Regional expression of endothelin-1, ANP, IGF-1, and LV wall stress in the infarcted rat heart

JAN P. LOENNECHEN, ASBJØRN STØYLEN, VIDAR BEISVAG, ULRIK WISŁOFF, AND ØYVIND ELLINGSEN. Regional expression of endothelin-1, ANP, IGF-1, and LV wall stress in the infarcted rat heart. Am J Physiol Heart Circ Physiol 280: H2902–H2910, 2001.—We hypothesized that myocardial infarction induces regional and temporal differences in endothelin-1 (ET-1), atrial natriuretic peptide (ANP), and insulin-like growth factor-1 (IGF-1) gene expression that correlate with left ventricular (LV) wall stress. Echocardiography and LV pressure measurements were performed in coronary artery-ligated or sham-operated rats. Gene expression was measured by competitive RT-PCR in the infarct, border zone, and remote area and in regionally isolated cardiomyocytes. ET-1 and IGF-1 expression was highest in the infarcted myocardium, whereas ANP expression was highest in noninfarcted myocardium. For all genes, remote area expression was highest after 7 days. At 42 days, ANP maintained maximum expression, ET-1 decreased to 50% of peak levels, and IGF-1 was normalized. Cardiomyocyte expression followed the same pattern as in the myocardium except for a markedly lower IGF-1 expression. Diastolic wall stress was the best hemodynamic variable to predict ET-1 and ANP expression in the remote area. We conclude that ET-1, ANP, and IGF-1 are expressed in different patterns in the infarcted heart in relation to time, functional regions, cellular distribution, and mechanical load.

CARDIOACTIVE HORMONES have become prime targets in the search for better treatment of heart failure. Since its discovery, endothelin-1 (ET-1) has been identified as an important signaling substance in acute and chronic heart failure (30, 35). Stimulation of the growth hormone insulin-like growth factor-1 (IGF-1) axis has been beneficial in several experimental studies (7, 18), but the role of growth hormone and IGF-1 in treatment of congestive heart failure has not been settled (11, 26). Atrial natriuretic peptide (ANP) causes vasodilatation and natriuresis in heart failure and is a marker of cardiac hypertrophy. Promising results have been obtained by combined angiotensin-converting enzyme (ACE) and neutral endopeptidase inhibitors that reduce ANP degradation (19). Detailed knowledge of the induction and expression patterns of genes involved is required to understand the effects of these therapies. Although several studies have demonstrated substantial upregulation of ET-1, ANP, and IGF-1 after myocardial infarction (4, 17, 23, 27, 36–38), regional expression and correlation with mechanical load in different phases of remodeling have not been studied in vivo. In cultured cardiomyocytes and endothelial cells, ET-1 and ANP expression are induced by stretch and by stimulation with hormones and cytokines (10, 14, 41). However, it is not clear which mechanism is more important for induction in the intact heart. Regulation of IGF-1 expression has been studied in endothelial cells, fibroblasts, smooth muscle cells, and skeletal muscle (12, 13, 34, 39), but the regulatory mechanisms in cardiomyocytes are uncertain. Some studies suggest that gene expression varies significantly with time and within the infarcted left ventricle (17, 23, 36–38). Three different regions can be distinguished by functional, mechanical, and morphological properties: the noncontractile infarcted area, the contractile but dysfunctional border zone, and the initially unaffected remote area (2, 16, 21, 24). In early heart failure, myocardial expression of cytokines is markedly elevated in both infarcted and noninfarcted areas. In chronic failure, expression is highest in noninfarcted tissue (25). Our current concepts of cellular and regional distribution of ET-1, ANP, and IGF-1 expression are mainly based on immunohistochemistry and in situ hybridization without quantification (6, 23, 36, 38). In general, mRNA and protein expression follow similar regional and temporal patterns. This has been clearly demonstrated for ET-1 and ANP, whereas data for IGF-1 are sparse (6, 20, 23, 29, 31, 36, 38).

The aims of the present study were to assess regional differences of ET-1, ANP, and IGF-1 gene expression in the left ventricle during early and late postinfarction remodeling in hearts with small and large infarctions; to determine whether mechanical loading was associated with changes in expression; and to identify which
hemodynamic measurement best predicted these changes.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats weighing 240 ± 15 g (Møllergaards Breeding Centre; Lille Skensved, Denmark) were housed under a 12:12-h light-dark cycle with free access to a pellet rodent diet and tap water. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996).

