Endothelin Receptors in the Failing and Nonfailing Human Heart

Klaus Pönicke, PhD; Magdalene Vogelsang; Moritz Heinroth; Karin Becker, PhD; Oliver Zolk, MD; Michael Böhm, MD; Hans-Reinhard Zerkowski, MD; Otto-Erich Brodde, PhD

Background—In patients with chronic heart failure (CHF), plasma endothelin-1 (ET-1) levels are increased. We studied whether the cardiac ET-receptor system is altered in CHF patients.

Methods and Results—We assessed ET-evoked inositol phosphate (IP) formation in slices from right atria and left ventricles from 6 potential heart transplant donors (NFH) and 15 patients with end-stage CHF; in membranes from the same tissues, we studied ET-induced inhibition of isoprenaline- and forskolin-stimulated adenylyl cyclase and ET-receptor density. ET (10⁻⁷ to 10⁻⁶ mol/L, ET-1 >>> ET-3) increased IP formation in right atria and left ventricles through ETA-receptor stimulation in a concentration-dependent manner; no difference in potency or efficacy between NFH and CHF hearts was observed. ET-1 (10⁻¹⁰ to 10⁻⁶ mol/L), via ETₐ-receptor stimulation, inhibited isoprenaline- and forskolin-stimulated adenylyl cyclase in right atria but not in left ventricles, whereas carbachol inhibited adenylyl cyclase in both tissues; again, the potency and efficacy of ET- or carbachol-induced adenylyl cyclase inhibition was not different between NFH and CHF hearts.

Conclusions—In the human heart, ETA and ETB receptors coexist; however, only ETA receptors are of functional importance. In right atria, ETA receptors couple to IP formation and inhibition of adenylyl cyclase; in left ventricles, they couple only to IP formation. In end-stage CHF, the functional responsiveness of the cardiac ETₐ-receptor system is not altered.

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Key Words: endothelin ■ heart failure ■ receptors ■ inositol phosphates

Endothelin-1, a 21 amino acid peptide, was originally isolated from porcine aortic endothelial cells as a potent vasoconstricting peptide. Subsequently, however, it became clear that ET-1 also exerts important cardiac effects. These include positive inotropic effects in the heart of various species (see Reference 2), including humans, and growth-promoting properties (see Reference 9). Thus, ET-1 might contribute considerably to the development of cardiac hypertrophy.

The physiological effects of ET-1 are mediated by at least two subtypes of ET receptors, designated ETA and ETB receptors. Both ETA and ETB receptors coexist in the human heart. Cardiac ET receptors in various species, including humans, couple via a G protein, presumably Gₛₐ₁₁.⁰ to the PLC/IP₃/DAG system as the major intracellular signaling pathway. However, in rat cardiomyocytes and human right atrium, ET receptors can also couple to inhibition of adenylyl cyclase activity, very likely via a pertussis toxin-sensitive G protein.

Recent studies have shown that in patients with CHF, plasma ET-1 levels were increased and the increase was positively correlated with the severity of the disease (judged by NYHA classification). Thus, it has been hypothesized that ET-1 might play a pathophysiological role in congestive heart failure. In favor of this idea are findings from Kiowski et al showing that treatment of CHF patients with the nonselective ETA/ETB receptor antagonist bosentan markedly improved hemodynamic parameters and increased cardiac index. Moreover, Sakai et al recently demonstrated that in a rat model of myocardial infarction, long-term treatment with the selective ETA receptor antagonist BQ-123 improved the survival rate of these rats.

The aim of the present study was to gain further insight into the properties of human cardiac ET receptors and their possible alterations in CHF patients. We therefore assessed ET receptor density and subtype distribution and the effects of ET-1 on IP formation and adenylyl cyclase activity in right atria and left ventricles from CHF patients compared with NFH.

