Deloading of the Left Ventricle by Ventricular Assist Device Normalizes Increased Expression of Endothelin ET<sub>A</sub> Receptors But Not Endothelin-Converting Enzyme-1 in Patients With End-Stage Heart Failure

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**Background**—Ventricular assist devices (VAD) are implanted in patients with end-stage heart failure for bridging the time until heart transplantation, resulting in hemodynamic unloading of the failing heart, improved cardiac contractile and mitochondrial function, and reversal of cardiac hypertrophy. It is unknown whether VAD unloading may affect the cardiac endothelin (ET) system, which has been proposed as one of the putative pathomechanisms of heart failure.

**Methods and Results**—With the use of standard-calibrated, competitive reverse-transcription–polymerase chain reaction mRNA expression of components of the ET system was analyzed in left ventricular myocardium from nonfailing donor hearts, from failing hearts without and with ACE inhibitor therapy, and from patients with end-stage heart failure at the time of VAD implantation and 103±15 days after VAD implantation during removal with subsequent heart transplantation. ET receptor A (ET<sub>A</sub>) was markedly upregulated in failing human myocardium. This increased ET<sub>A</sub> expression was not affected by ACE inhibitor treatment but was normalized by VAD unloading. ET<sub>A</sub> expression before or after VAD implantation did not correlate with duration of VAD implantation or suppression of Pro-ANP mRNA. ET<sub>B</sub> mRNA expression was unaffected by heart failure or VAD. In contrast, increased ET-converting enzyme-1 mRNA and ET-1 peptide levels in failing myocardium were partially normalized by ACE inhibition but not by VAD unloading.

**Conclusions**—We conclude that VAD implantation normalizes ET<sub>A</sub> expression in failing human left ventricular myocardium, probably as the result of the beneficial effects of VAD unloading. *(Circulation. 2000;102[suppl III]:III-188-III-193.)*

**Key Words:** endothelin ■ heart failure ■ heart-assist device

Ventricular assist devices (VAD) serve as mechanical support of the failing myocardium in patients with end-stage heart failure. The VAD has been used as a bridge to heart transplantation. Hemodynamic unloading by an implanted VAD may improve cardiac contractile and mitochondrial function, positive remodeling of heart chamber volume, and reduced cardiac hypertrophy. We recently showed that mechanical support of the failing heart by a VAD induces changes in myocardial gene expression compatible with a decreased susceptibility to apoptosis and partial improvement of calcium-regulatory determinants. However, it is unknown whether VAD unloading may affect the cardiac endothelin (ET) system, which has been proposed as one of the putative pathomechanisms of heart failure.

The potent vasoconstrictor ET-1 is generated by proteolytic cleavage of the precursor peptide big ET-1, catalyzed by the metalloprotease ET-converting enzyme-1 (ECE-1). ET-1 binds to 2 distinct receptors, ET receptors A and B (ET<sub>A</sub>, ET<sub>B</sub>), mediating regulation of the vascular tone, positive inotropic effects, and development of hypertrophy in the myocardium. In human left ventricular myocardium, only the ET<sub>A</sub> receptor has been shown to be of functional relevance.

ET-1 has been proposed to be involved in the development and progression of heart failure. A local ET-1 synthesis from the endocardium, myocardium, and coronary endothelium, suggesting an autocrine/paracrine action of ET-1 on cardiomyocytes, has been demonstrated. The upregulation of tissue and circulating levels of ET-1 has been described in experimental and human heart failure. A functional significance of ET-1 has been suggested by the positive correlation between elevated plasma ET-1 and increased mortality.
rates in human heart failure. Furthermore, in an experimental infarct model of heart failure, chronic ET-receptor antagonism was associated with a reduction in ventricular remodeling and mortality rates. In human myocardium, both ET,
and ET,
are expressed, but only ET,
has been shown to be of functional importance. Recently, upregulation of ET,
receptor-binding sites has been demonstrated in failing human myocardium of patients with dilated cardiomyopathy (DCM).

The expression of ECE-1 in failing human ventricular myocardium has been analyzed in less detail. In a recent study, no differences in ECE-1 expression could be detected in hearts of nonfailing donors, compared with patients with DCM. However, the impact of therapy by inhibitors of ACE, the gold standard in the treatment of heart failure, on the expression of genes of the ET system in failing human left ventricular myocardium is currently unknown. Angiotensin II and ET-1 act synergistically in the development of cardiac hypertrophy. In addition, ET-1 and Angiotensin II have been shown to mediate hypertrophic response in cultured cardiomyocytes exposed to mechanical stretch in vitro.

