Transient, isopeptide-specific induction of myocardial endothelin-1 mRNA in congestive heart failure in rats

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Three isoforms of endothelin (ET), ET-1, ET-2, and ET-3, have been identified, cloned, and characterized (10). ET-1 was originally isolated in 1988 as a very potent vasoconstrictor peptide from cultured porcine endothelial cells (33). It has subsequently been appreciated that ET is produced not only by vascular endothelial cells, but also by a number of other cell types, including vascular smooth muscle cells, bronchial and gastrointestinal epithelial cells, and monocytes/macrophages as well as cardiomyocytes (for review see Ref. 21). From this widespread distribution, it has become evident that the ETs may exert a wide variety of biologic effects in different target cell types. For example, in various myocardial preparations in vitro ET-1 exerts a positive inotropic response 30–60% of the maximal isoproterenol-stimulated inotropic response (13). ET-1 has also been shown to act as a potent mitogen or growth factor in several cells in vitro, including fibroblasts (28) and cardiomyocytes (25), and has been found to induce several of the molecular markers of hypertrophy of cardiomyocytes (25). In a previous study we showed by in situ hybridization analysis that cardiomyocytes may synthesize preproendothelin-1 (ppET-1) mRNA after acute ischemia in pigs (29). Cardiomyocytes also express ETA and ETB receptors at fairly high levels (16). Thus, clearly, the molecular basis for an autocrine/paracrine action of ET-1 in the myocardial tissue is present. Furthermore, in a recent study, chronic administration of the ETA-selective antagonist BQ-123 prevented cardiac hypertrophy due to pressure overload by aortic banding in rats (12).

Several studies have demonstrated increased levels of plasma ET during congestive heart failure (CHF) in humans (20) and in experimental animal models (2). The increase in plasma ET does not appear to be related to the etiology of CHF (1, 22). However, plasma ET correlated closely to the degree of heart failure in patients categorized according to the classification of the New York Heart Association (20). Furthermore, in a recent report by Omland and colleagues (18), plasma ET concentrations in the subacute phase after myocardial infarction were very strong and independent predictors of 1-yr mortality. These observations have led investigators to suggest a pathophysiological role for ET in CHF. However, plasma ET in normal subjects is extremely low, and even the modest elevations associated with CHF can hardly cause a significant activation of the ET receptor signaling pathways (3). Thus investigations have been aimed at identifying local synthesis of ET and testing the hypothesis of an autocrine/paracrine ET system in the heart. Induction of a myocardial ET system in the failing heart would be particularly intriguing on the basis of the growth regulatory properties of ET found in vitro. Very recently, induction of ppET-1 mRNA in some experimental models of CHF has been demonstrated (1, 22, 34). However, several important aspects of the putative myocardial ET system have not been studied. First, it is not known to what extent upregulation of the myocardial ET system is an early or late response to myocardial infarction and CHF, nor is it known how long the ET system remains elevated. Furthermore, it is not known how the different isoforms of ET are being regulated. The cells that synthesize ET in the myocardial tissue of failing hearts have not been identified.
addition, the localization of these cells in the ischemic area as well as in the nonischemic myocardial tissue needs to be established. To address these issues, we have used a rat model of CHF secondary to ligation of the left coronary artery (LCA). Hence, our first aim was to perform a time study of the regulation of ppET mRNA in CHF rats during the first 6 wk of healing and ventricular remodeling after induction of myocardial infarction. Thus, performing ribonuclease (RNase) protection assays, we have investigated how the three ET isoforms are regulated in the infarcted zone and in nonischemic myocardial tissue. Second, to identify and localize the cells containing immunoreactive ET-1, immunohistochemical analysis was performed.

**MATERIALS AND METHODS**

Animal preparation. We have employed a well-established rat model of CHF secondary to ligation of the LCA. Male Wistar rats (250–315 g) were subjected to ligation of the proximal portion of the LCA and infarction of the left ventricular free wall according to the method of Selye et al. (24) and the modifications of Pfeffer et al. (19). Briefly, the rats were anesthetized with 3% halothane, intubated, and maintained by a rodent ventilator with 30% O2-70% N2O-1% halothane. A left thoracotomy was made at the fifth intercostal space, and the pericardium was gently torn. The heart was exteriorized, and the proximal portion of the LCA was rapidly ligated by an intramural suture (6-0 silk). The heart was subsequently returned to its normal position, and LCA occlusion was ascertained by paling of the left ventricular free wall before the thoracotomy was closed. Except for ligation of the LCA, the sham-operated rats underwent the same procedure. Surgical mortality was ~40% in the rats that underwent ligation of the LCA. The procedure outlined above generally resulted in transmural infarction of the left ventricular free wall comprising 40–50% of the ventricular circumference (as judged macroscopically and after preparation of histological sections of the hearts). The animal experiments, procedures, and housing were approved by the Hospital Board for Animal Research in accordance with the Norwegian Council for Animal Research.