Methods

Study Patients
Myocardial tissue was obtained from the Essen, Germany, cardiac transplant program from 1989 through 1993. Atrial and ventricular...
human myocardium was obtained at the time of explantation from 15 heart transplant recipients. Six hearts were removed from patients with end-stage DCM and 9 from patients with end-stage ICM. All patients had given written informed consent before surgical procedures were performed. They were classified in NYHA functional class III to IV and IV with an ejection fraction of 21.3±1.6%, left ventricular end-diastolic pressure of 21.1±3.7 mm Hg, and a cardiac index of 2.2±0.2 L · min⁻¹ · m⁻². Tissues samples taken at the time of explantation were either immediately frozen in liquid nitrogen and stored at −80°C until use or immediately used for experiments. Ten patients were treated with digoxin, 11 with ACE inhibitors, 10 with diuretics, 8 with nitrates, and 6 with calcium antagonists; in addition, they were treated occasionally with lipid-lowering drugs (n=3), antiarythmics (n=4), and aspirin (n=3). Patients who had received catecholamines or β-adrenocceptor antagonists were withdrawn from the study. General anesthesia was performed with fentanyl, fentanyl, and pancuronium bromide with enflurane. Cardiac surgery was performed on cardiopulmonary bypass. The cardioplegic solution used was a Bretschneider-HTK solution.

**Control Group**

Right atrial appendages were obtained from 18 patients (12 male, 6 female; mean age, 59.8±2.5 years) undergoing coronary artery bypass grafting who were in NYHA functional class I. No patient suffered from acute myocardial failure or had been treated with catecholamines or β-adrenocceptor antagonists for at least 3 weeks before the operation. In addition, left ventricular myocardial tissues from 6 nonfailing control hearts were obtained from organ donors whose hearts could not be used for transplantation because of the lack of a suitable recipient. These patients (4 men aged 23, 33, 43, and 46 years and 2 women aged 29 and 35 years) died from injury to the brain. Patient histories revealed no evidence for heart disease. This study was approved by the ethical committee of the University of Essen.

**Radioligand Binding Studies**

Tissues were minced with scissors and homogenized in 10 vol of ice-cold 50 mmol/L Tris-HCl buffer, pH 7.4, containing 1 mmol/L EGTA and 10 μg/mL aprotinin with an Ultra Turrax (Ika-Werke) for 10 seconds at full speed and twice for 20 seconds at half-maximal speed in 1-minute intervals. The homogenate was diluted to 20 mL with homogenization buffer and centrifuged at 750g for 15 minutes. The supernatant was passed through four layers of cheesecloth and centrifuged at 50 000g for 30 minutes. The resulting pellets were washed once by resuspension and recentrifugation and finally resuspended in incubation buffer (50 mmol/L Tris-HCl, pH 7.4, containing 10 mmol/L MgCl₂, 0.1% BSA, and 1 mg/mL soybean trypsin inhibitor) to yield a protein concentration of 35 to 50 μg/mL. Protein content was determined by the method of Bradford using bovine IgG as a standard. Membranes (~15 μg of protein) were incubated with 15 different concentrations of ET-1 ranging from 10⁻¹³ to 10⁻⁶ mol/L and ~10 000 cpm of [¹²⁵I]ET-1 in siliconized polypropylene tubes in a total volume of 1 mL.²¹ Tubes were incubated for 60 minutes at 37°C in a shaking water bath. Bound ligand was separated by vacuum filtration over Whatman GF/C filters coated with 4% BSA followed by washing with 2×10 mL of incubation buffer. The radioactivity of the wet filters was determined in a γ-counter (Cobra Autogamma, Packard) at an efficiency rate of 80%. Nonspecific binding was defined as binding not displaced by 1 μmol/L boventan.²² To assess the relative amount of ETₐ and ETₐ receptors, membranes were incubated with ~10 000 cpm of [¹²⁵I]ET-1 and 13 concentrations (ranging from 10⁻¹¹ to 10⁻¹ mol/L) of the selective ETₐ receptor antagonist BQ-123,²³ and specific binding was determined as described above. Details have been described previously.²⁴ ET-1 and BQ-123 competition curves were analyzed by the iterative curve-fitting program InPlot (GraphPad software). Statistical analysis was performed using the F test to measure the goodness of fit of the competition curves for either one or two sites. From the ET-1 competition curves, Bₐmax and Kᵣ values were calculated as recently described.²¹

**Adenylyl Cyclase Determination**

Adenylyl cyclase activity was assessed as previously described in detail.²⁵ Membranes (30 to 40 μg of protein) were incubated for 10 minutes at 30°C in a final volume of 100 μL containing 40 mmol/L HEPES buffer (pH 7.4), 5 mmol/L MgCl₂, 1 mmol/L EDTA, 10 μmol/L GTP, 500 μmol/L ATP, ~1 000 000 cpm [³²P]-cAMP, 100 μmol/L cAMP, and an ATP regenerating system (5 mmol/L phosphocreatine and 50 U/mL creatine phosphokinase) in the presence or absence of isoproterenol (10 μmol/L), forskolin (10 μmol/L), and various concentrations of ET-1 (10 pmol/L to 1 μmol/L) or carbachol (10 nmol/L to 100 μmol/L). Recovery was assessed by trace labeling with [³²P]-cAMP was recovered by the column technique of Salomon et al.²⁶ Column recovery was usually 70% to 80%.