Therefore, we investigated the effect of hemodynamic unloading by the VAD on the expression of components of the human ET system in myocardium of patients with end-stage heart failure. In these studies, we obtained left ventricular specimens from the same patients with heart failure during VAD implantation (maximal hemodynamic overload) and afterward during heart transplantation with VAD removal (substantial temporal hemodynamic unloading by the VAD). In addition, we analyzed left ventricular myocardium of patients with and without ACE inhibitor therapy (without previous VAD support before heart transplantation) for comparison. In these human ventricular specimens, we analyzed the impact of hemodynamic unloading and of ACE inhibitor therapy on the expression of ET,
, ET,
, ECE-1, and ET-1 in failing human myocardium.

Methods

Patient Population

Left ventricular specimens were obtained after informed consent from 10 male patients (8 with DCM; 2 with ischemic cardiomyopathy, ICM) undergoing VAD implantation as a bridge to transplantation at the time of surgery and at removal of the VAD with subsequent orthotopic heart transplantation. At the time of VAD implantation, patients were 53 ± 4 years of age. The hemodynamic data were obtained from the date where the last echocardiographic determination of the ejection fraction (24 ± 1%) before VAD implantation was performed. For left ventricular unloading, either TCI HeartMate (Thermo Cardiosystems, Inc, n = 3), Novacor (Baxter Healthcare Corp, n = 6), or Thoratec (Thoratec Laboratories, Inc, n = 1) assist devices were used. For biventricular support in the case of TCI or Novacor use, an additional right ventricular assist device of Thoratec (n = 2) was implanted. The average time on VAD was 103 ± 15 days (range 36 to 169 days). Cardiac index was improved by hemodynamic unloading from 1.9 ± 0.2 L/min/m² before VAD implantation (before VAD; n = 10) to 2.9 ± 0.3 L/min/m² after VAD implantation (after VAD in those patients in whom measurements were possible immediately before transplantation; n = 4) (P < 0.05 versus before VAD). Pulmonary capillary wedge pressure was reduced by VAD from 29 ± 2 mm Hg before VAD to 14 ± 3 mm Hg after VAD (P < 0.05 versus before VAD). Mean aortic pressure was increased by VAD support from 69 ± 3 mm Hg before VAD to 78 ± 3 mm Hg after VAD, without reaching statistical significance (P = 0.058 versus before VAD).

In addition, explanted left ventricular specimens of patients transplanted by the Halle and Hamburg Cardiac Transplant Program (6 men, 56 ± 2 years old) with end-stage heart failure (ICM) and treated with β-blockers (n = 1), nitric oxide donors (n = 1), calcium antagonists (n = 2), and diuretics (n = 5) but not with ACE inhibitors or VAD unloading were investigated, with an average ejection fraction of 22 ± 4%. Finally, 11 patients (9 men, 2 women) with end-stage heart failure (8 DCM, 3 ICM; 53 ± 3 years old) treated with ACE inhibitors (n = 11), β-blockers (n = 1), nitric oxide donors (n = 2), calcium antagonists (n = 2), and diuretics (n = 5) but not with VAD unloading were studied. Long-term ACE inhibitor treatment before surgery was assessed in a retrospective manner. The ACE inhibitor dosages prescribed by the referring physicians were 31 ± 5% of respective target dosages in recent heart failure megatrials. The mean ejection fraction of this group with ACE inhibition was 28 ± 3%. Left ventricular tissues from 7 organ donors without structural abnormalities (4 men, 3 women; 35 ± 6 years old) served as controls. These hearts were not transplanted for technical reasons. The local ethics committee approved the study of these human cardiac tissues.

RNA Preparation

Left ventricular specimens of patients were dissected immediately after explantation, snap-frozen, and stored in liquid nitrogen until RNA preparation. Total RNA from myocardial tissue was isolated by guanidinium thiocyanate/cesium chloride centrifugation as described. The RNA was analyzed by standard agarose gel electrophoresis, and RNA concentration was determined by UV spectrophotometry.

Standard-Calibrated, Competitive Reverse Transcripase–Polymerase Chain Reaction

Human ECE-1, ET,
, ET,
, and Pro-ANP mRNA was quantified by standard-calibrated reverse transcriptase–polymerase chain reaction (RT-PCR). For human ECE-1 mRNA, a new standard-calibrated, competitive RT-PCR was established. In brief, a human ECE-1–specific cDNA fragment of 215 bp was amplified by PCR with the use of the following primers: ECE-1 sense primer: 5'-GAA GCC GCT GGT GGT GTT GGT G3' and ECE-1 antisense primer: 5'-GTT TCG TTA TGA AGC-3', respectively. The ECE-1–specific cDNA fragment was subsequently cloned into the pCR II TOPO vector (Invitrogen), and its identity was confirmed by DNA sequencing (ABI PRISM® Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS, Perkin-Elmer Co; ABI 373 DNA Sequencer). Second, an internal deleted ECE-1 cDNA standard (133 bp) was constructed by modification of a linker primer method. The identity of the standard was confirmed by cloning and DNA sequencing. Third, internal deleted cDNA standard was transcribed in vitro into cRNA (RNA Transcription Kit, Stratagene), and standard cRNA was quantified spectrophotometrically.

