Functional Effects of Endothelin and Regulation of Endothelin Receptors in Isolated Human Nonfailing and Failing Myocardium

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Background—An activated endothelin (ET) system may be of pathophysiological relevance in human heart failure. We characterized the functional effects of ET-1, ET receptors, and ET-1 peptide concentration in left ventricular myocardium from 10 nonfailing hearts (NF) and 27 hearts in end-stage failure due to idiopathic dilative cardiomyopathy (DCM).

Methods and Results—Inotropic effects were characterized in isolated muscle strips (1 Hz; 37°C). ET-1 0.0001 to 0.3 μmol/L significantly increased twitch force by maximally 59±10% in NF and by 36±11% in DCM (P<0.05 versus NF). Preincubation with propranolol 1 μmol/L and prazosin 0.1 μmol/L did not affect the response to ET-1, but the mixed ET receptor antagonist bosentan and the ETα receptor antagonist BQ-123 shifted the concentration-response curves for ET-1 rightward. The ETβ receptor agonist sarafotoxin S6c 0.001 to 0.3 μmol/L had no functional effects. The inotropic response to ET-1 was not associated with increased intracellular Ca2+ transients, as assessed in aequorin-loaded muscle strips. ET receptor density (Bmax; radioligand binding) was 62.5±12.5 fmol/mg protein in NF and 122.4±24.3 fmol/mg protein in DCM (P<0.05 versus NF). The increase in Bmax in DCM resulted from an increase in ETα receptors without change in ETβ receptors. ET-1 peptide concentration (radioimmunoassay) was higher in DCM than in NF (14 447±2232 versus 4541±1340 pg/mg protein, P<0.05).

Conclusions—ET-1 exerts inotropic effects in human myocardium through ETα receptor–mediated increases in myofibrillar Ca2+ responsiveness. In DCM, functional effects of ET-1 are attenuated, but ETα receptor density and ET-1 peptide concentration are increased, indicating an activated local cardiac ET system and possibly a reduced postreceptor signaling efficiency. (Circulation. 1999;99:1802-1809.)

Key Words: endothelin ▪ cardiomyopathy ▪ contractility ▪ receptors ▪ Ca2+ handling

Endothelin-1 (ET-1) is an endogenous vasoconstrictive peptide that increases blood pressure as well as the plasma concentrations of vasopressin, renin, aldosterone, epinephrine, norepinephrine, and atrial natriuretic peptide.1 In addition, ET-1 acts as a growth factor inducing hypertrophy.2 Furthermore, ET-1 exerts positive inotropic effects in rat,3,4 rabbit,5,6 and ferret7 but not in guinea pig8 or dog9 myocardium. Recently, a local ET production in the heart has been demonstrated; it is secreted from the endocardium, the myocardium, and the coronary endothelium,9,10 thereby acting in a paracrine and autocrine fashion on myocytes. ET plasma levels are elevated in patients with chronic heart failure.11,12 and increased ET-1 levels participate in the maintenance of cardiac contractile function in rats with congestive heart failure.13 Endogenous ET-1 contributes to the maintenance of vascular tone in healthy men14 and to vasoconstriction in severe chronic heart failure.15,16 However, a possible functional role of ET-1 in the human heart remains to be elucidated. Despite controversial reports on the effects of ET-1 in mammalian myocardium, little is known about its direct actions in human myocardium. Qiu et al17 showed a Ca2+-sensitizing effect of ET-1 in myocytes from failing human hearts, and Schömisch-Moravec et al18 demonstrated a slight increase in force in muscle strips from failing hearts. However, both studies investigated only one concentration of ET-1 and made no comparison between nonfailing and failing myocardi...
myocardium. Autoradiographic studies have shown the existence of ET receptors in human atrial and ventricular myocardium but did not address the question of ET receptor regulation in heart failure.19,20 Recently, Pönické et al21 demonstrated both ET_A and ET_B receptors in human cardiac tissue by radioligand binding studies. ET-1 peptide has been detected in human myocardium, but no comparison between nonfailing and failing tissue was made.22

Therefore, the goal of the present study was to characterize the inotropic effects of ET-1, the ET receptor subtype involved in this effect, and the subcellular mechanism of action of ET-1 in isolated myocardium from nonfailing and end-stage failing human hearts. Furthermore, possible alterations of ET receptors and ET-1 peptide concentrations in idiopathic dilative cardiomyopathy (DCM) were characterized.

