Early activation of cardiac and renal endothelin systems in experimental heart failure

Sophie Motte,1,* Ronald van Beneden,2,* José Mottet,3 Benoit Rondelet,1 Myrielle Mathieu,1 Xavier Havaux,4 Pascale Lause,2 Cécile Clercx,3 Jean-Marie Ketelslegers,2 Robert Naeije,1 and Kathleen McEntee1

1Laboratory of Physiology, Faculty of Medicine, Free University of Brussels, Brussels B-1070; 2Unit of Diabetes and Nutrition and 4Unit of Cardiac Physiology and Pathology, Catholic University of Louvain, Brussels B-1200; and 3Department of Clinical Sciences for Companion Animals, Faculty of Veterinary Medicine, University of Liège, Liege B-4000, Belgium

Submitted 13 May 2003; accepted in final form 6 August 2003


HEART FAILURE IS CHARACTERIZED by altered ventricular function and neurohumoral activation, with associated renal vasoconstriction and sodium and water retention. There is evidence that the endothelin system participates in the neurohumoral activation of heart failure. Circulating ET-1 is increased in heart failure (19, 20) and correlates inversely to functional state and survival (34). Cardiac myocytes express prepro-ET-1 (ppET-1) and synthesize and secrete mature ET-1 (40). The protease that catalyzes the conversion of ET-1 from its precursor Big ET-1, ET-1-converting enzyme (ECE-1), is expressed in the endocardium and myocardium (46). The potent inotropic and chronotropic effects of ET-1 (9), as well as its mitogenic actions (26), are mediated through two receptor subtypes, the ET<sub>A</sub> and ET<sub>B</sub> receptors, both of which are expressed on cardiac myocytes (22). ET-1 has been reported to act as a local autocrine and paracrine factor in heart failure (8).

The expression of cardiac ppET-1 has been found to be increased in various experimental animal heart failure models (10, 15, 17, 29, 37, 50) as well as in patients with end-stage ischemic cardiomyopathy (27), whereas this has not been confirmed in patients with end-stage dilated cardiomyopathy despite increased myocardial immunoreactive ET-1 (23, 51). On the other hand, the expression of cardiac ECE-1 has been reported to be increased (23, 24, 27) or unchanged (15, 47, 50, 51), and the expression of both cardiac ET<sub>A</sub> and ET<sub>B</sub> receptors increased (15, 27, 47), unchanged (ET<sub>A</sub>), or decreased (ET<sub>B</sub>) (23, 51), according to model or clinical circumstance. These discrepancies may reflect variability in models and methods and the fact that previous studies were based on unique myocardial tissue samplings (or a limited number), without documenting the temporal evolution of the activation of the different components of the endothelin system. In addition, there has been no previous attempt to correlate the expression of cardiac endothelin system genes to altered ventricular function activation during progressive heart failure.

It was previously shown that the renal endothelin system is activated in heart failure (17, 7, 21). This is known to contribute to salt and water retention (16), maintaining flow output of failing venticles through...
an increased venous return. However, the gene expression of the different components of the renal endothelin system during the progression from mild to severe heart failure has not been previously investigated.

We therefore studied the expression of the cardiac and renal tissue endothelin systems, in relation to circulating and urinary endothelin, cardiac function, and clinical course, by using repetitive myocardial and renal biopsies together with clinical and echocardiographic examinations in rapid pacing-induced heart failure in dogs.

METHODS

Animal Preparation

The investigation was approved by the institutional Animal Care and Use Committee of the Free University of Brussels and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996).

Eleven male beagle dogs were included in the study. Under general anesthesia, a bipolar pacemaker lead (Thin Line EZ model 438-10, Intermedics, Brussels, Belgium) was surgically inserted in the right jugular vein and implanted in the right ventricular apex under fluoroscopic control. A multiprogrammable pulse generator (Dart model 292-05, Intermedics) was inserted in the subcutaneous tissues of the cervical region and connected to the pacemaker lead. Myocardium tissue harvested from five normal beagle dogs used in preliminary experiments served for peptide content control measurements.

