ET-1 in the myocardial interstitium: relation to myocyte ECE activity and expression

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Ergul, Adviye, C. Allyson Walker, Aron Goldberg, Simona C. Baicu, Jennifer W. Hendrick, Mary K. King, and Francis G. Spinale. ET-1 in the myocardial interstitium: relation to myocyte ECE activity and expression. Am J Physiol Heart Circ Physiol 278: H2050–H2056, 2000.—Increased plasma levels of endothelin-1 (ET-1) have been identified in congestive heart failure (CHF), but local myocardial interstitial ET-1 levels and the relation to determinants of ET-1 synthesis remain to be defined. Accordingly, myocardial interstitial ET-1 levels and myocyte endothelin-converting enzyme (ECE)-1 activity and expression with the development of CHF were examined. Pigs were instrumented with a microdialysis system to measure myocardial interstitial ET-1 levels with pacing CHF (240 beats/min, 3 wk; n = 9) and in controls (n = 14). Plasma ET-1 was increased with CHF (15 ± 1 vs. 9 ± 1 fmol/ml, P < 0.05) as was total myocardial ET-1 content (90 ± 15 vs. 35 ± 5 fmol/g, P < 0.05). Paradoxically, myocardial interstitial ET-1 was decreased in CHF (32 ± 4 vs. 21 ± 2 fmol/ml, P < 0.05), which indicated increased ET-1 uptake by the left ventricular (LV) myocardium with CHF. In isolated LV myocyte preparations, ECE-1 activity was increased by twofold with CHF (P < 0.05). In LV myocytes, both ECE-1a and ECE-1c mRNAs were detected, and ECE-1a expression was upregulated fivefold in CHF myocytes (P < 0.05). In conclusion, this study demonstrated compartmentalization of ET-1 in the myocardial interstitium and enhanced ET-1 uptake with CHF. Thus a local ET-1 system exists at the level of the myocyte, and determinants of ET-1 biosynthesis are selectively regulated within this myocardial compartment in CHF.

congestive heart failure; endothelin-1; endothelin-converting enzyme; interstitial fluid

ENDOTHELIN (ET)-1 belongs to a family of peptides that influences a number of cardiovascular processes. Although ET-1 was first isolated from the endothelial cell, various cell types including myocytes and vascular smooth muscle cells have been shown to synthesize ET-1 (21, 27, 35). This peptide is generated from a precursor protein, preproET-1, and the final step involves the conversion of Big ET-1 to ET-1 by endothelin-converting enzyme (ECE) (35). The processing by ECE is an important step in yielding fully functional ET-1 and has been proposed to be a rate-limiting step in the ET-1 biosynthetic pathway (9). There are two isoforms of ECE, ECE-1 and ECE-2, and the physiologically important enzyme ECE-1 has three subisoforms that are generated by alternative splicing (28, 29). However, the activity and expression of ECE-1 subisoforms in left ventricular (LV) myocytes remain to be defined. In addition to potent and prolonged vasoconstrictive effects, ET-1 can influence a number of neurohormonal pathways as well as myocardial contractile function (15, 30). Thus the synthesis of ET-1 and the biological effects of this peptide in the setting of congestive heart failure (CHF) have received significant attention (2, 16, 23, 25). Specifically, plasma ET-1 levels have been shown to increase in patients and animal models of CHF (12, 25, 27, 32). Although ET-1 may exert a positive inotropic effect under normal conditions (8, 14), the peptide may contribute and/or exacerbate contractile deficiency with CHF (18, 26, 27). Although circulating levels of ET-1 have been shown to be elevated in CHF, the local dynamics of ET-1 within the myocardial interstitial compartment are not known. Furthermore, whether LV myocytes possess the capacity to synthesize ET-1 via the conversion of Big ET-1 by ECE-1 and whether alterations occur with CHF remain to be established. Accordingly, the goals of the present study were to investigate ET-1 concentration in the myocardial interstitium as well as ECE-1 subisoform expression and activity in normal and CHF LV myocytes.