**IP Determination**

Preparation of myocardial tissue usually began within 5 to 20 minutes of surgical removal in oxygenated Krebs-Henseleit solution at room temperature. Right atrial and left ventricular tissue samples were chopped into 250×250-μm slices with a McIlwain tissue chopper (Bacchus). The slices were suspended in Krebs-Henseleit buffer of the following composition (mmol/L): NaCl 108, KCl 4.7, CaCl₂ 1.3, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 24.9, glucose 11, and EDTA 0.001. The buffer was supplemented with 10 mmol/L LiCl to block IP degradation, 2 U/mL adenosine deaminase to remove from the assay adenosine that had possibly been liberated during tissue chopping, and 10 μmol/L propranolol. IP accumulation was determined in [H³]myoinositol-labeled slices during a 45-minute incubation at 37°C with detection of formed [H³]IPs by column chromatography as detailed elsewhere.²⁷

**Western Blotting**

Gₛ₁₁₁ protein α-subunits were quantified by immunoblotting as previously described in detail.²⁸ Briefly, α-subunits of Gₛ₁₁₁ were detected with the use of the antisem QU at a 1:600 dilution, followed by quantification of the blots with [¹²⁵I]protein A solution.

**Statistical Evaluation**

Data are presented as mean±SEM of n experiments. Experimental data were analyzes by computer-supported iterative nonlinear regression analysis using the InPlot program (GraphPAD Software). Data from ET-1–induced IP formation and carbachol– and ET-1–induced adenylyl cyclase inhibition were fitted to sigmoidal curves. In these calculations, the bottom of the curves was fixed at 0% stimulation or inhibition, respectively; stimulation of IP formation induced by 1 μmol/L ET-1 was taken as maximal stimulation and inhibition of adenylyl cyclase by 1 μmol/L ET-1 and 100 μmol/L carbachol as maximal inhibition; and the Hill slopes were kept variable. From these curves, EC₅₀ values were obtained that were not considerably different (maximal difference was a factor of two) from those calculated with a

**Selected Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>Ang II</td>
<td>angiotensin II</td>
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<tr>
<td>BQ-123</td>
<td>cyclo(-Asp-Pro-d-Val-Leu-d-Trp)</td>
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<tr>
<td>CHF</td>
<td>chronic heart failure</td>
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<tr>
<td>DAG</td>
<td>dacylglcerol</td>
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<tr>
<td>DCM</td>
<td>dilated cardiomyopathy</td>
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<tr>
<td>ET</td>
<td>endothelin</td>
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<tr>
<td>IP</td>
<td>inositol phosphate</td>
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<tr>
<td>IP₃</td>
<td>inositol trisphosphate</td>
</tr>
<tr>
<td>NFH</td>
<td>nonfailing human hearts</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
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<tr>
<td>PLC</td>
<td>phospholipase C</td>
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<td>sf</td>
<td>sarafotoxin</td>
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In right atrial slices from NFH, ET-1 (10⁻⁶ mol/L) increased [³H]IP formation in a concentration-dependent manner; increases at 10⁻⁶ mol/L were ≈90% above basal levels (Fig 1). Under these experimental conditions, the EC₅₀ value for ET-1 was 4.4±1.1 nmol/L (Table 1). However, we could not test higher concentrations of ET-1 and hence do not know whether 10⁻⁵ mol/L ET-1 causes maximal increases in [³H]IP formation. On the other hand, ET-3 even at 10⁻⁶ mol/L caused maximal increases in [³H]IP formation of only 35% (Fig 1). Among the sarafotoxins investigated, Sf6b (10⁻⁶ mol/L) was nearly equipotent to ET-1, whereas Sf6c, even at 10⁻⁶ mol/L, did not significantly affect [³H]IP formation.