Experimental Protocol

The experiment was a longitudinal repeated-measures study. The dogs underwent a modified pacing protocol with a stepwise increase of stimulation frequencies. The pacing was initiated after a 2-wk recovery period by activating the multiprogrammable pulse generator at 180 beats/min and continued for 1 week, followed by 200 beats/min over a second week, 220 beats/min over a third week, and finally 240 beats/min over the last 2 wk. The investigations were carried out at baseline (week 0) and once weekly throughout the pacing period (i.e., from week 1 to week 5) with exactly the same methods.

Measurements and Analytic Methods

Clinical examination. The clinical examination included cardiac and lung auscultation, observation of activity, and measurements of body weight, rectal temperature, heart rate (HR), respiratory rate (RR), and blood pressure. Systolic blood pressure was measured by Doppler sphygmomanometry (model 811-BTS, Parks Medical Electronics, Aloka, OR) on the forelimb of dogs placed in sternal recumbence. Blood pressure was calculated as the mean of three consecutive measurements.

Echo-Doppler examination of heart. Doppler echocardiography (Pandion, Fie Medical Benelux, Zaventem, Belgium) was performed under continuous ECG monitoring with a 3.5- to 5-MHz mechanical sector probe. Echocardiograms were recorded and analyzed according to the recommendations of the American Society of Echocardiography (36) and the Echocardiography Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine (43). All measurements were done in triplicate, irrespective of the respiratory phase. A right parasternal window was used to evaluate systolic cardiac function and systolic and diastolic volume indexes. Left ventricular internal end-diastolic (LVId) and systolic diameters (LVIDs) as well as systolic and diastolic left ventricular free wall (LVPWs and LVFWd) and interventricular septum thickness (IVSs and IVSd) were determined in right short-axis M-mode projections of the heart in a plane just below the mitral valves. E point to septal separation (EPS) was evaluated in the plane of mitral valves. A left caudal long-axis view of the left ventricle was used to obtain image of the aortic flow by pulsed-wave Doppler with the sample volume positioned just proximal to the valve. These velocity spectra were used to measure the preejection period (PEP) and left ventricular ejection time (LVET). From these data, left ventricular end-diastolic (EDV) and systolic volume (ESV), left ventricular ejection fraction (LVEF), and mean velocity of circumferential fiber shortening (MVCF) were calculated with the following formulas

\[
\text{EDV (ml/m}^2\text{)} = [(7/2.4 + \text{LVIDd}) \times \text{LVIDd}^3]/\text{BSA}
\]

\[
\text{ESV (ml/m}^2\text{)} = [(7/2.4 + \text{LVIDs}) \times \text{LVIDs}^3]/\text{BSA}
\]

\[
\text{LVEF} = [(\text{EDV} - \text{ESV})/\text{EDV}] \times 100
\]

\[
\text{MVCF (m/s)} = (\text{LVFS}/\text{LVET}) \times 10
\]

with left ventricular fractional shortening (LVFS; %) = \((\text{LVIDd} - \text{LVIDs})/\text{LVIDd}\) \times 100

\[
\text{EDV} = EDV/BSA \times 7/2.4
\]

\[
\text{ESV} = ESV/BSA \times 7/2.4
\]

\[
\text{LVET} = LVET/BSA \times 7/2.4
\]

\[
\text{LVFS} = LVFS/BSA \times 7/2.4
\]

\[
\text{LVEF} = (1 - \text{LVFS}/\text{LVET}) \times 100
\]

\[
\text{MVCF} = MVCF/BSA \times 7/2.4
\]

Circulating and urinary ET-1. Plasma (n = 11 dogs) and urine (n = 6 dogs) were drawn after each physical examination to evaluate ET-1 concentrations. Venous blood was collected into prechilled tubes containing 3 mM EDTA and 9 mM benzamidine. After centrifugation within 1 h, plasma was stored at –80°C until RIA. The extraction step of ET-1 in plasma was described in a previous study (35). Urinary ET-1 concentrations were determined without the extraction step and were expressed in picograms per milligram of urinary creatinine. Commercially available antibodies and standards (RAS 6901 and 6901, Peninsula, Belmont, CA) were used. The tracers were iodinated in our laboratory and purified by HPLC. The samples displaced the tracer parallel to the standard curve.