METHODS

Instrumentation and induction of pacing CHF. The present project employed a model of pacing-induced CHF in pigs that has been well described previously (11, 23). Pacemakers were implanted in nine pigs (20–25 kg) to induce rapid atrial pacing. The pigs were anesthetized with isoflurane (3% per 1.5 l/min) and nitrous oxide (0.5 l/min), intubated with auffed endotracheal tube, and ventilated at a flow rate of 22 ml·kg⁻¹·min⁻¹ and a respiratory rate of 15/min. Through a left thoracotomy, a shielded stimulating electrode was sutured onto the left atrium, connected to a modified programmable pacemaker (8329; Medtronic, Minneapolis, MN), and buried in a subcutaneous pocket. The pericardium was left open, the thoracotomy was closed, and the pleural space was evacuated of air. After a 7- to 10-day recovery from the surgical procedure, the pacemaker was activated to 240 beats/min for 3 wk. At the conclusion of the pacing period, the animals were returned to the laboratory for evaluation of LV function and measurements of plasma and myocardial ET levels. An additional 14 age- and weight-matched pigs served as con-
LV function measurements. The pigs were premedicated with 20 mg of benzodiazapam and initially sedated by mask delivery of 1.5% isoflurane. After placement of an intravenous catheter and establishment of an electrocardiogram (ECG), a bolus of 2 µg/kg of sufentanyl citrate was administered, and an endotracheal tube was placed. Anesthesia was maintained throughout the procedure by delivery of 3 mg·kg⁻¹·h⁻¹ of sufentanyl. In our preliminary studies, this anesthetic protocol provided stable hemodynamic profiles for up to 10 h in both normal and CHF pigs. An arterial line was placed with the use of a 7-F introducer in the carotid artery and connected to externally calibrated transducers (Statham P23 ID; Gould, Oxnard, CA). A sternotomy was performed, and the great vessels were isolated. A vascular ligature was placed around the inferior vena cava to perform transient caval occlusion. A microtipped transducer (7.5 F; Millar Instruments, Houston, TX) was placed in the LV through a small stab wound in the apex and sutured in place. The microtipped transducer was previously calibrated in vitro, and calibration was confirmed after placement by alignment of the aortic systolic pressure to LV peak pressure. A flow probe was placed around the ascending aorta and connected to a digital flow meter (HT1017, Transonics) for continuous measurement of LV stroke volume and cardiac output. A microdialysis probe was sutured in place in the myocardium in the midregion of the anterolateral aspect of the LV. This stabilized the probe and prevented stress being placed on the dialysis portion of the probe. Interstitial fluid collection was performed as described in the following paragraph. The ECG, pressure waveforms, and flow-probe signal were recorded with the use of a multichannel recorder (Hewlett-Packard, Houston, TX) as well as digitized on computer for subsequent analysis at a sampling frequency of 250 Hz (80386 processor; Zenith Data Systems, St. Joseph, MO). After the placement of the instrumentation, baseline hemodynamics were recorded. These measurements included arterial blood pressure, LV end-systolic and diastolic pressure, peak positive rate of rise in pressure (dP/dt), and stroke volume. All measurements were performed with the ventilator temporarily suspended to prevent respiratory artifact in the LV pressure recordings.

Microdialysis and sample collection. Microdialysis studies have been performed previously for ANG II, and the protocol reported was adapted for the present study (4, 31). On the basis of the low molecular weight of ET-1 (2,460 g/mol), a microdialysis probe that contained a 4-mm-long membrane with a 20-kDa molecular weight cutoff was used (CMA20, 8309570; CMA/Microdialysis, North Chelmsford, MA). The recovery of ET-1 through the membrane was initially evaluated using 125I-labeled ET-1 in vitro. The probe was immersed in perfusion buffer that contained Krebs buffer and 0.5% bovine serum albumin, the major component of plasma proteins, and various concentrations of 125I-ET-1 (40–100 fmol/ml). The probe was then perfused with the same buffer by a precision infusion syringe pump (Bioanalytical Systems, West Lafayette, IN) at various flow rates (1, 2.5, and 5 µl/min), and the effluent was collected from the outflow tube at 30-min sample periods. The relative recovery of 125I-ET-1 was calculated as the percentage of radioactivity (counts/min; cpm) in the perfusate to the radioactivity in the buffer. On the basis of these in vitro calibration experiments, the relative recovery of ET-1 at a 2.5-µl/min flow rate was calculated to be 15 ± 1% at various concentrations of 125I-ET-1. This recovery factor was constant in a wide range of ET-1 concentration (40–100 fmol/ml). This value was used as the correction factor for the recovery of the microdialysis samples.