Study protocol. The purpose of the first series of experiments was to investigate the course of myocardial ppET mRNA expression at various time points after induction of myocardial infarction during the processes of healing and ventricular remodeling. In rats that underwent ligation of the LCA, only those with left ventricular end-diastolic pressure (LVEDP) >15 mmHg were considered to have CHF and included in the study. The rats were allocated to 1 of 10 groups. Rats undergoing myocardial infarction by ligation of the LCA and to be killed after 1, 2, 7, 21, or 42 days were allocated to groups indicated by the various time points (n = 4 in each group). Sham-operated rats were allocated to groups to be killed at the same time points (n = 3 in each group, except at day 21, where n = 2). Four unoperated rats were included in a separate control group.

The purpose of the second series of experiments was to perform immunohistochemical analysis of the distribution of immunoreactive ET in the myocardial tissue. For these studies, animals were allocated to two groups: animals undergoing myocardial infarction by ligation of the LCA and to be killed after 7 days (n = 5) or after 42 days (n = 6). Two unoperated rats were included as controls.

**Hemodynamic measurements and tissue sampling.** On the day of the experiment the rats were anesthetized, intubated, and maintained by a rodent ventilator with 30% O2-70% N2O-1% halothane, as described above. Arterial blood pressure and LVEDP were recorded by a 2-F micromanometer-tipped catheter (model SPR-407, Millar Instruments, Houston, TX) inserted through the right carotid artery and connected to an amplifier and recorder (model 2600 S, Gould Instruments, Cleveland, OH). The rats were killed by exsanguination of the heart. The hearts were sectioned into right ventricle, left ventricle, and atrium. The left ventricles were subsequently sectioned, with the infarcted area separated from the noninfarcted myocardium; care was taken to avoid contamination of viable myocardial tissue with necrotic tissue. The tissue was rinsed briefly in physiological saline, snap frozen in liquid nitrogen, and stored at –70°C.

Hearts from the rats in the second series of experiments were perfused with Bouin’s solution (2% paraformaldehyde and 0.2% picric acid in phosphate-buffered saline (PBS)) before the animals were killed. The hearts were removed and further fixed in Bouin’s solution, embedded in paraffin wax, and stored at 4°C.

cDNA cloning and plasmid vector constructs. A 390-base pair (bp) SacI/PvuII restriction fragment of rat ppET-1 cDNA was subcloned into the pBluescript SK (+) vector (Stratagene, La Jolla, CA) between the restriction sites Sal and PvuI. The vector was subsequently linearized at the SacI site and blunt ended with T4 DNA polymerase to allow the synthesis of an antisense RNA probe from the T7 promoter site in pBluescript by in vitro transcription using T7 RNA polymerase. This probe protects a 300-bp fragment of ppET-1 mRNA in the ribonuclease (RNase) protection assay.

By use of the cDNA sequence information published for the rat ppET-2 cDNA (GenBank EMBL), a 156-bp fragment of the ppET-2 cDNA (bp 1–156) was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) from mRNA isolated from the small intestine, subcloned into pBluescript SK(+), and sequenced by the dyeoxy chain termination technique. A unique EcoRI restriction site was designed at one end of the amplified cDNA fragment was used to linearize the vector for riboprobe synthesis with T3 RNA polymerase. Similarly, a 531-bp fragment of the rat ppET-3 cDNA (bp 168–699) was amplified from mRNA from rat lung by RT-PCR, subcloned into pBluescript SK(+) (18), and sequenced by DNA sequence analysis. The plasmid construct containing the rat ppET-3 cDNA fragment was linearized with a unique BbsI site within the ppET-3 cDNA to synthesize an antisense RNA strand that protects a 218-bp fragment of rat ppET-3 mRNA. Rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) CDNA and rat cardiac α-actin cDNA were also isolated by RT-PCR from rat cardiac tissues, subcloned, and characterized by DNA sequence analysis using the dyeoxy chain termination method. The 536-bp fragment of the GAPDH cDNA (bp 458–994) was subcloned into pBluescript. This vector construct was linearized with a unique EcoO109I site for riboprobe synthesis (protected fragment 128 bp). The 533-bp fragment of rat cardiac α-actin cDNA (bp 453–986) in pBluescript was linearized with BseR I before in vitro transcription of the probe (protected fragment 222 bp). All the linearized vector constructs were extracted twice with phenol/chloroform to eliminate contaminating RNase activities before in vitro transcription reactions.