Real-time quantitative PCR analysis of cardiac and renal ET receptors, ECE-1, and ppET-1 mRNA. Myocardial and renal biopsies. Transvenous endomyocardial biopsies of the right ventricular septum were performed under anesthesia as previously reported (14). With a long sheath introducer and a disposable endomyocardial biopsy forceps (504–302L, Cordis, Miami, FL), five pieces for a total of 10–15 mg of tissue were obtained under angiographic guidance. Cortical biopsies of the caudal part of the left kidney were performed under echography with a percutaneous needle (EIZ 1809–15, Products Group International) as previously described (31) to obtain the same amount of tissue. Cardiac and renal biopsies were snap frozen in liquid nitrogen and stored at –80°C.

RNA extraction. Total RNA was extracted by the acid guanidinium thiocyanate-phenol-chloroform method (TRIZol reagent, Gibco-BRL Life Technologies, Paisley, UK) according to the manufacturer’s instructions. RNA concentrations were measured by absorbance at a wavelength of 260 nm and adjusted to 0.25 µg/µl.

RNA reverse transcription. One microgram of total RNA was reverse transcribed with 7.5 µM random hexanucleotides as primers by using a GeneAmp RNA PCR kit (Applied Biosystems, Foster City, CA) as previously described (35).

AJP-Heart Circ Physiol • VOL 285 • DECEMBER 2001 • www.ajpheart.org
Table 1. Sequences of upstream and downstream oligonucleotide primers and probes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers and Probe Sequences</th>
<th>Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppET-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>5'-ttctgctttctccgttaggtg-3'</td>
<td></td>
</tr>
<tr>
<td>Antisense</td>
<td>5'-gctgtgtgggtttggt-3'</td>
<td></td>
</tr>
<tr>
<td>ECE-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>5'-aaggtttgctgctctgtgtgtt-3'</td>
<td></td>
</tr>
<tr>
<td>Antisense</td>
<td>5'-ggggtggtctggtttggt-3'</td>
<td></td>
</tr>
<tr>
<td>ETa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>5'-gaaatgtagtgagcagagtc-3'</td>
<td></td>
</tr>
<tr>
<td>Antisense</td>
<td>5'-gaaatgtagtgagcagagtc-3'</td>
<td></td>
</tr>
<tr>
<td>ETb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>5'-atcagggatacgagctg-3'</td>
<td></td>
</tr>
<tr>
<td>Antisense</td>
<td>5'-atcagggatacgagctg-3'</td>
<td></td>
</tr>
<tr>
<td>ABL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>5'-caacagctgaaaataatac-3'</td>
<td></td>
</tr>
<tr>
<td>Antisense</td>
<td>5'-caacagctgaaaataatac-3'</td>
<td></td>
</tr>
</tbody>
</table>
| ppET-1, prepro-endothelin-1 (ET-1); ECE-1, ET-1-converting enzyme; ETa and ETb, endothelin receptors; ABL, Abelson.

PRIMERS AND PROBES. Sequences for primers were based on human (GenBank accession nos. S56805, S57498, and S57283) or porcine (49) sequences in region with high conserved homologies, because canine ET-1, ECE-1, ETa, and ETb cDNA sequences are not yet cloned and published. To obtain canine-specific sequence, cDNA were amplified with these primers and canine end-point PCR products were purified and sequenced with a Big Dye standard protocol. With primer express software (Applied Biosystems), primers and probes were designed to recognize canine cDNA sequences (Table 1). The probes contained a fluorescent reporter (6-carboxyfluorescein) at the 5' end and a fluorescent quencher (6-carboxytetramethylrhodamine) at the 3' end.

RTQ-PCR ANALYSIS. In TaqMan PCR, amplification was monitored by the fluorescence gain associated with the fluorescent reporter (6-carboxytetramethylrhodamine) at the 3' end. Gene expression was determined by the ΔΔCt method (ΔCt sample − ΔCt calibrator) using the baseline week as a calibrator for comparison of every unknown sample gene's expression levels. The conversion between ΔΔCt and relative gene expression is fold induction = 2−ΔΔCt.