Methods

Myocardial Tissue

Functional experiments were performed in isolated left ventricular muscle strips from 10 nonfailing human hearts that could not be transplanted for technical reasons and 27 end-stage failing hearts (DCM; ejection fraction, 22±1%). For biochemical analysis, transmucosalic specimens of left ventricular tissue (1 to 3 g), free of macroscopic fatty tissue and coronary arteries, were frozen in liquid nitrogen immediately after explantation and stored at –80°C. The study was approved by the Ethical Committee of the University Clinics of Freiburg.

Functional Measurements

Muscle Strip Preparation

Muscle strips were prepared as previously described,23 mounted in an organ chamber, superfused with modified Krebs-Ringer solution (37°C; pH 7.4), and attached to a force transducer (F30; Hugo Sachs Electronics). The solution contained (in mmol/L) Na⁺ 152, K⁺ 3.6, Cl⁻ 135, HCO₃⁻ 25, Mg²⁺ 0.6, H₂PO₄⁻ 1.3, SO₄²⁻ 0.6, Ca²⁺ 2.5, and glucose 11.2, and 10 IU/L insulin. The muscle strips were electrically stimulated by field stimulation and gradually stretched until maximum isometric twitch tension (mN/mm² cross-sectional area) was reached. Cross-sectional area was determined as the ratio of blotted muscle weight to length (average for nonfailing, 0.43±0.07 mm² and for DCM, 0.48±0.05 mm², P<NS).

Experimental Protocol

After complete mechanical stabilization, cumulative concentration-response curves for ET-1 0.0001 to 0.3 μmol/L or the specific ET_B receptor agonist sarafotoxin S6c 0.001 to 0.3 μmol/L were established. Additional experiments were performed after muscle strips had been preincubated for 30 minutes with prazosin 0.1 μmol/L, propranolol 1 μmol/L, the mixed ET receptor antagonist bosentan 3 and 30 μmol/L, or the specific ET_A receptor antagonist BQ-123 0.03 and 0.3 μmol/L.

Aequorin Measurements

To assess the effects of ET-1 on intracellular Ca²⁺ transients, muscle strips were loaded with the photoprotein aequorin as described previously.23 Briefly, aequorin 1 to 3 μL was microinjected through a fine-tipped glass micropipette into the quiescent muscle. Changes in aequorin light emission, reflecting changes in intracellular Ca²⁺ transients, were detected by a photomultiplier (XP 2802, Philips). Light emission (mV photomultiplier output) and isometric force (mN) were recorded simultaneously on an oscilloscope with signal-averaging function (Nicolet PRO 10C, Nicolet Instrument Corp) and on a chart recorder (WR 3310, Graphtec). After complete stabilization of aequorin light and force signals, cumulative concentration-response curves for ET-1 0.0001 to 0.3 μmol/L or extracellular Ca²⁺ ([(Ca²⁺)]_o) 1.25 to 4 mmol/L were performed in nonfailing and end-stage failing myocardium.

Radioligand Binding

Membrane Preparation

Microsomal membrane preparations and radioligand binding studies were performed as described in detail elsewhere.24 Briefly, myocardial tissue was homogenized by use of a Polytron homogenizer PT-K (Brinkman Instruments) for 2 minutes in ice-cold buffer of the following composition (in mmol/L): Tris/HCl 5, MgCl₂, 1, and sucrose 250; pH 6.4. After repeated centrifugation and rehomogenization steps, the resulting microsomal membrane homogenate was suspended in 1 mL incubation buffer (Tris/HCl 75 mmol/L, MnCl₂ 25 mmol/L, sucrose 250 mmol/L, chymostatin 2 mg/L, leupeptin 4 mg/L, bacitracin 40 mg/L, pH 7.4), divided into aliquots, and stored at –80°C.