Study design. The animals were randomized to either ligation of the left coronary artery or sham operation. Animals with large infarctions were euthanized on one of the following days: day 3 (n = 3), 5 (n = 3), 7 (n = 7), and 42 (n = 5); animals with small infarctions were euthanized on one of the following days: day 7 (n = 6) and 42 (n = 7); and sham-operated animals were euthanized on one of the following days: day 7 (n = 6) and 42 (n = 7) after surgery. Left ventricular (LV) pressure measurement, echocardiography, infarct size assessment, and cardiac tissue isolation were performed on all animals. Cardiomyocytes were isolated from a separate set of animals with large infarctions (n = 6) and from sham-operated animals (n = 6) 7 and 42 days after surgery.

Myocardial infarction. Animals were anesthetized with 3% halothane (Fluothane, Zeneca; Macclesfield, UK) in a 70% O2-30% N2O mixture and intubated and ventilated on a volume-controlled ventilator (model 655, Harvard Apparatus; Edenbridge, UK) with 1% halothane in a 70% O2-30% N2O mixture. After a left thoracotomy was performed, the pericardium was opened, and the left coronary artery was ligated with a polyester suture (Ethibond 6-0, needle RB-2, Ethicon; Norderstedt, Germany). Sham-operated animals were subjected to the same surgical procedures except the coronary artery ligation. The investigation conforms to the Guide for the Care and Use of Laboratory Animals.

LV pressure and echocardiography. Animals were anesthetized as previously described. Measurements were performed in a 0.5% halothane in 70% O2-30% N2O mixture anesthesia after a 5-min stabilization period. LV pressure was measured with a microtip catheter transducer, model SPR 407 2-F (Millar Instruments; Houston, TX), introduced through the right carotid artery. End-diastolic and peak systolic pressures were calculated as the mean of measurements of five consecutive pressure cycles.

Echocardiography was performed immediately after LV pressure measurements with a GE Vingmed Ultrasound System Five ultrasound scanner and with a 10-MHz linear array probe (GE Vingmed Ultrasound; Horten, Norway). After the rat’s chest was carefully shaved, the transducer was placed gently against the chest. Diastolic and systolic LV wall thickness and cavity diameters were calculated as the mean of measurements in five consecutive cardiac cycles in M-mode long-axis recordings following the recommendations of the American Society of Echocardiography (28).

Infarct size estimation and tissue sampling. After echocardiographic recordings were made, the heart was excised and sectioned into atria and ventricles, including the interventricular septum in the left ventricle. The left ventricle was cut open from base to apex along the middle of the septum, pinned to a plate with endocardium upward, and photographed (Fujicolor Reala, 100 ASA). Infarcted and noninfarcted LV areas were determined from the photo. The excised heart was kept in ice-cold sterile water and handled with autoclaved instruments. The left ventricle was divided into the infarcted area, border zone (0–2 mm outside the infarct), and the remote area (Fig. 1). Five hematoxylin, erytosin, and saffron-stained specimens demonstrated adequate detection of border between infarction and border zone with 2–6% infarct tissue in the border zone. All tissue specimens were frozen in liquid nitrogen within 15 min after excision and kept at −80°C.

Cardiomyocyte isolation. Animals were anesthetized with diethyl ether and heparinized (0.2 ml heparin 1,000 IU/ml iv, Novo Nordisk; Copenhagen, Denmark). To balance day-to-day variation, one infarcted and one sham-operated heart were isolated each day. Hearts were rapidly removed from the animals and connected to an aortic cannula of a standard Langendorff retrograde perfusion system. Cardiomyocytes were isolated using collagenase as previously described (15). During the isolation procedure, LV tissue was cut into infarcted, border zone (0–2 mm outside the infarct), and remote areas. Total number of cells and fraction of rod-shaped cells without morphological alterations were counted. Lysis buffer (2 ml per 106 cells, Dynal; Oslo, Norway) was added, and the lysate thoroughly mixed before being frozen at −80°C.