Because of the limited amount of tissue in left ventricular myocardium from NFH, we only studied the effects of ET-1 and ET-3 on [³H]IP formation. ET-1 (10⁻⁹ to 10⁻⁶ mol/L) increased [³H]IP formation in a concentration-dependent manner; the maximal increase at 10⁻⁵ mol/L was ≈70% (Fig 2); the EC₅₀ value was 3.6±0.8 nmol/L. On the other hand, 10⁻⁵ mol/L ET-3 increased [³H]IP formation by only 21% (Fig 2).

However, concentration-response curves for ET-1-induced [³H]IP formation in right atrial and left ventricular slices of CHF hearts were nearly superimposable with those from NFH (Fig 2); thus, no differences in potency or efficacy could be observed between NFH and CHF hearts (Fig 2; Table 1). The same held true when results obtained in CHF hearts were examined separately for ICM and DCM hearts (data not shown).

In addition, we were able to study ET-1 effects in a few left atria from CHF hearts. In these left atrial slices, the maximal increase in [³H]IP formation induced by 10⁻⁶ mol/L ET-1 was ≈80% (Fig 2); in these atria, too, 10⁻⁶ mol/L ET-3 increased [³H]IP formation by only 24% (Fig 2).

In some tissues, we could test the effects of the ETA receptor antagonist BQ-123 (1 μmol/L) on ET-1–induced [³H]IP formation; as shown in Fig 3, BQ-123 nearly completely suppressed 0.1 μmol/L ET-1–induced [³H]IP formation in right atrial and left ventricular slices at this concentration.

### Adenylyl Cyclase Response

In right atrial and left ventricular membranes of CHF hearts, adenylyl cyclase activation by GTP and isoproterenol was significantly reduced whereas that of NaF was unchanged compared with NFH. Forskolin stimulation of adenylyl cyclase was not tested in these experiments.

### Results

#### IP Formation

**Figure 1.** Effects of ET-1, Sf6b, ET-3, and Sf6c on IP formation in slices from nonfailing human right atria. Ordinate: [³H]IP formation in percent of control (100%); Abscissa: molar concentrations of the ET agonists. Values are mean±SEM. Number of experiments: ET-1, 9 to 12; Sf6b, 5; ET-3, 3; Sf6c, 3. Basal [³H]IP formation was ≈1% to 2% of the incorporated radioactivity and amounted to 1288±279 cpm (n=15).

**Figure 2.** Effects of ET-1 and ET-3 on IP formation in slices from right and left atria and left ventricles from nonfailing (NFH) and severely failing human hearts (CHF). Ordinate: [³H]IP formation in percent of control (100%); Abscissa: molar concentrations of the ETs. Values are mean±SEM; number of experiments is given in parentheses.

**Table 1. EC₅₀ Values for ET-1–Induced IP Formation in Myocardial Slices From Nonfailing and Failing Human Hearts**

<table>
<thead>
<tr>
<th>Location</th>
<th>Nonfailing Hearts</th>
<th>Failing Hearts</th>
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<tbody>
<tr>
<td>Right atrium</td>
<td>4.4±1.1 (9)</td>
<td>7.0±1.5 (14)</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>3.6±0.8 (5)</td>
<td>3.8±1.6 (13)</td>
</tr>
<tr>
<td>Left atrium</td>
<td>ND</td>
<td>4.5±1.3 (4)</td>
</tr>
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</table>

ND indicates not determined. EC₅₀ values were calculated from the data presented in Figs 1 and 2 as described in “Methods.” Values are means±SEM; number of experiments is given in parentheses.
ET-1 (10^{-11} to 10^{-7} mol/L) inhibited 10 μmol/L isoprenaline- and 10 μmol/L forskolin-stimulated adenylyl cyclase activity in right atrial membranes in a concentration-dependent manner (in agreement with our recently published data\(^\text{15}\)) but not in left ventricular membranes (Figs 5 and 6). The inhibitory effect of 10^{-2} mol/L ET-1 was inhibited by the ETA receptor antagonist BQ-123 in a concentration-dependent manner; the K_d value for BQ-123 was 3.3±1.1 nmol/L (n=3; data not shown). The muscarinic receptor agonist carbachol (10^{-8} to 10^{-4} mol/L), on the other hand, inhibited isoprenaline- and forskolin-stimulated adenylyl cyclase activity, respectively, in both tissues with a similar potency and efficacy (Figs 5 and 7; Table 2).