In competitive RT-PCR experiments, equal amounts of total RNA from human myocardium (200 ng) were incubated in separate reactions with defined amounts of serial 1:3 dilutions of ECE-1 standard cRNA for 3 minutes at 70°C and subsequently reverse transcribed into cDNA with random hexamer primers and Superscript II Reverse Transcriptase (Life Technologies) for 1 hour at 42°C. Afterward, 20% of each reverse transcription reaction was amplified in separate reactions with 20 pmol/L ECE-1 sense and antisense primers by the following PCR protocol: 30 seconds 95°C, 30 seconds 62°C, 30 seconds 72°C (36 cycles). PCR primers compete for sample-specific and standard molecules in the amplification reaction. The PCR reactions were separated by standard agarose gel electrophoresis, stained with ethidium bromide, and documented by photography with Polaroid film type 665. The optical density of standard and sample-specific PCR fragment was estimated by a Personal Densitometer (Molecular Dynamics). Optical density of standard PCR fragments was normalized with a correction coefficient (ECE-1: 215 bp/133 bp = 1.62) and logarithm of the
quotient of normalized standard and sample-specific PCR fragment density was graphically plotted versus amount of standard RNA molecules, with the SigmaPlot scientific graphing software (Jandel Corp). In the graph, equal amounts of RNA molecules in sample and standard were present at equivalence point.

The mRNA expression of human ET_A, ET_B, and Pro-ANP was quantified by standard-calibrated competitive RT-PCR as described previously. The characteristics of quantitative mRNA analyses in human left ventricular myocardium by competitive RT-PCR (PCR protocol: 30 seconds at 95°C, 30 seconds at primer-specific annealing temperature, and 30 seconds at 72°C) are summarized in the Table.

### Tissue Endothelin Protein Content

Total protein from left ventricular tissues was isolated as described. Protein concentration was determined by the Bio-Rad protein assay. Tissue ET-1 content was determined with isoform-specific rabbit antibodies against synthetic ET-1 (Peninsula Laboratories) and reverse-phase high-performance liquid chromatography as described previously.

### Statistics

Data are given as mean±SEM. The ANOVA procedure followed by Bonferroni’s method (multiple comparison) or Student’s t test was used for statistical comparison as appropriate (SigmaStat software, Jandel Corp). The correlation coefficient r of the linear regression analysis for significance was tested by a 2-sided test. A value of \( P<0.05 \) was considered statistically significant.

### Results

#### Normalization of Increased ET_A Expression by VAD in Failing Human Myocardium

The mRNA expression of genes of the human endothelin system was determined by standard-calibrated, competitive RT-PCR in RNA from left ventricular myocardium (Figure 1). Myocardial ET_A expression was upregulated in patients with heart failure (20±2 versus 12±1 amol/µg RNA in donor hearts, \( P<0.05 \)) (Figure 2A). This augmented ET_A expression in failing human myocardium was not affected by concomitant ACE inhibitor therapy (20±2 without versus 19±3 amol/µg RNA with ACE inhibition). Hemodynamic unloading by VAD normalized increased ET_A expression in the myocardium of patients with end-stage heart failure and ACE inhibitor therapy before implantation of the VAD (before VAD: 18±2 amol/µg RNA) compared with myocardium from the same patient after transplantation and VAD removal (after VAD: 12±1 amol/µg RNA, \( P<0.05 \) versus before VAD) (Figure 2B). ET_A expression was reduced in 7 of 10 patients, whereas the remaining 3 patients with initially lower ET_A levels showed no further decrease of expression.

However, although we observed reduced left ventricular Pro-ANP expression after VAD-induced hemodynamic unloading (before VAD: 28±9 amol/µg RNA, \( P<0.05 \); donor hearts: 22±12 amol/µg RNA) and although a role of ET_A in volume load-induced cardiac ANP release has been described, no correlation of reduced ET_A and Pro-ANP expression by VAD was found. Furthermore, duration of VAD implantation did not correlate with ET_A expression (data not shown).