Mean numbers of isolated cells were 3.4 ± 0.6 106 in left ventricles of sham-operated animals, 1.2 ± 0.1 106 in border zone, and 0.6 ± 0.1 106 in remote area and were related to the different weights of the sampled tissues. The yields of rod-shaped cells were 57 ± 6% in left ventricles of sham-operated animals, 41 ± 4% in border zone, and 41 ± 6% in remote area.

mRNA isolation. Myocardial mRNA was isolated with Dynabeads Oligo(dT)25 (Dynal) according to manufacturer's instructions. Myocardial tissue (50 mg) was homogenized in 1 ml of cold lysis-binding buffer with an Ultra-Turrax T25 (Janke & Kunkel IKA-Labortechnik; Staufen, Germany), and the supernatant was mixed with 250 µl of Dynabeads Oligo(dT)25. Lysate (1 ml) from isolated cardiomyocytes was mixed with 250 µl of Dynabeads Oligo(dT)25. After five washing procedures, the mRNA was eluted in 25 µl of elution buffer per 50 mg of tissue or per 1 × 106 cardiomyocytes. Eluates were frozen at −80°C.

![Fig. 1. Short-axis section of heart with large myocardial infarction illustrating right ventricle (RV), left ventricle (LV), infarction (INF), border zone (BZ) 2-mm outside infarction, and remote area (REM).](http://aphelium.medphys.org/)
Competitive RT-PCR. Competitive RT-PCR was performed in a Perkin-Elmer GeneAmp 2400 PCR system using rTth DNA polymerase (Perkin-Elmer/Roche Molecular Systems; Bronchburg, NJ) according to manufacturer’s instructions. Reverse transcription for ET-1, ANP, and IGF-1 was performed at 50°C for 5 min and 61°C for 40 min followed by 3 min denaturation at 95°C.

ET-1 PCR amplification was performed for 27 cycles at 94°C for 20 s, 55°C for 15 s, and 72°C for 30 s. For each sample of ET-1 mRNA, RT-PCR was performed in three tubes, each with the same amount of sample mRNA together with 0.05, 0.15, and 0.45 attomol of DNA competitor, respectively. The DNA competitor consisted of the 313-bp endothelin gene sequence targeted by the PCR primers listed below, including a 149-bp coding sequence and an intercalated 164-bp intron. The DNA competitor was cloned into a pCR2 vector and transformed into an Escherichia coli strain using Original TA Cloning Kit (Invitrogen; Groningen, Netherlands). Prepro-ET-1-specific sense and antisense primer sequences were 5′-GCTGTCACCCCAAAGACAGC-3′ (bp 519–538) and 5′-CAGCTGCTGATAGATACACTTC-3′. Sequencing of RT-PCR products confirmed amplification of the expected templates.

ANP PCR amplification was performed for 27 cycles at 94°C for 20 s, 55°C for 15 s, and 72°C for 30 s. To quantify one sample of ANP mRNA, RT-PCR was performed in three tubes, each with the same amount of mRNA together with 0.11, 0.44, and 1.76 fmol of DNA competitor, respectively. The DNA competitor consisted of the 472-bp fragment, including a 149-bp coding sequence and an intercalated 164-bp intron. The DNA competitor was cloned into a pCR2 vector and transformed in an INV vector and transformed in an INV strain of Escherichia coli, and sequenced. The DNA competitor was cloned into a pCR2 vector and transformed in an INV strain of Escherichia coli, and sequenced. The DNA competitor was cloned into a pCR2 vector and transformed in an INV strain of Escherichia coli, and sequenced.

ET-1 and ANP, the logarithm of the sample-to-competitor fluorescence ratio was plotted against the logarithm of the known amount of competitor added to each tube, resulting in a linear relationship. The line of best fit was calculated using least sum of squares method. Sample mRNA content was calculated by setting the logarithm of sample-to-competitor fluorescence ratio to 0 in the linear equation. For IGF-1, a standard curve was calculated for each set of PCR by plotting the logarithm of the sample to standard fluorescence ratio against the six amounts of IGF-1 cDNA added to tubes. IGF-1 mRNA content in each sample was assessed by fitting the logarithm of the sample-to-competitor fluorescence ratio into the standard curve equation. To assess intraassay variation, six samples from the same mRNA isolation were analyzed in parallel. Reproducibility was good for ET-1 and IGF-1 (coefficients of variation 0.11 and 0.19, respectively). ANP reproducibility was good at high levels (coefficient of variation 0.18). For ANP levels in controls, which were near the detection limit, the coefficient of variation was 0.38, implying that the concentrations in normal hearts may not be as accurately detected as in failing hearts. However, these inaccuracies would not affect comparisons with failing hearts because those concentrations were 10–45 fold higher. Accuracy of the mRNA analysis for ET-1, ANF, and IGF-1 is shown in Fig. 2.