**Synthesis of radiolabeled RNA probes.** Contiguously labeled antisense RNA probes for RNase protection assay were generated by in vitro transcription using T3 or T7 RNA polymerase and [α-32P]CTP (3,000 Ci/mmol; DuPont-New England Nuclear). Generally, linearized template DNA was transcribed in the presence of 40 mmol/l tris(hydroxymethyl) aminomethane-HCl, pH 7.5, 6 mmol/l MgCl2, 2 mmol/l sper
midine, 10 mmol/l NaCl, 10 mmol/l dithiothreitol, 500 µmol/l each of the unlabeled ribonucleotides (ATP, GTP, and UTP), 3 µmol/l (α-32P)CTP (specific activity ~800 Ci/µmol), 20 U of the RNase inhibitor RNasin (Promega), and 20 U of T3 or T7 RNA polymerase. After the transcription reaction was completed, the DNA template was removed by digestion with RQ1 deoxyribonuclease I. The radiolabeled RNA was subsequently extracted with phenol-chloroform and purified by polyacrylamide gel electrophoresis and electroelution. By use of the conditions described above, the RNA probes were labeled to high specific activity typically from 0.7 to 1.0 x 10^6 cpm/µg.

RNase protection assay. Total RNA from rat myocardial tissues was prepared by acid-phenol extraction in the presence of chaotropic salts (Trizol, Life Technologies, Gaithersburg, MD) and subsequent isopropanol-ethanol precipitation. Total RNA (40–50 µg/assay) was mixed with the antisense ET probe and GAPDH probe, coprecipitated with ethanol, and dissolved in hybridization buffer (80% deionized formamide, 100 mmol/l sodium citrate, 300 mmol/l sodium acetate, 1 mmol/l EDTA, pH 6.4). The hybridization was performed overnight in a 43°C incubator. The samples were subsequently treated with RNase (RNase A/RNase T) at 37°C for 30 min. This reaction was terminated with a commercial RNase inactivation/precipitation mixture (RPA II kit, Ambion). The precipitated RNA was dissolved in 80% formamide, 0.1% xylene cyanol, 0.1% bromphenol blue, and 2 mmol/l EDTA and subjected to electrophoresis on a 5% denaturing polyacrylamide gel. Autoradiography of the gel was performed using storage phosphor plates and a scanning phosphor imager (model 445 SI, Molecular Dynamics). Densitometric analysis of the bands was obtained with the Image-Master software package (Pharmacia Biotech). The assays were repeated three times to ensure reproducibility of the results. All time points of the time course studies were performed in the same assay. The data were subsequently corrected for variations in RNA loading by normalizing the ppET mRNA expression levels to the GAPDH mRNA levels.

Immunohistochemistry. Sections (10 µm) of paraffin-embedded heart tissue were made on a sliding microtome. The sections were subsequently dewaxed in xylene and rehydrated in descending concentrations of ethanol. To block the presence of endogenous peroxidase activity, the slides were preincubated with 0.3% hydrogen peroxide in methanol for 30 min at room temperature. Before they were immunostained, the sections were blocked with 3% normal goat serum. The sections were subsequently incubated with a rabbit polyclonal anti-ET-1 antisera (catalog no. IHC-6901, Peninsula, Belmont, CA) at a 1:1,000 dilution in 1.5% normal goat serum for 30 min at room temperature. After washing in PBS for 10 min, and immunostained with an avidin-biotin-peroxidase system (Vectastain Elite kit, Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions. Briefly, the sections were incubated with biotinylated goat anti-rabbit immunoglobulin G for 30 min at room temperature, washed in PBS, and incubated with the avidin-biotin-peroxidase complex for 30 min. After a final wash in PBS the slides were incubated with diaminobenzidine as the chromagen in a commercial metal-enhanced system (Pierce Chemical). The sections were counterstained with hematoxylin. Nonimmune normal rabbit serum or ET-1 antisera preabsorbed with biotinylated ET-1 and streptavidin-coated paramagnetic particles were used as negative control.