In right atria from CHF hearts, maximal inhibition of isoprenaline- and forskolin-stimulated adenylyl cyclase by ET-1 was not significantly different from that in NFH (Fig 5); however, concentration–response curves for ET-1 were ≈5-fold shifted to the right (Fig 6; Table 2). On the other hand, carbachol-induced inhibition of isoprenaline- and forskolin-stimulated adenylyl cyclase was nearly identical in right atrial and left ventricular myocardium of CHF hearts and NFH (Figs 5 and 7; Table 2). As described for IP formation, no significant differences between ICM and DCM hearts were observed for adenylyl cyclase inhibition.

### Endothelin Receptors
In NFH hearts, ET receptor density was 167.7±19.7 fmol [^{125}\text{I}]ET-1 specifically bound/mg protein in right atria (n=6) and 113.1±27 fmol/mg protein in left ventricular membranes (n=5); the K_d values for [^{125}\text{I}]ET-1 were 19.9±2.5 pmol/L in atria and 19.7±1.1 pmol/L in ventricular membranes. In both tissues, the ETA receptor antagonist BQ-123 inhibited [^{125}\text{I}]ET-1 binding with biphasic competition curves, resulting in an ETA/ETB receptor ratio of 62.5±5%;37.5±5% in atrial and 66±3.3%;34±3.3% in ventricular membranes.

In CHF hearts, mean ET receptor densities showed the tendency to increase in right atria (230.5±44 fmol/mg protein, n=13; K_d value, 18.8±2.8 pmol/L) and left ventricular membranes (147.5±44 fmol/mg protein, n=10; K_d value, 23.3±3.1 pmol/L); however, the differences to NFH did not reach statistical significance. The same held true when data for ICM (right atrium: 209.5±69 fmol/mg, n=7; left ventricle:
81.9±12 fmol/mg, n=5) and DCM hearts (right atrium: 248±62 fmol/mg, n=6; left ventricle: 213±82 fmol/mg, n=5) were examined separately. Similarly, ETₐ:ETₐ receptor ratios (atria, 66.2±2.3%;33.8±2.3%; ventricles, 68.3±2.8%;31.7±2.8%) in the CHF hearts were not significantly different from those in the NFH.

**Gq/11 Protein**

Finally, we studied whether Gq/11, the G protein most likely coupling the ET receptor to the PLC/IP₃/DAG system, might be altered in left ventricular membranes of CHF hearts. The Gq/11-specific antiserum QL detected a single band with an apparent molecular weight of 42.7 kD. However, no significant difference in the amount of [125I]protein A bound in this band could be detected between NFH and CHF hearts (Fig 8).

**Discussion**

In the present study, we have quantified and subclassified ET receptors in the human heart with the use of [125I]ET-1 radioligand binding studies. The results show that in both right atrial and left ventricular myocardium of NFH, ETₐ and ETₐ receptors coexist, a finding consistent with other studies. Moreover, mRNA for both ETₐ and ETₜ receptors has been identified in atrial and ventricular myocardium having a similar distribution. The density of ET receptors, however, appears to be 1.5-2-fold higher in atrial tissue than in ventricular myocardium (present study).

Despite the coexistence of ETₐ and ETₜ receptors in the human heart, only ETₐ receptors appear to be of functional importance. As shown in Fig 2, ET receptor agonists induced IP formation in atrial and ventricular myocardium with an order of potency ET-1 ≫ ET-3, which is the typical one for an ETₐ receptor. Moreover, ET-1-induced IP formation in human myocardial slices was nearly completely suppressed by the ETₐ receptor antagonist BQ-123 in a concentration (1 μmol/L) that under these experimental conditions occupies ≈99% of ETₐ receptors but <5% of ETₜ receptors. In addition, Meyer et al recently showed in human right atrial preparations that the ET receptor mediating the positive inotropic effect of ET-1 is an ETₐ receptor. Finally, the growth-promoting effect of ET-1 in rat cardiac myocytes was exclusively mediated by ETₐ receptor stimulation.