On the basis of the results reported by Dell'Italia and colleagues (4), after a 20-min perfusion period to allow the probe to equilibrate, microdialysis of the myocardial interstitium was carried out for an additional 30 min at a flow rate of 2.5 µl/min, and the dialysate was collected into chilled microcentrifuge tubes. In preliminary experiments, an additional microdialysis sample was collected at 60 min from three control animals. The mean ET-1 values at 30- and 60-min time points were 24 ± 7 and 25 ± 8 fmol/ml, respectively. Nonparametric statistical analysis (Mann-Whitney test) of these data indicated no significant difference in ET-1 levels between two groups (P = 0.82). Thus, in subsequent experiments, interstitial fluid samples were collected for only 30 min. At the end of the microdialysis procedure, an arterial blood sample (20 ml) was withdrawn into prechilled EDTA tubes, and plasma was separated immediately. After the final set of measurements, the heart was quickly removed and placed in chilled Krebs solution. The region served by the left anterior descending artery was rapidly cut into 5 × 5-cm cubes and frozen in liquid nitrogen for subsequent total myocardial ET measurements. The region of the LV free wall incorporating the circumflex artery (5 × 5 cm) was used for the myocyte isolation, as described previously (11). The isolated LV myocytes were suspended in Krebs solution, allowed to form a pellet, and then immediately frozen at −70°C. The region of the LV containing the microdialysis probe was immersed in a buffered Formalin solution for a minimum of 3 days. Next, a 3 × 3-cm full thickness transmural section of the fixed LV in which the probe was maintained in situ was prepared for histological analysis.

ET-1 measurement. Myocardial ET-1 content was determined with the use of an extraction method described previously (23). Plasma, dialysate, and LV extracts were first eluted over a cation exchange column (C-18 Sep-Pak; Waters Associates, Milford, MA) and then dried by vacuum centrifugation. Recovery from the extraction procedure was 70 ± 5%, based on spiked plasma and microdialysis standards (0.003–32 fmol/ml). Myocardial ET-1 values were corrected for the extraction efficiency (80%). The samples were reconstituted in 0.02 mol/l borate buffer, and a high-sensitivity RIA was performed (RPA 545; Amersham). After the incubation of samples with 125I-ET-1 and an ET-1 specific antibody, a secondary antibody was added and bound and free label was separated by magnetic separation. To support the range of ET-1 within the samples, two sets of standards, a low range (0.003–1 fmol/ml) for microdialysis samples and a higher range (0.25–32 fmol/ml) for plasma and tissue samples, were prepared by serial dilution of the standard ET-1 peptide. A standard curve was generated in which the known concentrations of ET-1 were linearized by use of regression analysis. The optimized RIA standards for the microdialysis samples were linear with respect to the ET-1 concentrations relevant to the present study (y = −9.9128x + 8.89439, r = 0.9946). The interassay variation was 10 and 9% for the plasma and microdialysis ET-1 RIA procedure, respectively. Plasma and myocardial interstitial ET-1 values were determined as femtomoles per milliliter, and LV ET-1 content was expressed as femtomoles per gram. The cross-reactivity of the antibody employed in the RIA with ET-1, ET-3, and Big ET-1 was 100, <0.001, and <0.001%, respectively.

Myocyte membrane preparation. In a subset of experiments, myocytes (107 cells) isolated from control (n = 5) and CHF (n = 5) pigs were resuspended in cold phosphate-buffered saline. The LV myocyte pellet was homogenized in...
buffer A (20 mM Tris·HCl, pH 7.4, 20 μM pepstatin A, 1 mM phenylmethylsulfonyl fluoride, and 250 mM sucrose). The homogenate was then centrifuged at 1,000 g for 10 min, and the resulting supernatant was centrifuged at 100,000 g for 60 min. The crude membrane fraction was solubilized in homogenization buffer containing 2.5% polyoxyethylene-10-lauryl ether for 30 min. The mixture was then centrifuged at 50,000 g for 60 min. Solubilized LV membrane were aliquoted and stored at −70°C. The protein content in the membrane preparation was measured with the use of the Bradford protein assay from Bio-Rad Laboratories (Hercules, CA).