Statistical analysis. Values are means ± SE. Statistical analysis for intratreatment group variations was performed by one-way analysis of variance and post hoc analysis with Student-Newman-Keuls procedure for multiple comparisons. Between-treatment group variations were assessed by unpaired t-test. P < 0.05 was considered to be statistically significant.

RESULTS

Hemodynamic measurements. Mean arterial pressure (MAP) and LVEDP of all the rats in the study, grouped according to surgical procedure (ligation of LCA-induction of CHF or sham operation), are shown in Table 1. The hemodynamic measurements demonstrate substantial left ventricular dysfunction in the CHF rats. As shown in Table 1, MAP was significantly lower and LVEDP significantly increased in the CHF than in the sham-operated rats (P < 0.05).

ppET-1 mRNA expression. RNase protection assays were performed to investigate the regulation of ppET-1 mRNA levels at various time points after induction of myocardial infarction. In the RNase protection assay, low levels of ppET-1 mRNA could be confidently identified in myocardial tissues in unoperated rats. An increased expression of ppET-1 mRNA was observed in the left ventricle after induction of myocardial infarction, reaching maximal expression after 7 days (Fig. 1). The most dramatic upregulation was observed in the infarcted area of the failing left ventricle. In this region, ppET-1 mRNA levels were elevated 25-fold above the levels in the sham-operated rats 7 days after the induction of myocardial infarction (P < 0.05). Compared with the levels in the control group (unoperated rats), the increase was 48-fold (P < 0.05). The induction of ppET-1 mRNA expression was an early response after the infarction. A significant increase of ppET-1 mRNA was found as early as 1 day after ligation of the LCA. At this time, a sevenfold elevation of ppET-1 mRNA was found in the infarcted zone relative to the sham-operated group (P < 0.05). After 7 days, ppET-1 mRNA expression declined gradually and approached the expression levels in the sham-operated groups. However, at 42 days after induction of myocardial infarction, the ppET-1 mRNA levels in the infarcted zone were still 17-fold above the levels in the sham-operated group (P < 0.05, unpaired t-test).

The mRNA levels were also significantly elevated in the noninfarcted region of the left ventricle, albeit to a more moderate extent. The ppET-1 mRNA expression in the noninfarcted area displayed a transient profile very similar to that observed in the infarcted region. The expression levels of ppET-1 mRNA were significantly increased as early as 1 day after ligation of the LCA (3-fold, P < 0.05), reaching maximal levels 7 days after induction of myocardial infarction. At this time the ppET-1 mRNA levels were sixfold above the levels observed in the sham-operated group (P < 0.05). After

<table>
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<tr>
<th>Group</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>LVEDP, mmHg</th>
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<tr>
<td>Sham</td>
<td>14</td>
<td>118 ± 2</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>CHF</td>
<td>20</td>
<td>84 ± 3</td>
<td>19 ± 1</td>
</tr>
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Values are means ± SE; n, no. of rats. Sham, sham operated; CHF, congestive heart failure; MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure.
42 days, however, the ppET-1 mRNA level in the noninfarcted region was essentially similar to that in the sham-operated group.

We also investigated ppET-1 mRNA expression in myocardial tissue from the right ventricle, a region of the heart not subjected to ischemic damage after ligation of the LCA. Induction of ppET-1 mRNA expression was also observed in the right ventricle (Fig. 2). Two days after ligation of the LCA, ppET-1 mRNA levels displayed a twofold increase compared with the sham-operated group (P < 0.05). The ppET-1 mRNA expression reached maximal levels 7 days after induction of myocardial infarction, increasing fourfold above the levels measured in the sham-operated group (P < 0.05), and remained significantly elevated even after 42 days (P < 0.05).