Competitive Radioligand Binding

Incubation suspensions were prepared by addition of 100 μL of microsomal membrane homogenates (final protein concentration, 200 μg/mL), 50 μL of radioactive [¹²⁵I]-labeled ET-1, and 100 μL of the competitive cold ligand ET-1, ET-3, or BQ-123 at increasing concentrations. The concentration of [¹²⁵I]-labeled ET-1 was 32 pmol/L, equivalent to ~30 000 cpm. For displacement of the iodinated ligand, ET-1 was added in 15 different concentrations, ranging from 10⁻¹¹ to 10⁻⁶ mol/L. To assess the relative amount of ET_A and ET_B receptors, displacement experiments were performed with increasing concentrations of ET-3 (showing a relative selectivity for the ET_B receptor) and the selective ET_A receptor antagonist BQ-123. All experiments were performed in triplicate and started by adding the membrane homogenate, followed by 180 minutes of incubation at 25°C. Incubation was terminated by filtration of the suspension through a Whatman GF/C glass filter. Filters were washed, and membrane-bound radioactivity remaining in the filter was measured by scintillation gamma counter. Specific binding was determined by subtracting nonspecific binding, as assessed with ET-1 0.1 μmol/L, from total binding. The individual binding experiments were analyzed for receptor distribution, receptor density (Bₘₐₓ), and ligand affinity (K_D) by use of the LIGAND program.

Endothelin-1 Peptide Expression

ET-1 peptide concentration in tissue homogenates was measured by radioimmunoassay (RIA) as described by Löffler and Maire.25 Frozen tissue samples were thawed on ice and homogenized in a 10-fold wet weight volume of 0.9% NaCl solution and kept at 0°C to 4°C. Aliquots of 100 and 200 μL were mixed with 1 mL methanol. Precipitated protein was sedimented at 3000g for 10 minutes. The methanol/water phase was evaporated to dryness and redissolved in RIA buffer, and ET-1 was determined as described.25 ET extraction efficacy, as measured by spiking of homogenates with ET-1 10 to 100 pg, was determined to be >90%.

Materials

ET-1 (porcine/human; Sigma Chemical Co) was dissolved in deionized water in a concentration of 10 μmol/L and stored at –80°C until use. BQ-123, sarafotoxin S6c, and isoproterenol hydrochloride were also obtained from Sigma. Bosentan was obtained from Hoffmann–La Roche.

Statistical Analysis

Average values are given as mean±SEM. Comparison within one group of myocardium was performed by use of paired t test and Bonferroni-Holms equation. Comparisons between different groups were performed by ANOVA followed by Student-Newman-Keuls test. For analysis of binding data, the average from triplicate experiments under each condition was used. Differences were considered significant at P<0.05.
**Results**

**Positive Inotropic Effects of ET-1 in Isolated Human Myocardium**

ET-1 exerted clear positive inotropic effects in human myocardium. Figure 1 shows original recordings from typical experiments. In a nonfailing preparation (top panel), isometric force increased from 6.5 to 11.4 mN. In a preparation from a failing heart (bottom panel), the positive inotropic effect was less pronounced (increase from 4.9 to 6.4 mN). There was no initial depression of tension before the rise in twitch force, as has been previously described in atrial preparations.26

The concentration-dependent inotropic response to ET-1 is shown in Figure 2. In nonfailing myocardium, the effect started at a concentration of 0.001 μmol/L and was maximal at 0.1 μmol/L ET-1. At that concentration, force had increased by 5.3±1.2 mN/mm², or 52±11% (P<0.05; n=10).