Measurement of ECE-1 activity. Enzyme activity was measured by incubating various concentrations of solubilized LV myocyte membrane (25–200 μg) with Big ET-1 (0.1 μM/l) in 50 μl of reaction mixture containing 0.1 M sodium phosphate buffer, pH 6.8, and 0.5 M NaCl for various time points (1–4 h) at 37°C. On the basis of these results, reaction conditions were set for the hydrolisis to be in the linear range with respect to time and protein concentration, and the enzyme assays were repeated with the use of various concentrations of Big ET-1 (0.01–2 μM/l) with 100 μg of membrane protein for 1 h. In addition, to eliminate the possibility of nonspecific cleavage of Big ET-1 by the membrane fractions, enzyme activities were repeated in the presence of phosphoramidon (100 μM), a metalloprotease inhibitor that inhibits ECE activity, and thiorphan (100 μM), another metalloprotease inhibitor that does not affect ECE activity (34). The reaction was terminated with 50 μl of 5 M EDTA, and the mixture was assayed for ET-1 by an enzyme-linked immunoassay kit designed for ET-1-rich samples (Amersham Life Sciences). The data were analyzed using the Prism enzyme kinetics software (GraphPad Software, San Diego, CA) to calculate maximal velocity (Vmax) and Michaelis-Menten constant (Km) values. All assays were performed in triplicate.

RT-PCR of ECE-1 isoforms. RNA was extracted from the LV myocyte preparations with the use of GlassMax RNA microlization system (Gibco, Long Island, NY). First-strand cDNA synthesis was performed with 1 μg of total RNA and oligo(dT)12–18 primers by use of SuperScript reverse transcriptase II (Gibco). The PCR was carried out in a reaction mixture containing 20 mM Tris·HCl (pH 8.5), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM/l each dNTP, 500 mM/l of each sense and antisense primer, 10 ng first-strand cDNA, and 2.5 units of Taq DNA polymerase. The amplification primers for ECE-1a (product expected 108 base pairs) were 5’-AGTGTCCCGGGGCGGATAG-3’ (sense, amino acid residues 1–8 of bovine ECE-1a) and 5’-TTTACCTGAGGGAGGAGGCA-3’ (antisense, amino acid residues 29–36). The primers used for ECE-1c (product expected 93 base pairs) were 5’-AGTGTACCTAACCACCGGCAA-3’ (sense, amino acid residues 1–8 of bovine ECE-1c) and 5’-TTTACCTGACGTGTTGGGT-3’ (antisense, amino acid residues 24–31). To determine the linear range for the generation of desired PCR products, initially, various PCR cycles (20–40) of 95°C and 60 s denaturation, 60°C and 90 s annealing, and 72°C and 90 s extension steps were employed for amplification. On the basis of these results, in the subsequent PCR runs, amplification step was set for 30 cycles. To ensure that equivalent CDNA template was used in each reaction, amplification of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control. The sequences for GAPDH primers were (sense) 5’-GCAAATTCCTGGAAGCCGGTCA-3’ and (antisense) 5’-GTCATACCAAGAATGAGCTTT-3’. The PCR products were electrophoresed on 2% agarose gels, and image was captured with the use of Image-Pro software (Media Cybernetics, Silver Spring, MD). The densities of the PCR products were then analyzed by Gel-Pro analyzer software (Media Cybernetics) and expressed as the ratio of ECE to GAPDH.

RESULTS

LV function with pacing-induced CHF. LV function and hemodynamics for the control and chronic pacing group are summarized in Table 1. Resting ambient heart rate was increased, and mean arterial pressure, LV stroke volume, and pressure development were reduced with chronic rapid pacing. Thus, as reported previously, chronic rapid pacing caused LV pump dysfunction consistent with the physiological phenotype of CHF (11, 23).

Table 1. LV function and hemodynamics with rapid pacing heart failure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CHF</th>
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<tbody>
<tr>
<td>Resting heart rate, beats/min</td>
<td>82 ± 5</td>
<td>133 ± 8*</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>99 ± 3</td>
<td>76 ± 4*</td>
</tr>
<tr>
<td>LV Function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>33 ± 2</td>
<td>16 ± 2*</td>
</tr>
<tr>
<td>End-diastolic pressure, mmHg</td>
<td>7.5 ± 1</td>
<td>17 ± 2*</td>
</tr>
<tr>
<td>Peak + dp/dt, mmHg/s</td>
<td>1,769 ± 97</td>
<td>1,200 ± 121*</td>
</tr>
<tr>
<td>Sample size</td>
<td>14</td>
<td>9</td>
</tr>
</tbody>
</table>

Values are means ± SE. For congestive heart failure (CHF), 21 days of rapid pacing at 240 beats/min. LV, left ventricular; P, pressure; dp/dt, peak positive rate of rise in pressure; t, time. *P < 0.05 vs. control.