GAPDH mRNA levels were measured to normalize for intra-assay variations in total RNA per sample. GAPDH mRNA levels have previously been shown not to be regulated during CHF. Nevertheless, we also measured cardiac α-actin mRNA expression levels, another mRNA species not supposed to be subject to regulation during CHF. We found that the two mRNA markers correlated closely in the ischemic region as well as in the nonischemic myocardial tissue. Thus GAPDH mRNA levels and cardiac α-actin mRNA levels served as equivalent markers of intra-assay variations in total RNA (data not shown).

ppET-2 and ppET-3 mRNA expression. Low levels of ppET-2 mRNA could be identified in the myocardial tissues by RNase protection assay (Fig. 3). However, the ppET-2 mRNA levels in myocardial tissue from CHF rats were not statistically different from the ppET-2 mRNA levels in the sham-operated animals. ppET-3 mRNA could clearly be identified in total RNA from rat lung using the RNase protection assay.
However, ppET-3 mRNA was not detectable in the left ventricular tissues; i.e., no bands migrating similar to ppET-3 in the lung could be confidently identified.

Immunohistochemistry. Immunohistochemical analysis of the failing myocardium revealed the presence of ET-1-like immunoreactivity (ET-1-ir) in the myocardial tissue of the left and right ventricles (Figs. 4 and 5). Figure 4, A–C, shows slightly stronger ET-1-ir in the nonischemic myocardium of CHF rats 7 and 42 days after induction of myocardial infarction than in the same region of control rats. Higher powers of magnification reveal that the cardiomyocytes contain ET-1-ir (Figs. 4D and 5B). However, in sections from hearts obtained 7 days after induction of myocardial infarction, a marked increase in the intensity of anti-ET-1 immunostaining was observed in the ischemic region (Fig. 4E). High-power photomicrographs of the same sections demonstrate heavy immunostaining of the granulation tissue replacing the necrotic myocardial cells (Fig. 5, C and D). In this area, macrophages and vascular endothelial cells displayed strong ET-1-ir. In addition, cells most likely representing proliferating fibroblasts and endothelial cells also showed intense immunostaining. At 42 days after the induction of myocardial infarction, the necrotic area was totally replaced by scar tissue. The fibrotic area displayed little ET-1-ir (Figs. 4F and 5E and F). Immunoreactivity in this area was localized to smaller vessels and a few remaining macrophages, especially at the border zones of the infarction. Figure 5E demonstrates ET-1-ir at the transition between viable myocardial tissue and fibrotic tissue. Note the robust staining of cardiomyocytes compared with the weak staining of the scar tissue. Sections of hearts incubated with nonimmune rabbit serum or preabsorbed antisemur (Fig. 5A) did not demonstrate immunostaining of any of the

Fig. 2. Ribonuclease protection assay of ppET-1 and GAPDH mRNA expression in right ventricle of sham-operated and CHF rats after ligation of left coronary artery. A total of 40 µg of total RNA were used in each hybridization reaction. A: autoradiograph was exposed (72 h for ppET-1 and GAPDH) and analyzed by phosphor imaging. B: densitometric analysis of scanning data. Data are ratios of levels of ppET-1 mRNA to levels of GAPDH mRNA. Values are means ± SE of 2 sham-operated rats (●) and 4 CHF rats (■). Statistical analysis was performed as described in Fig. 1 legend. *P < 0.05.

Fig. 3. Ribonuclease protection assay of ppET-2, ppET-3, and GAPDH mRNA expression in left ventricle of sham-operated (n = 2) and CHF rats (n = 4) after ligation of left coronary artery. A total of 50 µg of total RNA were used in each hybridization reaction. mRNA expression is shown in infarcted and noninfarcted zone of left ventricle. Autoradiograph was exposed for 72 h and analyzed by phosphor imaging. Colon and lung tissue were used as positive control to demonstrate presence of protected bands corresponding to expected position of ppET-2 and ppET-3 mRNA.
cellular elements of the myocardial tissue, demonstrating specificity of the ET-1 antiserum. The results from the immunohistochemical analysis are summarized in Table 2.

**DISCUSSION**

Specificity and time course of ppET regulation. The present study demonstrates for the first time the profile and time course of ppET mRNA regulation during CHF in rats. Substantial elevations of ppET-1 mRNA were measured in the infarcted myocardium as well as in the nonischemic myocardial tissue of the left and right ventricles. ppET-2 mRNA could be confidently identified in the myocardial tissue but did not appear to be regulated during the 42 days of observation. On the other hand, ppET-3 mRNA was below the limit of detection in myocardial tissue as analyzed by the RNase protection assay using 50 µg of total RNA in each hybridization reaction. However, Firth and Ratcliffe (8) demonstrated minute amounts of ppET-3
mRNA in rat myocardial tissue analyzed by RNase protection assay using 100 µg of total RNA in each sample reaction. We therefore conclude that although ppET-3 mRNA may be detectable in rat myocardial tissue, ppET-1 mRNA is by far the dominant isoform and the only isoform substantially regulated during CHF.