In DCM, the effect of ET-1 was likewise maximal at 0.1 μmol/L. However, ET-1 increased force only by 3.0±0.5 mN/mm², or 36±11% (P<0.05 versus baseline; n=14). At concentrations >0.01 μmol/L, the positive inotropic effect was significantly more pronounced in nonfailing than in failing myocardium. Despite the reduced effectiveness of ET-1 in failing myocardium, its potency remained unchanged: the EC₅₀ was 4.28 nmol/L (CI, 1.25 to 14.67 nmol/L) in nonfailing and 3.42 nmol/L (CI, 1.52 to 7.66 nmol/L) in failing myocardium (P=NS). ET-1 prolonged both time to peak and relaxation time in a concentration-dependent manner (Table 1).

α-Adrenergic receptor blockade with prazosin 0.1 μmol/L and β-adrenergic receptor blockade with propranolol 1 μmol/L did not affect the inotropic response to ET-1 in end-stage failing myocardium (increase in force by 31.7±9.9%; n=8; P=NS versus data without antagonists, not shown). In contrast, the mixed ET receptor antagonist bosentan 3 and 30 μmol/L shifted the concentration-response curves for ET-1 to the right, and at 30 μmol/L bosentan, the maximal inotropic response to ET-1 was attenuated at the concentrations of ET-1 used (Figure 3, left).

To test whether the inotropic effect of ET-1 is mediated via ETₐ or ETₐ receptors, experiments were performed after selective blocking of ETₐ receptors with BQ-123. BQ-123 at 0.03 and 0.3 μmol/L shifted the concentration-response curve for ET-1 to the right, and with 0.3 μmol/L BQ-123, the maximal inotropic effect of ET-1 was reduced (Figure 3, right). Furthermore, the selective ETₐ receptor agonist sarafotoxin S6c 0.001 to 0.3 μmol/L did not elicit any inotropic effects in nonfailing or failing myocardium (Figure 4), whereas ET-1 at 0.1 μmol/L applied after sarafotoxin S6c increased force to 181±20% and 139±18%, respectively (P<0.05 versus baseline for both groups). These data indicate that the functional effects of ET-1 are mediated exclusively via ETₐ receptors.

**Aequorin Experiments**

ET-1 increased twitch force with only slight changes in aequorin light emission. This can be seen from superimposed original tracings in aequorin-loaded muscle strips from a nonfailing (Figure 5, left) and a failing heart (Figure 5, middle). In contrast, a similar increase in twitch force in a muscle strip from a nonfailing heart after [Ca²⁺], was raised to 4 mmol/L was associated with a substantial

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**Table 1. Influence of ET-1 0.1 μmol/L on Time Parameters of the Isometric Twitch**

<table>
<thead>
<tr>
<th>Time, ms</th>
<th>Nonfailing</th>
<th>DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPT</td>
<td>Baseline</td>
<td>ET-1</td>
</tr>
<tr>
<td>167±10</td>
<td>177±109</td>
<td>188±6</td>
</tr>
<tr>
<td>138±10</td>
<td>143±10</td>
<td>140±5</td>
</tr>
<tr>
<td>345±34</td>
<td>356±38</td>
<td>309±9</td>
</tr>
<tr>
<td>Total time</td>
<td>512±40</td>
<td>531±39</td>
</tr>
</tbody>
</table>

TPT indicates time to peak tension; RT₅₀, time to 50% relaxation; and RT₉₅, time to 95% relaxation.