**Table 2. EC₅₀ Values (nmol/L) for Carbachol- and ET-1–Induced Inhibition of 10 μmol/L Isoprenaline- and 10 μmol/L Forskolin-Induced Activation of Adenylyl Cyclase Activity in Myocardial Membranes From Nonfailing and Failing Human Hearts**

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<th></th>
<th>Nonfailing Hearts</th>
<th>Failing Hearts</th>
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<tr>
<td></td>
<td>ISO-AC</td>
<td>FOR-AC</td>
</tr>
<tr>
<td>Carbachol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right atrium</td>
<td>350±44</td>
<td>1060±111 (9)</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>780±98</td>
<td>2760±351 (5)</td>
</tr>
<tr>
<td>ET-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right atrium</td>
<td>0.11±0.02</td>
<td>0.31±0.04 (10)</td>
</tr>
</tbody>
</table>

ISO indicates isoprenaline; AC, adenylyl cyclase; and FOR, forskolin.

EC₅₀ values were calculated from the data presented in Figs 6 and 7 as described in “Methods.” Values are means±SEM; number of experiments is given in parentheses.
We recently demonstrated that in human right atria, ET-1 not only increases IP formation but also inhibits isoprenaline- or forskolin-stimulated adenylyl cyclase activity. This effect is also mediated by ET\(_\alpha\) receptors because it is induced by ET receptor agonists with an order of potency ET-1 >> ET-3 and is inhibited by BQ-123 with a \(K_i\) value (3.3 nmol/L) that is well within its range of affinity for ET\(_\alpha\) receptors. In ventricular myocardium, however, stimulation of ET\(_\alpha\) receptors does not inhibit adenylyl cyclase. This is not due to an inability of ventricular receptor stimulation to inhibit adenylyl cyclase, because the muscarinic receptor agonist carbachol inhibited isoprenaline- and forskolin-stimulated adenylyl cyclase activity in atrial and ventricular myocardium with similar potency and efficacy (present study; see References 34 through 36). Taken together, these results show that in human right atria, ET\(_\alpha\) receptors couple to IP formation and inhibition of adenylyl cyclase, whereas in human left ventricles, they couple only to IP formation.

In CHF patients, plasma ET-1 concentrations are increased. Thus, in CHF, cardiac ET receptors are chronically exposed to high concentrations of ET-1, and it could be expected, therefore, that they might be downregulated and/or desensitized. However, this is obviously not the case. In the present study, ET\(_\alpha\) receptor density was decreased neither in right atrium nor in left ventricle of CHF patients but rather showed a tendency to increase (cf “Results”); similarly, preliminary data from Pieske et al\(^8\) described increased left ventricular ET\(_\alpha\) receptors in CHF patients. In addition, ET\(_\alpha\) receptor-mediated IP formation was not different in atrial and ventricular tissues of CHF patients versus that in NFH. Moreover, the immunodetectable amount of G\(_{q/11}\) was not significantly different between CHF hearts and NFH. Thus, the ET\(_\alpha\) receptor in CHF patients shows a similar pattern as the \(\alpha_1\)-adrenoceptor, another presumably G\(_{q/11}\)-coupled receptor in the human heart: receptor density is unchanged or slightly increased, and IP formation is unchanged compared with NFH. Moreover, the carbachol-induced IP formation (which very likely also involves G\(_{q/11}\)) is not different in NFH and CHF hearts. On the other hand, the positive inotropic effect evoked by \(\alpha_1\)-adrenoceptor stimulation in vitro (in isolated right and left ventricular preparations) and in vivo is decreased in CHF hearts. In addition, preliminary results indicate that the ET-1–induced positive inotropic effect in left ventricular preparations of CHF hearts is also decreased. Taken together, it appears that G\(_{q/11}\)-coupled receptors undergo very similar changes in CHF in the human heart: the number is unchanged or increased, G\(_{q/11}\) is unchanged, IP response is unchanged, and the positive inotropic effect is (presumably) decreased. This indicates that human cardiac G\(_{q/11}\)-coupled receptors appear to be uncoupled from the physiological response in end-stage CHF. We do not know why cardiac ET\(_\alpha\) receptors (and \(\alpha_1\)-adrenoceptors) are not decreased but rather increased in CHF in the face of elevated plasma ET-1 (and norepinephrine) levels and can only speculate as to the reason. One possible mechanism might be related to cross-regulation phenomena, because it has been shown that chronic activation of the adenylyl cyclase/cAMP system (as in CHF) can upregulate mRNA levels for ET\(_\alpha\) receptors\(^9\) and \(\alpha_1\)-adrenoceptors. Another possibility might be that ET-1 that is generated and secreted locally in the heart is more important for regulation of cardiac ET\(_\alpha\) receptors than circulating ET-1. It has been proposed that Ang II plays an important role in maintaining local ET-1 concentrations in the heart, presumably via AT\(_1\) receptor stimulation on cardiac fibroblasts. Because cardiac AT\(_1\) receptors are downregulated in CHF (see below), it might be that Ang II fails to induce production of sufficient amounts of local ET-1 to downregulate the cardiac ET\(_\alpha\) receptor.