Tissue ET-1 Protein Content

After euthanasia by an overdose of barbiturates, cardiac and renal tissue samples were immediately harvested, snap frozen in liquid nitrogen, and stored at −80°C. Four-hundred-milligram samples were pulverized in liquid nitrogen and transferred at 4°C in 4 ml of 1 M acetic acid-20 mM HCl-9 mM benzamidine (5, 11). Samples were homogenized at high speed with a Ultra-Turrax T-25 (IKA, Staufen, Germany), incubated for 10 min at 4°C, and then centrifuged at 27,000 g and 4°C. The supernatant was processed following the same procedure as for plasmatic ET-1 RIA.

Histology and Immunohistochemistry

Immunolocalizations and semiquantitative protein content evaluations were realized on routine 5-μm-thick sections of paraffin-embedded formalin-fixed left ventricular free wall specimens. After deparaffinization and rehydration, sections were reacted for 15 min with 3% hydrogen peroxide in 100% methanol to inhibit endogenous peroxidase activity. No pretreatment and no blocking by normal goat serum were required. After rehydration with PBS (pH 7.4, 10 min), sections were incubated for 1 h with homemade rabbit anti-ET-1 antiserum diluted 1/100 in PBS supplemented with 0.1% bovine serum albumin. After three successive washings with PBS supplemented with 0.1% bovine serum albumin, sections were incubated with the Envision Rabbit system (no. K4002, Dako, Carpinteria, CA) for 30 min in a black box. After washings as described above, the peroxidase activity was revealed by an 3-amino-9-ethylcarbazole commercial substrate kit (AE+, no. K3461, Dako) and the sections were counterstained with Mayer’s hematoxylin and mounted with aqueous mounting.

Table 2. Clinical examination and circulating and urinary ET-1 in tachycardia-induced heart failure

<table>
<thead>
<tr>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, kg</td>
<td>13.8 ± 0.9</td>
<td>14.0 ± 0.9</td>
<td>14.0 ± 0.8</td>
<td>14.1 ± 0.8</td>
<td>14.6 ± 0.9</td>
</tr>
<tr>
<td>T, °C</td>
<td>38.7 ± 0.1</td>
<td>39.0 ± 0.1</td>
<td>39.0 ± 0.1</td>
<td>38.7 ± 0.1</td>
<td>38.9 ± 0.1</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>106 ± 6</td>
<td>111 ± 7</td>
<td>113 ± 6</td>
<td>125 ± 7*</td>
<td>134 ± 9*</td>
</tr>
<tr>
<td>RR, cycles/min</td>
<td>22 ± 2</td>
<td>22 ± 2</td>
<td>24 ± 2</td>
<td>29 ± 3*</td>
<td>28 ± 5*</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>179 ± 11</td>
<td>172 ± 11</td>
<td>168 ± 13</td>
<td>158 ± 12*</td>
<td>140 ± 14*</td>
</tr>
<tr>
<td>Circulating ET-1, pg/ml</td>
<td>7.1 ± 0.4</td>
<td>6.8 ± 0.6</td>
<td>6.9 ± 0.6</td>
<td>8.3 ± 0.8</td>
<td>10.2 ± 0.8*</td>
</tr>
<tr>
<td>Urinary ET-1, pg/mg UC</td>
<td>84 ± 4</td>
<td>81 ± 6</td>
<td>86 ± 15</td>
<td>93 ± 9</td>
<td>113 ± 11*</td>
</tr>
</tbody>
</table>

Results are means ± SE values during development and progression of tachycardia-induced heart failure in dogs. BW, body weight; T, rectal temperature; HR, heart rate; RR, respiratory rate; SAP, systolic arterial blood pressure; UC, urinary creatinine. *P < 0.05 vs. baseline week (week 0); †P < 0.01 vs. baseline week; ‡P < 0.001 vs. baseline week.
medium (Faramount, no. S3025, Dako). Negative controls (omission of primary antiserum and incubation with preimmune antiserum of rabbit as a first layer) were prepared by the method described above in the absence of staining.

For estimation of myocardial ET-1 peptide content, we established a relative optical gradient between 0 and 3 based on the intensity and localization of immunostaining, with preimmune antiserum as zero calibrator. The sections were analyzed in duplicate in a blinded fashion by the same analyst, and the mean value for each was used for statistical analysis.