Wall stress estimation. Wall stress in the remote area was estimated using an equation by Falsetti and Sandler (8, 32): Meridional wall stress \( \sigma_m = PR/2h(1 + h/2R) \), where \( P \) is LV pressure, \( R_i \) is LV inner radius, and \( h \) is wall thickness. This expression gives a representative estimate of wall stress in the remote area provided that wall thickness is fairly constant, the tissue is fairly homogenous within the area analyzed, and there is axisymmetry of the radius. Analysis of long- and short-axis recordings confirmed a more spherical LV shape in failing hearts than in control hearts. The ratio of diastolic anteroposterior versus laterolateral diameter was 1.15 ± 0.13 (means ± SD) for control hearts and 1.03 ± 0.05 and 1.03 ± 0.06 in hearts with large infarctions after 7 and 42 days, respectively. Corresponding results for long-axis
versus anteroposterior diameter were 1.55 ± 0.12, 1.19 ± 0.09, and 1.12 ± 0.06. These data indicate that the inaccuracy in diastolic wall stress estimates due to nonsphericity was smallest in hearts with large infarctions.

Statistical analysis. Differences in gene expression among groups were analyzed with Friedman test for related observations and Kruskal-Wallis test for unrelated data, applying appropriate procedures for multiple comparisons (5). The reported association of gene expression with hemodynamic measurements was analyzed by forward stepwise multiple linear regression (1). Similar results were obtained using ranks (data not shown). P < 0.05 was considered statistically significant.

RESULTS

Gene expression. Myocardial infarction markedly induced LV ET-1 expression with increased ET-1 mRNA in all regions of the infarcted left ventricle at all examined time points after small and large infarctions (Fig. 3, A and D). Expression was time and region dependent with a marked peak in all regions at day 7, with ET-1 mRNA 19 times control in the infarct, 10 times control in the border zone, and 4 times control in the remote area after large infarctions. The regional differences were reproduced in cardiomyocytes isolated 7 and 42 days after large myocardial infarctions, as shown in Fig. 3G.

Myocardial expression of ANP increased in a different pattern (Fig. 3, B and E). ANP mRNA was strongly induced in all regions of the infarcted left ventricle with highest expression in the border zone and remote area. After large infarctions, there was no distinctive peak but a sustained high mRNA level at days 7 and 42 in the border zone (37 and 47 times control) and remote area (39 and 38 times control). In isolated cardiomyocytes, the high level of ANP mRNA was reproduced (Fig. 3H). However, regional differences changed during remodeling. After 7 days ANP expression was highest in cardiomyocytes from the border zone, whereas after 42 days it was highest in cardiomyocytes from the remote area.

IGF-1 gene expression was transient, with high levels in the early phase and low levels in the late phase after infarction. IGF-1 mRNA levels were highest in all regions at 7 days (Fig. 3, C and F) with 14 times control in the infarct, 8 times control in the border zone, and 3 times control in the remote area after large infarctions.

Fig. 3. ET-1, ANP, and IGF-1 mRNA expression measured by competitive RT-PCR in myocardial specimens from sham-operated rats (Sham), infarct, border zone, and remote area after small myocardial infarctions (A–C) and after large myocardial infarctions (D–F). G–I: gene expression in cardiomyocytes isolated from border zone and remote areas of hearts with large myocardial infarctions and left ventricles of sham-operated controls. Values are means ± SE. *P < 0.05 vs. sham; †P < 0.05 vs. remote area; ‡P < 0.05 vs. border zone; §P < 0.05 vs. large infarctions.
After 42 days, IGF-1 expression was normalized in the remote area but still moderately upregulated in the border zone and infarct. Expression in isolated cardiomyocytes after infarction differed from that in myocardial tissue (Fig. 3I), with considerably lower IGF-1 mRNA levels compared with control. After 7 days there was only a moderate upregulation in the remote area but still moderately upregulated in the border zone (1.4 and 2.4 times control), whereas there was no significant upregulation after 42 days.

Comparison of gene expression after small and large infarctions revealed different results for ET-1, ANP, and IGF-1. Large and small infarctions induced similar patterns of IGF-1 expression in the left ventricle. However, large infarctions induced higher ET-1 and ANP expression in the remote area than did small infarctions.