#### Myocardial ET_B Expression

No significant changes of left ventricular ET_B receptor expression could be found in failing hearts from patients with or without ACE inhibitor therapy (donors: 17±2, explanted without ACE inhibitor: 22±4, explanted with ACE inhibitor: 23±4 amol/µg RNA, \( P=0.5 \), NS) (Figure 3A). Furthermore, hemodynamic unloading by VAD had no effect on left ventricular ET_B expression (before VAD: 17±2 versus after VAD: 20±4 amol/µg RNA, \( P=0.45 \), NS) (Figure 3B).

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**Figure 1.** Quantification of mRNA expression of genes of human ET system by standard-calibrated, competitive RT-PCR. Method compares amplification of ECE-1, ET_A, or ET_B receptor cDNA fragment from reverse-transcribed total RNA of human left ventricular myocardium (upper lane, longer fragment) vs different concentrations of internally deleted and reverse-transcribed cRNA standard (lower lane, shorter fragment) by PCR. PCR fragments were separated on agarose gels and stained with ethidium bromide. Serial 1:3 dilution of appropriate cRNA standard was used in lanes 1 to 6.
Upregulation of ECE-1 in Failing Human Myocardium

In left ventricular myocardium of patients with heart failure and medical treatment without ACE inhibitors, determined in a retrospective manner, we found a significant upregulation of ECE-1 expression (58±6 versus 34±7 amol/µg RNA in donor hearts, P<0.05) (Figure 4A). This increased ECE-1 expression in failing hearts was partially reduced by ACE inhibitor therapy (48±2 amol/µg RNA). A similar regulation was seen in tissue ET-1 peptide content in myocardium from patients with heart failure before and after ACE inhibitor therapy (48±2 amol/µg RNA). ECE-1 mRNA is upregulated in left ventricle of patients with heart failure (A). This increased expression can be normalized by hemodynamic unloading with VAD (B). Data are expressed as mean±SEM (amol/µg RNA).

Discussion

This study for the first time demonstrates normalization of increased ET<sub>A</sub> receptor expression in failing left ventricular myocardium of patients by hemodynamic unloading with VAD support. Our data of increased ET<sub>A</sub> expression in failing left ventricular myocardium are in agreement with recent reports describing an upregulation of ET<sub>A</sub>-specific binding sites in myocardium of patients with heart failure. In human left ventricular myocardium, only ET<sub>A</sub> receptors have been shown to be of functional importance. These data not only support a role for the cardiac ET system in heart failure but suggest furthermore that mechanical left ventricular off-loading by VAD downregulates the augmented expression of components of the ET system in failing human hearts. ET-1 stimulates hypertrophy, contractility, protein synthesis, and phosphoinositide hydrolysis in cardiac myocytes by ET<sub>A</sub> receptors. In experimental studies, chronic endothelin receptor blockade improved cardiac function, attenuated progressive ventricular dilation, and reduced mortality rates in an infarct model of heart failure. Recently, chronic ET<sub>A</sub> receptor blockade was shown to attenuate left ventricular hypertrophy independent of blood pressure effects in hypertensive rats. The augmented ET<sub>A</sub> expression found in failing human myocardium may provide the target of a new therapeutic strategy in the treatment of heart failure by ET<sub>A</sub> receptor antagonism. It is important to note that in the patients in our study, no effect of ACE inhibition, the standard therapy in the treatment of heart failure, on ET<sub>A</sub> expression in left ventricular myocardium of patients with heart failure was observed.

Because of the limitations in tissue availability, in this study only ET<sub>A</sub> expression studies on mRNA level could be performed. We cannot determine whether in left ventricular homogenates ET<sub>A</sub> upregulation was due to expression in cardiac myocytes or nonmyocytes (eg, endothelial cells, smooth muscle cells, fibroblasts). However, we and others
In addition, an increased ETα receptor density in failing heart failure patients with heart failure was noted. Therefore, the observed normalization of increased ETα receptor expression by the VAD might explain the differences of our data, with a putative beneficial effect of ECE inhibition by the VAD. The left ventricular ETα receptor expression was not changed in patients with heart failure with or without ACE inhibitor therapy. ETα binding studies in nonfailing and failing left ventricular myocardium of patients with DCM are controversial. While one group found a downregulation of ETα binding sites, 14 other groups found no significant changes in ETα binding sites in failing human myocardium, supporting our ETα expression data. 10 The observed differences might reflect differences in the patient population studied. Hemodynamic unloading by the VAD did not affect ETα expression. This result further supports the importance of the observed normalization of increased ETα receptor expression by the VAD.