Forty-two days after induction of myocardial infarction, the necrotic area is totally replaced by fully differentiated scar tissue in the rat. Interestingly, the ppET-1 mRNA levels in the infarcted region showed a marked peak at 7 days after ligation of the LCA and subsequently declined progressively toward the end of the study. Thus the ppET-1 mRNA approached the levels in the sham-operated groups parallel to the differentiation of the scar tissue. A similar transient induction was observed in the nonischemic myocardial tissue of the left ventricle. Although the infarcted region of the left ventricular free wall was carefully excised and separated from the nonischemic myocardial tissue, it could be argued that the increase of ppET-1 mRNA in the viable region of the left ventricle...
was due to contamination with necrotic tissue. However, such a contamination could not account for the increased expression of ppET-1 mRNA measured in myocardial tissue from the right ventricle, since this region of the heart does not suffer ischemic damage after ligation of the LCA, and the induction of ppET-1 mRNA in right ventricular tissue did not exhibit a transient profile but remained elevated. Furthermore, the increase in the nonischemic region of the left ventricular myocardium was of the same magnitude as previously reported in other models of CHF (1, 34). Obviously, cell types involved in the increased expression of ppET-1 in the necrotic area must be different from those involved in the nonischemic, viable myocar-
dium. Thus we believe that an important distinction should be made regarding the induction of ppET-1 mRNA in these two regions of the failing myocardium.

The regulation of ppET-1 mRNA in the nonischemic myocardial tissue is supported by increased amounts of ET-1-ir in sections of this area. In this respect, our results diverge from those previously reported by Wei et al. (31). In the latter study, no significant differences in the activity of ET were found in myocardial tissues of normal and failing human hearts, as analyzed by immunohistochemistry and radioimmunoassay. To the extent that two different species can be compared, lack of proper normal control tissue from the right and left ventricles of the human heart might preclude the authors from identifying such modest changes as reported in the rat model. Our results are in agreement with a recently published report by Sakai et al. (22) demonstrating a fivefold increase in myocardial ET content 3 wk after induction of myocardial infarction in rats.

Myocardial localization of ET. Cardiomyocytes (21) as well as vascular endothelial cells (33) have been shown to synthesize ET-1 in vitro. The significant elevations of ppET-1 mRNA levels in the nonischemic myocardium of CHF rats demonstrated in the present study suggest that cardiomyocytes may also produce ET-1 in vivo. We recently reported data from in situ hybridization analysis demonstrating expression of ppET-1 mRNA in cardiomyocytes in vivo (29). Indeed, as demonstrated in the present study, cardiomyocytes also contain ET-1-ir. However, a number of other cell types not normally present in the myocardial tissue to any substantial degree may also produce ET-1 and may contribute to the increased expression during CHF. These include cells of the monocyte/macrophage system (21) and proliferating fibroblasts (28). Interestingly, in our study the most substantial increase in ppET-1 mRNA expression took place in the infarcted region of the left ventricular free wall. This increase was transient and correlated with the process of wound healing, suggesting that the cellular elements that constitute the granulation tissue contribute to the dramatic elevations in ppET-1 mRNA. This hypothesis is supported by another finding in this study demonstrating heavy immunostaining with anti-ET-1 antiserum in the granulation tissue. Macrophages, proliferating fibroblasts, and endothelial cells displayed strong immunoreactivity to ET-1. Macrophages have previously been shown to produce and release ET-1 after cytokine stimulation in vitro (21). Also, proliferating endothelial cells in vitro have been shown to synthesize large amounts of ET-1, implicating ET-1 in angiogenesis (9). This is of interest in myocardial infarction, because as many as 30% of the proliferating cells in the ischemic area have been reported to be endothelial cells (14). Furthermore, ET-1 has been shown to be mitogenic (28), to stimulate fibroblast proliferation and collagen synthesis (28), and to exert proinflammatory properties in vitro (21). These are all processes that are integral to the mechanism of wound healing.