*P<0.05 vs baseline.
increase in aequorin light emission (Figure 5, right). Figure 6 shows concentration-response curves for ET-1 in aequorin-loaded muscle strips from 5 nonfailing (left) and 7 DCM (right) hearts. In nonfailing myocardium, ET-1 resulted in a pronounced increase in force but only slight increases in aequorin light emission. In DCM, the maximal inotropic effect of ET-1 was smaller, and there were only minor changes in aequorin light emission. In contrast, [Ca\(^{2+}\)]\(_o\) (1.25 to 4 mmol/L; data related to basal values at [Ca\(^{2+}\)]\(_o\) = 2.5 mmol/L) concentration-dependently increased twitch force from 69±7% to 154±9% in nonfailing (n=6) and from 60±8% to 153±8% in failing (n=6) myocardium, with parallel increases in aequorin light emission (from 68±8% to 138±18% in nonfailing and from 70±8% to 160±13% in failing myocardium, respectively; P<0.05 versus baseline; data not shown). Time to 50% decline of the aequorin light transient (baseline versus 0.1 mmol/L ET-1) increased from 64±7 ms to 98±12 ms (P<0.05) in nonfailing and from 91±6 to 98±12 ms in failing myocardium (P=NS). Time to 90% decline increased from 194±16 to 213±22 ms (P=NS) and from 199±11 to 215±12 ms (P=NS) respectively.

For comparing the subcellular mechanism of action of ET-1 and [Ca\(^{2+}\)]\(_o\), the relation between the concentration-dependent change in twitch force and aequorin light emission for both interventions is plotted in Figure 7 for nonfailing myocardium (according to Reference 27). It becomes obvious...
that the increase in force after [Ca\textsuperscript{2+}], is raised is associated with a proportional increase in aequorin light emission, whereas similar changes in force after ET-1 are associated with only minor increases in aequorin light emission.

**Radioligand Binding Studies**

ET receptors in human myocardium were characterized by competitive radioligand binding using \textsuperscript{125}I-labeled ET-1 and unlabeled ET-1, which binds with similar affinity to ET\textsubscript{A} and ET\textsubscript{B} receptors, ET-3 with \textapprox100-fold higher affinity for the ET\textsubscript{B} receptor, and the selective ET\textsubscript{A} receptor antagonist BQ-123. Figure 8 shows average values from displacement experiments for increasing concentrations of ET-1, ET-3, and BQ-123 in left ventricular myocardial membrane preparations from 5 nonfailing and 7 DCM hearts. In DCM, compared with nonfailing myocardium, the remaining \textsuperscript{125}I-labeled ET-1 binding in the presence of BQ-123 is significantly decreased, from \textapprox40\% to 25\%. Accordingly, the upper part of the ET-3 displacement curve is shifted rightward, indicating a low affinity of the ET\textsubscript{B}-specific ligand ET-3. LIGAND analysis of the individual experiments revealed both ET\textsubscript{A} and ET\textsubscript{B} receptors in human cardiac tissue, and the relative proportion of the ET\textsubscript{A} receptor increased from 63\%±5\% in nonfailing to 73\%±3\% in failing myocardium (Table 2). This was the result of an increased absolute number of ET\textsubscript{A} receptors. ET\textsubscript{A} receptor density was 38.1±6.3 fmol/mg protein in nonfailing and 88.3±17.4 fmol/mg protein in DCM hearts (P<0.05 versus nonfailing). ET\textsubscript{B} receptor density was lower than ET\textsubscript{A} receptor density in both types of myocardium and was not altered in DCM. Total ET receptor density (B\textsubscript{max}) was 62.5±12.5 fmol/mg protein in nonfailing myocardium and increased to 122.4±24.3 fmol/mg protein in DCM (P<0.05 versus NF) because of the selective upregulation of ET\textsubscript{A} receptors. There were no significant differences in the K\textsubscript{D} values for ET-1, ET-3, and BQ-123 between nonfailing and failing myocardium. B\textsubscript{max} values and binding characteristics of both ET\textsubscript{A} and ET\textsubscript{B} receptors are summarized in Table 2.

**Endothelin-1 Peptide Tissue Concentration**

ET-1 peptide concentration was measured in left ventricular homogenates from 5 nonfailing and 5 end-stage failing hearts. ET-1 peptide concentration in nonfailing myocardium was 4541±1340 pg/mg protein (1.8±0.5 fmol/mg). ET-1 peptide concentration was significantly increased in DCM to 14 447±2232 pg/mg protein (5.8±0.9 fmol/mg; P<0.05 versus nonfailing).