The upregulation of ECE-1 expression in left ventricular myocardium of patients with heart failure suggests a novel therapeutic strategy in the treatment of heart failure by ECE inhibition. A similar upregulation of ECE-1 has been found in failing human atrial myocardium (Morawietz, unpublished data, 1999). A local increased ECE-1 expression in failing human myocardium could promote increased local ET synthesis in cardiomyocytes and in increased local proteolytic cleavage of systemically elevated big ET-1 levels in patients with heart failure. Our tissue peptide measurements indicate an increase of local ET synthesis in failing human myocardium. Interestingly, we found a partial normalization of elevated ECE-1 expression in failing human myocardium of patients with ACE inhibitor therapy. These data suggest an additional beneficial effect of ACE inhibitor therapy. However, ECE-1 expression was still slightly elevated in left ventricular myocardium of patients with ACE inhibitor therapy, suggesting a putative beneficial effect of ECE inhibition in the treatment of heart failure. The effect of ACE inhibitor therapy might explain the differences of our data, with a recent study showing no significant change in ECE-1 expression in failing human left ventricular myocardium. 14 The myocardial ECE-1 expression was not changed during VAD unloading. Therefore, reduced ETα expression is not counterbalanced by altered ECE-1 expression in myocardium of parameters was avoided. However, the decrease in pulmonary capillary wedge pressure and the increase in the cardiac index (determined in 4 of 10 patients shortly before orthotopic heart transplantation by pulmonary artery catheter or transthoracic echocardiography) showed a partial improvement by hemodynamic unloading with a VAD. Furthermore, no change in the expression of indicators of inflammatory cell infiltration during time on VAD was found in left ventricular myocardium of our patients.3

ET-1 has been shown to be involved through ETα receptors in the overload-induced release of ANP. 21 In left ventricular myocardium, we found a normalization of increased Pro-ANP expression during VAD unloading. Therefore, we hypothesized a regulatory effect of ETα on Pro-ANP expression. However, we could not find a correlation of both parameters. In addition, we detected in previous studies a time-dependent change in myocardial mRNA expression of potential apoptosis-mediating genes Bcl-xL and FasExo6Del/Fas on the VAD, 3 but ETα expression did not correlate with duration of VAD support.

Figure 4. Upregulation of ECE-1 mRNA expression in failing left ventricular myocardium. ECE-1 mRNA (with standard-calibrated, competitive RT-PCR) (A) and tissue ET-1 levels (B) were measured in nonfailing donor hearts and explanted (expl.) myocardium of patients with heart failure with and without ACE inhibitor therapy. ECE-1 mRNA is augmented in left ventricle of patients with heart failure without ACE inhibitor therapy. Increased ventricular ECE-1 expression can be partially normalized by ACE inhibition (A). Similar trend can be seen for tissue ET-1 peptide levels (B). In contrast, no effect of hemodynamic unloading with VAD on ventricular ECE-1 mRNA expression in patients with end-stage heart failure before VAD implantation (pre VAD, white bar) and after VAD support (post VAD, dashed bar) could be found (C). Data are expressed as mean±SEM (amol/μg RNA).

have shown that endothelial cells express ETβ receptors only. 19 The ET receptor number on smooth muscles and fibroblasts does not exceed ET receptor number on myocytes. In addition, an increased ETα receptor density in failing human myocardium has been described. 11 Therefore, the increased ETα mRNA levels most likely reflect increased ETα receptor protein expression and function on myocytes of patients with heart failure.

It is reasonable to speculate that the normalization of ETα expression by hemodynamic unloading with a VAD was a consequence of partial recovery of failing myocardium. The overload-induced distension of the myocytes is considered to be a critical factor promoting the progression of heart failure. In our study, VAD implantation resulted in putative beneficial effect on cardiac function and improvement of the highly critical hemodynamic state before VAD implantation. The putative improvements could not be exactly quantified because the potentially risky procedure of temporarily turning off the VAD for measurements of the genuine hemodynamic
patients under VAD support, further supporting a beneficial effect of normalization of ET\textsubscript{A} receptor levels.

In conclusion, hemodynamic unloading by the VAD normalized ET\textsubscript{A} receptor expression in the left ventricular myocardium of patients with end-stage heart failure. This effect might contribute to the beneficial effects of the VAD on phenotypic and functional changes in failing human myocardium. Furthermore, we found an interaction of the human renin-angiotensin and the ET system on the level of myocardial ECE-1 expression. Pharmacological therapy of locally elevated ET\textsubscript{A} and ECE-1 expression by ET\textsubscript{A}-specific receptor blockade or ECE inhibition might provide new therapeutic perspectives in the treatment of heart failure.

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