LV function in plasma, myocardial interstitium, and myocardium. Postmortem evaluation confirmed the placement of the microdialysis probe in the LV midmyocardium. A schematic of the microdialysis system and representative photomicrographs of a probe in situ are shown in Fig. 1. Microscopic examination of the myocardium containing the microdialysis probe demonstrated that the insertion of the probe caused minimal trauma to the surrounding parenchyma, as evidenced by no extravasation of red blood cells or acute inflammatory response. ET-1 levels were determined in the plasma and myocardial interstitial samples obtained by microdialysis and in whole myocardial homogenates in the control and CHF preparations, and the results are summarized in Fig. 2. Both plasma and myocardial levels were increased, whereas myocardial interstitial ET-1 concentration was decreased with the development of CHF.

ECE-1 activity. ECE-1 activity in isolated LV myocytes obtained from control and CHF pigs increased in a linear fashion with the protein content (Fig. 3A). Analysis of enzyme kinetics revealed that there was no significant change in Km values (103 ± 13 vs. 85 ± 15 nM), whereas the Vmax increased from 7,789 ± 235 in control myocytes to 10,880 ± 284 fmoI ET-1·mg protein−1·h−1 in CHF myocytes (Fig. 3B; P < 0.05 vs.
control). In the presence of 100 µM phosphoramidon, enzyme activity was reduced by 87% in both control and CHF membranes. Addition of thiorphan did not cause any change in the enzyme kinetics. These results indicated that Big ET-1 was processed by ECE-1 in the myocyte membrane fractions.

ECE-1 expression. RT-PCR analysis of isolated LV myocytes demonstrated that both ECE-1a and ECE-1c isoforms were expressed (Fig. 4A). The densitometric analysis of PCR products revealed that mRNA for ECE-1a isoform was increased fivefold in CHF compared with control myocytes (Fig. 4B), with no detectable change in ECE-1c expression.

**DISCUSSION**

Whereas systemic plasma ET-1 levels are elevated with CHF, the local ET-1 concentrations that the LV myocytes are exposed to and the extent to which endogenous ET-1 biosynthesis is altered in CHF remained unclear (2, 12, 16, 27, 30, 32). Thus we measured ET-1 levels in the interstitial space surrounding myocytes with the use of a microdialysis technique in a model of pacing CHF. In addition, ECE-1 subisoform expression and activity in myocytes were quantified. The important findings were twofold. First, myocardial interstitial ET-1 levels appeared to be higher than the circulating plasma ET-1 concentrations in the control animals, and with the development of CHF, plasma and total myocardial ET-1 content was increased, but interstitial concentrations were decreased. Second, total ECE activity was increased with CHF and was accompanied by a selective increase in ECE-1a expression in CHF myocytes. These unique results demonstrate the presence of a local ET system at the level of the LV myocyte that is selectively regulated within this myocardial compartment in CHF.

The local myocardial regulation of the ET system with the development of CHF is an area of active investigation. There have been a number of experimen-
and clinical studies that have reported increased myocardial levels of ET-1 (10, 23, 25). However, the localization of ET-1 within the myocardial compartment with CHF remained unclear. The present study is the first report on the measurement of ET-1 levels in the myocardial interstitial fluid. Moreover, our results demonstrate that total myocardial ET-1 was increased with pacing CHF, whereas ET-1 levels in the interstitial compartment were decreased. These results lead to the conclusion that increased cellular uptake of ET-1 from the myocardial interstitium must occur with pacing CHF. There are several lines of evidence that support the concept of cellular uptake of ET-1. In a recent study using a 125I-ET-1 infusion technique, it has been demonstrated that ET-1 has a prolonged terminal half-life, and this finding is due to the high degree of cellular uptake as indicated by the large steady-state volume distribution of the radiolabeled ET-1 (20). The authors have also proposed that extensive uptake of ET-1 in various organ systems has important implications for the pathophysiology of ET-1 in disease states. Pacing CHF causes significant hemodynamic, contractile, and neurohormonal changes consistent with the clinical spectrum of CHF (11, 23). The progression of this CHF process likely contributed to the alterations in both systemic and local ET-1 levels observed in the present study. The specific hemodynamic and neurohormonal factors, which contribute to the changes in systemic and local ET-1 with pacing CHF, remain to be established. Clearance of ET-1 by binding to ET receptors has been demonstrated in many tissue beds, providing additional evidence for cellular uptake of ET-1 (6, 7). Moreover, Azevedo and colleagues (1) reported that coronary sinus ET-1 levels were lower than arterial ET-1 concentrations in patients with CHF compared with subjects with normal LV function, further indicating heightened ET-1 extraction by the myocardium in CHF. The present study did not provide information on ET-1 concentrations in the coronary venous circulation. Nevertheless, results from past studies as well as the present report suggest that alterations in the distribution of ET-1 in the myocardial compartment may occur with CHF. In a study by Zolk et al. (36), the increased myocardial ET-1 content in end-stage human CHF was not
accompanied by an absolute increase in preproET-1 mRNA levels. This past study suggests that the increased ET-1 levels within the myocardium may not be accompanied by an absolute increase in preproET-1 mRNA levels. This past study suggests that the increased ET-1 levels within the myocardium may not be due to increased ET-1 synthesis but to an acceleration of conversion to active ET-1 as well as enhanced uptake. Circulating Big ET-1 levels are also elevated in CHF and are strongly related to survival of patients (19, 24). ECE-1 is the major enzyme involved in the biosynthesis of ET-1 (28, 34). Therefore, the increased ECE-1 activity detected in this study may contribute to the conversion of inactive Big ET-1 to active ET-1 leading to elevated peptide levels. Thus results from past studies as well as the present report suggest that increased ET-1 production and uptake may occur with CHF.