**Discussion**

The main findings of this study are that (1) ET-1 exerts direct inotropic effects in human ventricle that are reduced in failing compared with nonfailing myocardium, (2) functional responses are mediated via ET\textsubscript{A} receptors and are not associated with significant increases in intracellular Ca\textsuperscript{2+} transients, and (3) ET\textsubscript{A} receptor density and ET-1 peptide concentration are increased in DCM.

**Functional Effects of ET-1**

Direct positive inotropic effects of ET-1 have been described in rat, rabbit, and ferret but not in guinea pig or dog myocardium. Recently, a direct inotropic effect of ET-1 has been reported for human atrial myocardium, and a single concentration of ET-1 showed inotropic responses in muscle strips.
ET A and ET B receptors were demonstrated in human myocardium, and ET A receptor mRNA was recently found in human cardiac muscle. 28 However, the exact characterization of the functional effects of ET-1 in nonfailing and failing human ventricles and the ET receptor subtype involved in this effect remained to be determined.

From this study, it is evident that ET-1 exerts a concentration-dependent inotropic response in nonfailing and failing myocardium. This effect is comparable to β-adrenergic receptor stimulation but is only 20% to 30% of the maximal inotropic effect that can be obtained with β-adrenergic receptor stimulation in human cardiac muscle. As assessed by α- and β-adrenergic receptor blockade, the inotropic response to ET-1 is independent of activation of these receptors. In contrast, the mixed ET receptor antagonist bosentan as well as the selective ET A receptor antagonist BQ-123 shifted the concentration-response curve for ET-1 to the right. In contrast, selective ET B receptor stimulation with sarafotoxin S6c did not elicit any inotropic response. These data suggest that the inotropic effect of ET-1 is mediated specifically and exclusively via ET A receptors located on ventricular myocytes. This is in contrast to the rat heart, in which ET B receptor-mediated inotropic effects of ET-1 have been reported. 29

**TABLE 2. Characterization of Endothelin Receptors**

<table>
<thead>
<tr>
<th>Nonfailing (n=5)</th>
<th>DCM (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bmax Total</td>
<td>62.5±12.5</td>
</tr>
<tr>
<td>Bmax ET A</td>
<td>38.1±6.3</td>
</tr>
<tr>
<td>Bmax ET B</td>
<td>24.6±6.5</td>
</tr>
<tr>
<td>% ET A</td>
<td>63±5</td>
</tr>
<tr>
<td>% ET B</td>
<td>37±5</td>
</tr>
<tr>
<td>ET A</td>
<td></td>
</tr>
<tr>
<td>Ke ET-1, pmol/L</td>
<td>12.7±2.7</td>
</tr>
<tr>
<td>Ke ET-3, nmol/L</td>
<td>7.8±3.7</td>
</tr>
<tr>
<td>Ke BQ-123, nmol/L</td>
<td>1.5±0.5</td>
</tr>
<tr>
<td>ET B</td>
<td></td>
</tr>
<tr>
<td>Ke ET-1, pmol/L</td>
<td>12.7±2.7</td>
</tr>
<tr>
<td>Ke ET-3, pmol/L</td>
<td>21.8±5.9</td>
</tr>
<tr>
<td>Ke BQ-123, μmol/L</td>
<td>11.7±4.2</td>
</tr>
</tbody>
</table>

Bmax indicates maximal binding, in fmol/mg protein; Total, ET A+ET B receptors.  
*P<0.05 vs nonfailing.

**Endothelin Receptor Densities**

Elevated ET plasma levels in patients with congestive heart failure 11, 12, 15 might influence ET receptor regulation. In contrast to observations in the β-adrenergic receptor system, 30 but similar to α-adrenergic receptors, 31 the present study shows increased cardiac ET receptor densities in DCM without changes in the affinity of the receptors for their ligands. The upregulation of ET receptors was due to a selective upregulation of ET A receptors with no change in ET B receptor density.