**Statistical Analysis**

All values are reported as means ± SE. Statistical analysis of gene expression was performed by one-way ANOVA for repeated measures, followed by modified Student's t-tests (44). Coefficients of weighted linear regression (r) and associated probabilities (P) were determined to examine the relationship between relative cardiac ET-1 mRNA expression (y-axes) and various echocardiographic measurements (x-axes). The weighting factor was based on the standard deviation.
deviations of the measurements. A value of $P < 0.05$ was considered statistically significant.

**RESULTS**

Clinical examination showed an increase in HR and RR and a decrease in blood pressure starting at the third week, with no significant change in temperature or in weight (Table 2). Mitral or tricuspid regurgitant murmurs were detected in two dogs from week 3 and in an additional dog from week 4. At week 5, one dog presented with ascites, one with a 2-kg weight loss and anorexia, and two with lethargy. Circulating and urinary ET-1 were increased at weeks 4 and 5 (Table 2).

The echo-Doppler examination of the heart showed progressive decreases in LVEF and MVCF and increased PEP/LVET ($0.34 \pm 0.02$ in week 0 vs. $0.55 \pm 0.04$ in week 5; $P < 0.001$) starting at week 1, together with progressive increases in left ventricular volumes and in EPSS starting at week 2 (Fig. 1). Various measurements of systolic and diastolic wall thickness decreased starting from weeks 1–3 (Fig. 2).

The immunohistochemical study showed staining for ET-1 in endothelial cells, vascular smooth muscle cells, and cardiac myocytes. Intensities in cardiac myocytes were higher in failing hearts than in control hearts (Fig. 3). Myocardial ET-1 peptide content was increased in all cardiac regions studied (Fig. 4). The expressions of cardiac ppET-1 and ECE-1 (Fig. 5) increased progressively, and this was already significant at week 1, whereas the expression of cardiac ET$_A$ and ET$_B$ receptors decreased from week 2 and remained unchanged, respectively (Fig. 5).

Renal ET-1 peptide content was increased compared with that in control dogs (Fig. 6), whereas the expression of ppET-1 (Fig. 6) and ECE-1 (Fig. 7) remained unchanged during development and progression of the disease. The expressions of renal ET$_A$ and ET$_B$ receptors increased from week 2 (Fig. 7).

There were tight correlations between the expressions of cardiac ppET-1 and renal ET$_A$ receptor and the alteration of indexes of cardiac systolic function and volumes (Figs. 8 and 9). Moreover, cardiac ppET-1 expression was correlated to wall thickness ($r = -0.94$ and $-0.91$ for IVSs and IVSd, respectively ($P < 0.001$) and $r = -0.87$ and $-0.86$ for LVFWs and LVFWd, respectively ($P < 0.001$)). Renal ET$_A$ expression was correlated to wall thickness ($r = -0.98$ for IVSs as well as IVSd ($P < 0.001$) and $r = -0.99$ and $-0.97$ for LVFWs and LVFWd, respectively ($P < 0.001$)). Cardiac ET$_A$ expression was correlated to LVEF ($r = -0.90$; $P < 0.001$) and to MVCF ($r = -0.94$; $P < 0.001$).

![Fig. 3. Myocardial endothelin-1 (ET-1) peptide content was evaluated by immunostaining in LVFW. Intensity of ET-1 staining in cardiomyocytes was higher in heart of chronic heart failure dogs (B) than in heart of control (Ctr) dogs (A). There was an increase in quantitatively assessed immunostained ET-1 peptide content (means ± SE; ***$P < 0.001$; C).](image)

![Fig. 4. Myocardial ET-1 peptide content measured by RIA in various cardiac regions of chronic heart failure and control dogs. RA, right atrium; LA, left atrium; RVFW, right ventricular free wall. Values are expressed as means ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. Ctr dogs.](image)
Cardiac ECE-1 expression was correlated to EPSS ($r = 0.89; P < 0.001$), LVFWd ($r = -0.9; P < 0.001$), LVFWs ($r = -0.89; P < 0.001$), and IVSd ($r = -0.92; P < 0.001$). No correlations were found between circulating ET-1 and renal ETB expression and indexes of cardiac systolic function and volume.