Echocardiography, LV pressure, weights, and infarct size. As shown in Table 1, infarct size was a strong predictor for heart failure and remodeling. Large myocardial infarctions lead to a progressive eccentric hypertrophy of the left ventricle with 70% increase in diastolic and 151% increase in systolic LV diameter at day 42. After small infarctions, the corresponding dilatation was 35% and 86%, without statistically significant progression from day 7 to day 42. The noninfarcted cardiac chambers hypertrophied markedly after large infarctions (Table 2). Left atrial hypertrophy was observed 3 days after infarction and increased to 240% after 42 days, whereas right atria and ventricles hypertrophied with 250% and 123% at 40 days, respectively. Mechanical load increased progressively after large infarctions, with a ninefold increase in diastolic and a threefold increase in systolic posterior wall stress. Infarct size, body weights, and chamber weights are presented in Table 2.

Association of gene expression and mechanical forces. End-diastolic posterior wall stress was the best hemodynamic predictor of myocardial ET-1 and ANP expression in the remote area both at days 7 and 42 (Fig. 4). When it was included in a stepwise multiple linear regression model together with systolic wall stress, peak systolic pressure, LV end-diastolic pressure, diastolic LV diameter, systolic LV diameter, and posterior wall thickening, none of the other variables added significantly to the linear regression model. End-diastolic wall stress did not correlate with ET-1 and ANP expression in the border zone or infarcted area except for ANP in the border zone at day 42 (r = 0.77, P = 0.003). For myocardial IGF-1 expression, there was no statistically significant correlation with the hemodynamic parameters analyzed.

DISCUSSION

This study is the first to present that the cardioactive peptides ET-1, ANP, and IGF-1 are expressed in dis-

---

**Table 1. Hemodynamic measurements of rats with myocardial infarction and sham-operated controls**

<table>
<thead>
<tr>
<th></th>
<th>Large Infarctions</th>
<th>Small Infarctions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days</td>
<td>5 days</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>440 ± 9</td>
<td>388 ± 20*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>9 ± 1</td>
<td>16 ± 2*</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>147 ± 2</td>
<td>115 ± 2*</td>
</tr>
<tr>
<td>LVDd, mm</td>
<td>6.1 ± 0.2</td>
<td>7.9 ± 0.1*</td>
</tr>
<tr>
<td>LVDs, mm</td>
<td>3.7 ± 0.2</td>
<td>6.8 ± 0.2*</td>
</tr>
<tr>
<td>FS, %</td>
<td>40 ± 1</td>
<td>14 ± 1*</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>2.1 ± 0.1</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>LVPWs, mm</td>
<td>3.0 ± 0.1</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>PWSd, kdyn/cm²</td>
<td>7 ± 1.1</td>
<td>17 ± 4.0*</td>
</tr>
<tr>
<td>PWSs, kdyn/cm²</td>
<td>34 ± 2.8</td>
<td>79 ± 19.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. HR, heart rate; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular peak systolic pressure; LVDd, left ventricular diastolic diameter; LVDs, left ventricular systolic diameter; FS, fractional shortening; LVPWd, left ventricular posterior wall diastolic thickness; LVPWs, left ventricular posterior wall systolic thickness; PWSd, end-diastolic posterior wall stress; PWSs, systolic posterior wall stress. *P < 0.05 vs. sham; †P < 0.05 vs. large infarctions.

**Table 2. Body weight-corrected cardiac weights of rats with myocardial infarction and sham-operated controls**

<table>
<thead>
<tr>
<th></th>
<th>Large Infarctions</th>
<th>Small Infarctions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days</td>
<td>5 days</td>
</tr>
<tr>
<td>BW, g</td>
<td>259 ± 5</td>
<td>247 ± 4</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td>39 ± 1</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>Infarct, mg/g</td>
<td>0.71 ± 0.08</td>
<td>0.40 ± 0.02</td>
</tr>
<tr>
<td>LV/BW, mg/g</td>
<td>2.54 ± 0.06</td>
<td>2.54 ± 0.04</td>
</tr>
<tr>
<td>RV/BW, mg/g</td>
<td>0.71 ± 0.03</td>
<td>0.88 ± 0.06*</td>
</tr>
<tr>
<td>LA/BW, mg/g</td>
<td>0.10 ± 0.01</td>
<td>0.15 ± 0.03*</td>
</tr>
<tr>
<td>RA/BW, mg/g</td>
<td>0.10 ± 0.01</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals. BW, body weight; LV, left ventricle; RA, right ventricle; LA, left atrium; RA, right atrium. *P < 0.05 vs. sham; †P < 0.05 vs. large infarctions.
tinctly different patterns after myocardial infarction. Implications of the detected differences in regional and cellular distribution, temporal changes, and relation to mechanical forces are discussed below.