In animal models of heart failure, both upregulation and downregulation of ET receptors were reported. 32-33 ET receptor densities in this study in DCM are similar to results from autoradiographic studies by Molenar et al 10 in 4 failing human hearts. Corresponding to our data, a subtype distribution of 57% ET A and 43% ET B was observed. 19 Furthermore, autoradiographic experiments by Bax et al 20 in nonfailing human hearts revealed a subtype distribution of 53% ET A and 47% ET B receptors, but these data were not compared with failing myocardium.

In agreement with our binding data, Morawietz and Holtz (Department of Physiology, University of Halle, Germany) observed a significant upregulation of ET A receptor mRNA in end-stage failing compared with nonfailing human myocardium (personal communication). However, our results seem to be in contrast to receptor binding data by Pönicke et al 21 in human cardiac tissue. These authors found a total ET receptor density of 113±27 fmol/mg protein in nonfailing myocardium. Using pooled myocardium from end-stage failing hearts due to both idiopathic dilative and ischemic cardiomyopathy, the authors detected an increase in ET receptor density to 147.5±44 fmol/mg protein, which was not significant. Interestingly, in their study, separate analysis of ET receptors according to pathogenesis of cardiac disease revealed a decline of ET receptors in ischemic (81.9±12 fmol/mg protein; n=5) but a 2-fold increase in dilative cardiomyopathy (213±82 fmol/mg protein; n=5). We also found a decline of ET receptor expression in ischemic cardiomyopathy (43.2±5.7 fmol/mg protein; n=6; unpublished data). Therefore, it seems possible that ET receptor expression is differentially regulated in dilative versus ischemic cardiomyopathy, and pooling of myocardium with both diseases might contribute to the lack of significance for the ET receptor increase in the study by Pönicke et al. 21

Because radioligand binding studies in myocardial homogenates do not allow us to discriminate between receptors on...
myocytes and on nonmyocytes, the possibility cannot be excluded that upregulation of ET$_A$ receptors on fibroblasts, smooth muscle cells, or endothelium mask a downregulation on cardiac myocytes. However, ET$_A$ receptors are not expressed on human coronary endothelium.$^{24}$ ET receptor density is lower on fibroblasts than on myocytes,$^{25}$ and ET receptor number on smooth muscle cells does not exceed ET receptor number on myocytes.$^{26}$ Therefore, the observed marked upregulation of ET$_A$ receptors in DCM hearts most likely results from increased myocyte receptor density.

**ET-1 Tissue Concentration**

The existence of a local cardiac ET system has been postulated on the basis of the expression of preproET-1 mRNA$^{27}$ and peptide,$^{22}$ but no information about changes in peptide content in human failing myocardium is available. ET-1 exerts its local action in a paracrine and autocrine fashion$^{27}$ through secretion from endothelial cells toward their abluminal borders$^{28}$ and direct secretion from cardiac myocytes.$^{29}$ We detected a 3-fold increase in ET-1 peptide concentration in DCM, possibly indicating the activation of a local cardiac ET system. However, from our experiments in myocardial homogenates, we cannot localize ET-1 peptide overexpression to either endothelial cells, smooth muscle cells, or cardiac myocytes.

**Subcellular Mechanism of Action of ET-1**

It was previously reported that ET-1 induces phosphoinositide breakdown,$^{21}$ mediating protein kinase C activation and subsequent stimulation of Na$^+$/H$^+$ exchange.$^4$ This results in intracellular alkalization and sensitization of the myofilaments for Ca$^{2+}$. Consistently, we did not detect significant increases in intracellular Ca$^{2+}$ after ET-1. Furthermore, we demonstrated that inhibition of protein kinase C or Na$^+$/H$^+$ exchange prevented the inotropic response to ET-1 but not to isoproterenol in human atrial trabeculae.$^{28}$