**DISCUSSION**

The present study shows that it is possible to follow the temporal evolution of cardiac and renal tissue gene expression of humoral systems by serial biopsies in a large animal heart failure model and to correlate the findings to clinical state and cardiac function noninvasively evaluated by echocardiography. Current RTQ-PCR techniques requiring only small amounts of tissue, to $< 1$ mm$^3$, make this approach feasible. The main new findings reported here are an early expression of cardiac ECE-1 and ppET-1 genes, with ppET-1 mRNA tightly correlated to progressive alteration of cardiac function and volume. These changes were followed shortly thereafter by an increased expression of renal $\text{ET}_A$ and $\text{ET}_B$ receptor genes, with $\text{ET}_A$ receptor mRNA tightly correlated to altered cardiac function as well. All these changes occurred at a still clinically silent stage of the disease.

The overpacing-induced heart failure model is characterized by the rapid development, within a few weeks, of a dilated cardiomyopathy with impaired systemic and regional hemodynamics, broad neurohumoral activation, and avid sodium and fluid retention (39). In the present study, we started at a relatively lower pacing rate than previously reported (17) to be able to observe earlier stages of the syndrome. This strategy proved successful, because we recorded important alterations in systolic function and ventricular dimensions over 3 wk preceding any clinical manifestation of heart failure.

In the present experiments, the expression of cardiac ppET-1 was increased as soon as after the first week of tachycardia. This early increase in cardiac ppET-1 mRNA is in keeping with observations in rats with norepinephrine-induced cardiac hypertrophy (12) or...
Increased conversion of Big ET-1 by ECE-1, even in the presence of unaltered ppET-1 expression (51). In the present study, expression of cardiac ECE-1 was found to be unchanged in rats with hypertensive cardiomyopathy (47, 50) or with myocardial infarction-associated heart failure (15) and in patients with end-stage dilated cardiomyopathy (51). Different models and more or less advanced stages of the disease, with variable effects of counterregulatory systems, may explain these discrepancies. The renin-angiotensin system may be involved in the upregulation of ECE-1 expression, because angiotensin-converting enzyme inhibitor therapy has been reported to downregulate ECE-1 expression (23).

A decrease in myocardial ET-1 clearance could also result in increased tissue ET-1 peptide. In our experiments, gene expression of the ETB receptor, known to be involved in ET-1 clearance (1), was unchanged. This is in keeping with two other studies on failing human hearts (32, 33). However, a downregulation of the myocardial ETB receptor has been reported in a third study (51). The reasons for these discrepancies are unclear.

After 2 wk of tachycardia, expression of the cardiac ETA receptor started to decrease. This could be attributed to increased agonist-induced downregulation (3), but the absence of ETB receptor downregulation indicates that additional mechanisms might be involved. Intracellular cAMP and angiotensin II have been reported to upregulate ETA and ETB receptor expression, respectively (13, 28). On the other hand, our biopsies could only sample myocardial septal tissue and regional differences in the expression of the components of the endothelin system are not excluded. However, ET-1 peptide content was increased in all cardiac regions tested postmortem in the present study.

The decreased cardiac ETA receptor expression in our experiments may appear to be at variance with previous reports that selective ETA receptor antagonists improve hemodynamics (2) and prevent left ventricular remodeling (25) in patients and experimental heart failure models. Because it appears that neither the affinity nor the density of myocardial ET receptors is modified in rapid pacing-induced experimental heart failure (41), a decrease in cardiac ETA gene expression might thus be adaptive, although incomplete, still leaving room for additional improvement with pharmacological ETA receptor blockade.

In view of the positive inotropic properties of ET-1 in vitro (9), it may appear surprising that, in the present experiments, the cardiac expression of ppET-1 related closely to altered ventricular systolic function. However, although ET-1 indeed increases the contractility of normal myocardium strips, it decreases the contractility of myocardial strips from failing hearts (32). Negative inotropic effects of ET-1 have been observed in several heart failure models (10, 38, 42) and in clinical heart failure (18). Myocardial ET-1 content has been reported to be closely correlated to altered ventricular function in salt-sensitive rats with heart failure (10). Negative inotropic effects of ET-1 are incompletely understood but may be in part related in vivo to congestive heart failure caused by myocardial infarction (29). However, in these models, a normalization over time of ppET-1 mRNA expression was observed (29, 12), contrasting with a continuing increase in the present experiments. It may be that our overpaced dogs had not yet attained a stage at which this mechanism would be exhausted, either spontaneously or because of counterregulatory hormones, but this is unlikely, in view of the advanced clinical and echocardiographic heart failure reached at the end of the 5-wk observation period. Increased ppET-1 gene expression could explain the increase in cardiac tissue ET-1 protein content found in our dogs and also reported in patients with end-stage dilated cardiomyopathy (51).