In contrast to \(\alpha_1\)-adrenoceptors and ET\(_\alpha\) receptors, the AT\(_1\) receptor, which also presumably couples via G\(_{q/11}\) in the human heart, is decreased in ventricular myocardium of CHF patients; this has been found on a protein\(^{30,41}\) and mRNA level. The reason for this differential regulation of cardiac G\(_{q/11}\)-coupled receptors in CHF patients is not clear. However, the properties of human cardiac AT\(_1\) receptors differ from those of ET\(_\alpha\) receptors and \(\alpha_1\)-adrenoceptors: whereas norepinephrine and ET-1 cause positive inotropic effects in right atrial and left ventricular preparations of the human heart, several groups have convincingly shown that Ang II exerts positive inotropic effects in right atrial but not in left ventricular preparations of the human heart. This raises the question whether AT\(_1\) receptors are localized on cardiomycocytes or on myocyte cells in human ventricular myocardium; in rat heart, it has been shown that the AT\(_1\) receptor is localized predominantly on myocyte cells (mainly cardiac fibroblasts) and that the AT\(_1\) receptor on these cells plays a critical role in Ang II–mediated effects in neonatal rat cardiomycocytes. In human heart, AT receptors have been directly identified on cardiomycocytes but have been demonstrated to exist on fibroblasts. A large body of evidence has accumulated that shows that in end-stage CHF, the functional activity of the inhibitory G protein G\(_i\) is increased. This is also found in the present study: the GTP response of adenylyl cyclase (concomitant activation of G\(_i\), and G\(_\beta\)) was decreased whereas that of NaF (activating only G\(_i\), under these conditions) was unchanged, a typical pattern of adenylyl cyclase activation in severely failing human hearts. Despite the increase in the functional activity of G\(_i\), however, inhibition of isoprenaline- and forskolin-stimulated adenylyl cyclase activity by carbachol and ET-1 was not considerably different in NFH and CHF hearts. These results are in good agreement with data from the literature that show that in CHF, the number of muscarinic receptors is not changed and the negative inotropic effect of carbachol is unaltered.

A limitation of our study is that we have used myocardial homogenates that contain not only cardiomycocytes but also a variety of other cell types. However, in the human heart, ET-1 causes IP formation (present study) and positive inotropic effects via ET\(_\alpha\) receptor stimulation, and ET receptors mediating increases in contraction have been demonstrated on isolated human ventricular cardiomycocytes. Thus, at least some of the effects described in the present study are representative for ET\(_\alpha\) receptors on cardiomycocytes. Whether this holds true for ET\(_\beta\) receptors and on which cell type they are localized remains to be elucidated.

In conclusion, ET\(_\alpha\) and ET\(_\beta\) receptors coexist in the human heart; however, only ET\(_\alpha\) receptors appear to be of functional
importance. In atrial tissue, ET₄ receptors couple to IP formation (very likely via Gq/11) and inhibition of adenyl cyclase (very likely via Gi), whereas in ventricular myocardium, they only couple to Gq/11. In severely failing human hearts, ET₄ receptor density, the immunodetectable amount of Gq/11, and ET-induced IP formation is unchanged, a pattern similar to that found for human cardiac α₁-adrenoceptors. The fact that ET₄ receptor– and α₁-adrenoceptor–mediated IP formation are unchanged in severely failing human hearts might be of pathophysiological importance; it has been suggested that the PLC/IP₃/DAG pathway, with subsequent activation of protein kinase C, can increase the rate of protein synthesis and hence is involved in the hypertrophic response. Thus, in CHF patients with elevated endogenous norepinephrine and ET-1, long-term stimulation of ET₄ receptors and α₁-adrenoceptors might significantly contribute to development of cardiac hypertrophy, as is often seen in CHF patients.

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