Our findings of an ET-1–induced positive inotropic effect with only minor changes in intracellular Ca$^{2+}$ transients are in agreement with previous reports in animal experiments in ventricular tissue. ET-1 increases contractility but does not change intracellular Ca$^{2+}$ transients in isolated adult ventricular myocytes from rats$^3$ and rabbits.$^{39}$ In contrast, using isolated ferret papillary muscle, Wang and Morgan$^{40}$ demonstrated a slight, albeit significant, increase in [Ca$^{2+}$], after ET-1 associated with a 64% increase in twitch force. Furthermore, Qiu et al.$^{17}$ did not observe an increase in Indo-1 fluorescence associated with increased shortening after ET-1 in human myocytes. For human ventricular myocardium, our results suggest increased myofilament Ca$^{2+}$ responsiveness as the major mechanism of action of ET-1. However, we recently found a slight increase in aequorin light emission in human atrial trabeculae,$^{26}$ but different coupling of endothelin receptors in atrial compared with ventricular myocardium was described.$^{41}$ Therefore, we cannot exclude the possibility that small increases in intracellular Ca$^{2+}$, possibly related to increased transsarcomemal Ca$^{2+}$ influx$^{42}$ or mobilization of Ca$^{2+}$ from intracellular stores$^{43}$ contribute to the positive inotropic effect of ET-1 in human ventricular tissue.

**Possible Mechanisms Underlying the Reduced Functional Effect of ET-1 in DCM**

The present finding of a reduced inotropic effect of ET-1 in failing human myocardium is in agreement with a previous report in a rabbit heart failure model.$^{44}$ Downregulation of the ET$_A$ receptor is unlikely to contribute to reduced functional effects of ET-1, because we detected an increased expression and unchanged affinities of the ET$_A$ receptors in DCM. Accordingly, Pönické et al.$^{23}$ found unchanged inositol phosphate accumulation after ET-1 stimulation in failing compared with nonfailing myocardium. However, Freedman et al.$^{45}$ recently described a rapid homologous ET$_A$ receptor desensitization by ET-1–induced activation of G protein–coupled receptor kinases and phosphorylation of the receptor. This process may be of importance in the light of increased ET-1 plasma levels in heart failure. Furthermore, the effectiveness of ET-1 to stimulate Na$^+$/H$^+$ exchange activity was impaired in rat cardiac hypertrophy, resulting in blunted functional effects of ET-1.$^{46}$ This might indicate that alterations in subcellular mechanisms mediating functional responses of ET-1 could contribute to the reduced inotropic effect in diseased myocardium. However, few data regarding such defects were reported for human myocardium. Furthermore, because binding data in myocardial homogenates cannot provide final proof for an upregulation of ET$_A$ receptors on cardiomyocytes, the alterations underlying the reduced effectiveness of ET-1 in DCM deserve further investigation.

**Clinical Relevance of the Endothelin System in Heart Failure**

The relevance of an activated ET system for maintaining cardiac function has recently been shown for a rat infarct model of heart failure.$^{13}$ However, this may not completely translate into the situation of patients with chronic congestive heart failure, for several reasons: (1) in addition to its positive inotropic effects, ET-1 constricts coronary arteries and peripheral resistance vessels, thereby reducing coronary blood flow and increasing afterload, which might impair contractility$^{47,48}$; (2) the inotropic effect of ET-1 is small and accounts for only 20% to 30% of the maximal β-adrenergic receptor–mediated inotropic effect in nonfailing and failing myocardium; and (3) short-term treatment of patients with severe congestive heart failure with the nonselective ET receptor antagonist bosentan resulted in favorable acute hemodynamic effects,$^{15}$ and long-term treatment with bosentan increased survival in a rat model of chronic heart failure.$^{49}$ Therefore, the beneficial effect of ET-1 on contractile performance in the human heart may be offset by increased load, reduced coronary blood flow, and induction of cardiac hypertrophy and remodeling, and treatment of heart failure patients with ET receptor antagonists may be beneficial despite the loss of ET-1–related positive inotropy.

**References**


Functional Effects of Endothelin and Regulation of Endothelin Receptors in Isolated Human Nonfailing and Failing Myocardium
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