The analysis of ECE-1 cDNA cloned from several sources revealed that there are at least three isoforms of ECE-1 generated by alternative splicing: ECE-1a, ECE-1b, and ECE-1c (22, 28, 29, 34). ECE-1a and ECE-1c are the most commonly expressed subisoforms (29, 34), and, on the basis of the analysis of the promoter sequences, ECE-1a has been suggested to be the isoform that can be induced under pathophysiological conditions (28). A recent study has demonstrated that ECE expression can be influenced by a variety of cytokines, including tumor necrosis factor-α and interferon-γ (33). Several studies have shown that elevated circulating levels of these cytokines accompany the development of CHF (3, 5, 13, 17). Taken together, these factors can modulate ECE expression and activity with the development of CHF. Consistent with these observations, in the present study, ECE-1a was the predominant isoform in isolated LV myocytes, which was selectively upregulated with CHF. In a past study, Zolk and colleagues (36) have reported that there was no significant difference in ECE-1 expression in total LV myocardium in human CHF. The current study using isolated normal and CHF LV myocytes provided evidence that, at the level of myocyte, ECE-1a expression and activity are increased with the development of CHF.

There are several limitations to the present study that must be recognized. Increased volume retention and capillary leakage can occur with the development of CHF. Thus, in the present study, the ET-1 levels in the myocardial interstitium may have been subject to dilution, and this possibility was not directly addressed in this study. Furthermore, with the development of CHF, alterations in the determinants of the Starling hypothesis may have occurred. Specifically, changes in capillary perfusion and oncotic pressure with pacing CHF may have influenced myocardial interstitial ET-1 levels. The fundamental basis for the differences in myocardial interstitial ET-1 levels that occurred in this model of CHF warrants further study. Another limitation of the study is that the decrease in interstitial ET-1 levels with the development of CHF may be due to the increased interstitial degradation by ET-1 degrading enzymes such as neutral endopeptidase. Future studies employing the microdialysis technique for ET-1 developed in the present study and the use of selective inhibitors would be necessary to address this issue. Lastly, the current study employed a semiquantitative RT-PCR technique to study ECE-1 expression rather than a more quantitative method such as Northern or RNase protection assay. However, it should be noted that due to the high degree of sequence identity among ECE-1 subisoforms, the RT-PCR technique is the best method to differentiate these subisoforms (29). Nevertheless, the findings of the present study provide important information for the synthesis and distribution of ET-1 in CHF. Several studies have demonstrated that ET-1 increases contractile performance in normal myocyte preparations (8, 23, 27). However, with the development of CHF, ET-1 appears to exert a negative effect on contractile function. For example, Onishi and colleagues (18) demonstrated that ET-1 caused a negative effect on indexes of LV myocardial contractile function, which were ameliorated with the administration of an ET receptor antagonist. Thus, unlike in normal myocardium, the synthesis and release of ET-1 may contribute to the contractile dysfunction with CHF. The present study demonstrated that indexes of ET-1 biosynthesis and uptake were increased in LV myocardium with CHF, which may exacerbate the LV pump failure in this disease state.

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