Potential mechanism of myocardial ET regulation. An intriguing issue is which factor(s) induces ppET-1 mRNA expression in the failing myocardium. Induction of myocardial ppET-1 mRNA may be a general response to hemodynamic overload and cardiac failure. In support of this notion, cardiomyocytes in vitro have recently been shown to respond to mechanical stretch by releasing ET (32). Furthermore, ET-1 has been shown to exert potent inotropic effects on isolated myocardial preparations (13). Thus, to what extent could the increased expression of ppET-1 mRNA in the nonisch-
emiac myocardium contribute to maintain cardiac function in CHF rats? In a recent report by Sakai et al. (22), evidence was found that suggests a role for ET-1 in maintaining cardiac function during CHF. They reported that infusion of the ETA receptor antagonist BQ-123 in CHF rats decreased myocardial contractility. No effect on cardiac contractility was found after infusion of the same dose in normal rats. Thus they concluded that the increased levels of myocardial ET-1 found during CHF could contribute to maintain cardiac function during hemodynamic overload.

Neurohumoral mediators of ET induction may also be envisaged. During CHF the renin-angiotensin system and the sympathetic catecholamine system become activated (5, 26). Angiotensin II has been shown to induce ppET-1 mRNA expression and ET-1 synthesis in cardiomyocytes in vitro (11), and angiotensin II and catecholamines stimulate ppET-1 mRNA expression in endothelial cells in vitro (6). However, as recently reported by Sakai and colleagues (22), ppET-1 mRNA expression in the kidney was not induced during

Table 2. ET-1 immunostaining in normal and CHF hearts: summary of immunohistochemical analysis in Fig. 4

<table>
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<th>Region</th>
<th>Normal 7 days post-MI</th>
<th>CHF 42 days post-MI</th>
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<tbody>
<tr>
<td>LV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>+ (+)</td>
<td>++</td>
</tr>
<tr>
<td>RV</td>
<td>+ (+)</td>
<td>++</td>
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LV, left ventricle; RV, right ventricle; MI, myocardial infarction; ET-1, endothelin-1; +, weak staining intensity; ++, moderate staining intensity; ++++, strong staining intensity.
CHF, lending somewhat less credence to these neurohumoral factors. Thus it could be argued that the plasma levels of these hormones may not be sufficiently elevated to activate the cardiac receptor systems. Consequently, autocrine/paracrine mediators of ppET-1 mRNA induction should also be considered. A myocardial renin-angiotensin system has also been detected (4). Angiotensin II is another potent vasoconstrictor peptide that has been shown to act as a mitogen (23) as well as an inotropic agent (17). Angiotensinogen mRNA (15) has been shown to be induced in the noninfarcted left ventricle in CHF rats. Cytokines may also be involved in regulation of ppET-1 mRNA expression. Tumor necrosis factor-α (30) and transforming growth factor-β mRNA expression (27) are induced in failing hearts and have been shown to stimulate ET-1 production in vascular endothelial cells (3, 21).

In conclusion, the present study demonstrates a transient, isopeptide-specific induction of ppET-1 mRNA in the hearts of CHF rats. The most striking elevations were observed in the ischemic region. However, the induction of ppET-1 mRNA was not confined to this area. Significant upregulation of ppET-1 mRNA also took place in the nonischemic regions of the failing hearts. The pathophysiological role of ET-1 might be different in the ischemic area and in the nonischemic myocardium. In the ischemic region ET-1 most likely contributes to wound healing. The role of ET-1 in the nonischemic myocardium is less evident. A very important issue to be addressed in future studies is to what extent ET-1 is involved in the pathophysiological remodeling of the myocardial tissue associated with CHF. As previously mentioned, ET-1 has been shown to stimulate myocardial hypertrophy, fibroblast proliferation, and collagen synthesis in vitro. These are the key structural alterations that are known to impair the diastolic function of the heart in CHF. With ET antagonists available, these issues should be testable in experimental animal models like the CHF rats.

We thank Dr. Dag Sørensen for scientific advice and helpful discussions on the CHF rat model. The rat ppET-1 cDNA was kindly provided by Dr. Sawamura (University of Kyoto, Kyoto, Japan). These studies were supported by grants from the National Research Council, the Norwegian Council for Cardiovascular Diseases, Novo Nordic Foundation, the Blix Fund for the Promotion of Medical Science, Medinnova, and The National Hospital.

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Received 21 November 1996; accepted in final form 27 May 1997.

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