Another cause of increased tissue ET-1 could be an increased conversion of Big ET-1 by ECE-1, even in the presence of unaltered ppET-1 expression (51). In the present study, expression of cardiac ECE-1 also increased as soon as the first week, although less continuously compared with the expression of cardiac ppET-1. This result is in agreement with previous studies in patients with end-stage heart failure (23, 24, 27). However, the expression of cardiac ECE-1 was found to be unchanged in rats with hypertensive cardiomyopathy (47, 50) or with myocardial infarction-associated heart failure (15) and in patients with end-stage dilated cardiomyopathy (51). Different models and more or less advanced stages of the disease, with variable effects of counterregulatory systems, may explain these discrepancies. The renin-angiotensin system may be involved in the upregulation of ECE-1 expression, because angiotensin-converting enzyme inhibitor therapy has been reported to downregulate ECE-1 expression (23).
coronary vasoconstriction and relatively increased systemic vascular resistance (42, 30). Endothelin receptor blockade has been reported to improve ventricular function in various models of experimental heart failure (6, 25, 38, 42).

Previous studies have shown an activation of the renal endothelin system in heart failure (7, 17, 21). In overpacing-induced heart failure, renal ET-1 content has been reported to be increased either with (48) or without (17) associated increased expression of ppET-1. We found an increased renal ET-1 content, which could also be in keeping with the increased urinary ET-1 found in the present experiment. However, this was not associated with increased ppET-1.

Fig. 8. Scatterplots of relative cardiac ppET-1 mRNA expression (y-axis) vs. various echocardiographic measurements (x-axis). A: LVEF. B: MVCF. C: end-systolic volume (ESV) and EDV. D: EPSS. Regression equations and their coefficients of correlation (r) are shown, and all are significant (P < 0.001).

Fig. 9. Scatter plots of relative renal ETA mRNA expression (y-axis) vs. various echocardiographic measurements (x-axis). A: LVEF. B: MVCF. C: ESV and EDV. D: EPSS. Regression equations and their coefficients of correlation are shown, and all are significant (P < 0.001).
ECE mRNA or decreased ET_2 mRNA. It is possible that the kidneys could accumulate circulating ET-1.

In the present study, there was a tight correlation between renal ET_A receptor expression and altered cardiac function. This observation may support the notion of a participation of the renal endothelin system in the heart failure syndrome through ET_A receptor-mediated renal vasoconstrictive and salt retention (16). The findings also are in keeping with reported improvements in renal function with ET-1 receptor blockers in heart failure (4, 7).

In summary, our results demonstrate early activations of the cardiac endothelin system and of the renal endothelin receptors in experimental heart failure and may provide a rationale for ET receptor blocker therapy in patients with asymptomatic or minimally symptomatic alteration in echocardiographic indexes of ventricular function.

We thank Pascale Jespers for excellent technical assistance.

DISCLOSURES

This work was supported by Fund for First Doctorate Enterprise 991/4167 from Centre d’Economie Rurale (CER, Marloie, Belgium) and Ministère de la Région Wallonne, Division de la Recherche et de la Coopération Scientifique (Namur, Belgium), Grant 3.4516.02 from the National Funds for Scientific Research (FNRS) Belgium, the Foundation for Cardiac Surgery, Belgium, and a grant from the Intervet International. S. Motte is a research fellow “aspirant” of the FNRS, and M. Mathieu is a research fellow from the Foundation of Cardiovascular Research (FNRS) Belgium, the National Funds for Scientific Research and Ministère de la Région Wallonne, Division de la Recherche et de la Coopération Scientifique (Namur, Belgium).

REFERENCES


