefore, a systemic effect of circulating endothelin-1 in heart failure cannot be excluded. Moreover, circulating levels of endothelin may only indirectly reflect local vascular production,\textsuperscript{45} and concentrations in the vessel wall may be orders of magnitude greater than those measured in the plasma, thus well within the range for biological activity.

From the present report, it cannot be determined whether the increases in circulating endothelin-1 levels in congestive heart failure were directly linked to the hemodynamic disturbances of the failure state or occurred secondary to the action of other circulating vasoactive agents. In vitro, both norepinephrine and angiotensin II have been shown to increase the expression of preproendothelin-1 mRNA in cultured endothelial cells.\textsuperscript{1,46} In the present study, norepinephrine and plasma renin activity were indeed elevated in heart failure. In a recent report,\textsuperscript{47} however, the infusion of norepinephrine and other vasoactive mediators in healthy men did not modify plasma endothelin-1. In addition, it is possible that a decreased clearance of endothelin may have contributed to the elevation in circulating levels, as was suggested in a canine model of congestive heart failure.\textsuperscript{42} Both the lung\textsuperscript{3,32,48} and the kidney\textsuperscript{49} have been implicated in the clearance of endothelin. Pulmonary congestion or decreased renal perfusion, both of which are present in severe heart failure, might reduce the ability of these organs to clear endothelin.

To further assess the possible role of endothelin-1 in hemodynamic regulation in humans, we studied the effects of orthostatic stress on plasma levels in normal subjects and patients with heart failure. In normal subjects, there was a surprisingly sharp increase in endothelin-1 plasma concentration in response to 60° upright tilt, with a peak after only 5 minutes. This pattern of response agrees with an earlier report showing elevation of plasma endothelin during the cold pressor test after only 4 minutes.\textsuperscript{35} Such a rapid release, however, is not consistent with the view, based on the behavior of cultured endothelial cells, that stimulated endothelin secretion requires de novo gene expression and protein synthesis.\textsuperscript{26} The recent demonstration of endothelin-1 immunoreactivity in neuroendocrine cells of the lung\textsuperscript{50} raises the possibility that there may be a nonendothelial pool of endothelin-1 in vivo that can be rapidly released in response to postural change. The trend toward a second rise after 30 minutes of postural stress is more consistent with de novo endothelin synthesis in response to a sustained hemodynamic stimulus. Alternatively, the biphasic response may reflect an initial postural stress at the onset of tilt that is then effectively countered by the action of various neurohumoral compensatory mechanisms. A second rise in endothelin may be the result of an inability to sustain adequate circulatory compensation during prolonged upright tilt in the absence of activity of postural muscles.

The rapid increase in plasma endothelin in normal subjects at the onset of tilting preceded the peak increases in plasma concentration of most of the neurohumoral mediators studied. Therefore, endothelin release could not have been secondary to the action of epinephrine and norepinephrine (peaking at 15 and 30 minutes, respectively) or of aldosterone, plasma renin activity, and vasopressin (peak at only 30 minutes). Rather, these data suggest that the release of endothelin in response to postural change may be directly linked to hemodynamic alterations by some as yet unidentified mechanism. Of note, the biphasic pattern of response of endothelin to tilting in normal subjects was completely shared by dopamine (Figure 3A) and closely followed the biphasic response of systemic blood pressure (Figure 1). The tight association between plasma levels of endothelin-1 and dopamine in normal subjects raises the possibility that their release in vivo may be coupled. Alternatively, parallel plasma levels of endothelin and dopamine during tilting might reflect a similar rapid response to orthostatic stress, with an initial and late component, as evidenced by the blood pressure response.

In patients with congestive heart failure, there were marked increases in circulating levels of nearly all vasoactive agents studied, in agreement with earlier reports.\textsuperscript{37–40} This probably reflects an activation of compensatory mechanisms that are thought to be crucial for the maintenance of circulatory function in heart failure.\textsuperscript{37} Moreover, there was considerable blunting of response to tilting for each agent in proportion to the degree of basal elevation in plasma levels (Figure 5). Thus, aldosterone, plasma renin
activity, and atrial natriuretic peptide, all markedly elevated in heart failure, showed no significant further change in response to postural stress. In contrast, agents that showed no significant increases of basal levels in heart failure (epinephrine and dopamine) demonstrated intact or exaggerated responses to tilt. Accordingly, the markedly increased circulating levels of endothelin in heart failure patients were not further augmented by orthostatic stress. Unlike the close association between plasma levels of endothelin and dopamine observed during tilting in normal subjects, however, patients with heart failure showed markedly divergent responses (Figure 4A).

Thus, the alterations of plasma endothelin-1 in patients with congestive heart failure and in normal subjects in response to orthostatic stress were qualitatively and quantitatively similar to the alterations of known mediators of neurohumoral compensation. Moreover, the increase in plasma endothelin-1 on upright tilting was extremely rapid in the normal group, preceding the peak elevations in circulating levels of the other vasoconstrictor mediators studied. This is not consistent with endothelin release occurring as a secondary manifestation of increased circulating catecholamine or angiotensin levels but rather suggests a more direct link to changes in the hemodynamic state. The precise contribution of this potent vasoconstrictor peptide to vascular compensation in response to circulatory stress awaits the development of specific inhibitors of its action or release. The present data suggest, however, that endothelin-1 may act in concert with other constrictor agonists to maintain peripheral resistance and perfusion pressure in the face of compromised venous return or cardiac performance.

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