ET-1 gene expression. In the present study, we confirmed previous observations by immunohistochemistry and in situ hybridization, demonstrating highest expression in granulation tissue in infarcted area and in the adjacent myocardium (23, 38). Whether cardiomyocytes contribute to increased ET-1 expression after myocardial infarction has been uncertain. We found a similar pattern of regional ET-1 expression in isolated cardiomyocytes and myocardium, demonstrating that cardiomyocytes contribute to the increased ET-1 expression in the viable myocardium postinfarction and to the higher level in the border zone than in the remote area. After 7 days, border zone ET-1 expression increased 4-fold in isolated cardiomyocytes and 10-fold in myocardium, suggesting higher expression in nonmyocytes than in cardiomyocytes. This might be due to induction of ET-1 expression in nonmyocytes and higher content of nonmyocytes in the border zone.

Examination of hematoxylin, erytrosin, and saffron-stained samples revealed scarce islets of scar tissue (2–6%) in the border zone adjacent to the infarct. Immunohistochemistry has suggested high levels of ET-1 in macrophages, fibroblasts, endothelial cells, and remaining cardiomyocytes, and in situ hybridization indicates a high level of mRNA in granulation tissue (23, 38).

Peak ET-1 expression concurs with maximum myocardial infiltration of chronic inflammatory cells (9, 25) and peak plasma and myocardial levels of tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and transforming growth factor-β (TGF-β) (25, 42), which are known inducers of ET-1 expression, and suggest a role of ET-1 in myocardial inflammation and repair during early remodeling. This is supported by the detrimental effects of ET-1 antagonism early postinfarction with impaired scar healing and increased infarct thinning (22).

Stretch induces ET-1 in isolated cardiomyocytes (41) and cultured endothelial cells (40), but the role of mechanical load on myocardial ET-1 expression in vivo
has been unclear. The present study is the first to document a significant correlation between diastolic wall stress and ET-1 expression in the remote area of the infarcted heart. The results suggest that stretching of the myocardium during diastole contributes significantly to the induction of ET-1 in both acute and chronic postinfarction heart failure.

ANP gene expression. The persistent high levels and regional distribution of ANP expression quantified in the present study agree with results obtained by immunohistochemistry in humans and rats, demonstrating high levels in areas adjacent to the infarct, in the subendocardium of the infarct, and in viable cardiomyocytes within the infarct (17, 36).

As expected, but not previously reported, ANP expression correlated strongly with end-diastolic wall stress in the remote area. This observation supports the current opinion that stretch is the most important inducer of myocardial expression in vivo of ANP and other proteins such as β-cardiac myosin heavy chain (3, 33). Remote area and border zone ANP levels did not increase significantly from day 7 to day 42, despite the 71% increase in diastolic wall stress. This might be because the ANP response to mechanical load has already reached a maximum at day 7, or because other stimuli inducing ANP has declined, such as paracrine effects of ET-1 and other local mediators.

IGF-1 gene expression. Which types of cells are the main contributors to myocardial IGF-1 mRNA has been unsettled. Seven days after infarction, IGF-1 expression in isolated cardiomyocytes increased 1.4-fold in the remote area and 2.4-fold in the border zone, which is comparable to the results of Cheng and co-workers (4). However, in myocardial tissue, IGF-1 expression increased threefold in the remote area and eightfold in the border zone, suggesting that nonmyocytes are the main source of myocardial IGF-1 mRNA postinfarction. This is supported by the results of Dean and co-workers (6), who found IGF-1 mRNA predominantly in the infarct and the peri-infarct area, located in blood vessels, endocardium, and cellular infiltrates, with little expression in the hypertrophied remote area. The regulatory mechanisms of IGF-1 expression in cardiomyocytes are largely unknown. In endothelial cells, IGF-1 is downregulated by hypoxia (39) and upregulated by IL-1 (12), whereas expression is stimulated by stretch in isolated vascular smooth muscle cells (34) and skeletal muscle cells (13). In the present study, IGF-1 expression was normal in the remote area despite a ninefold increase in diastolic wall stress, and there was no correlation between myocardial IGF-1 mRNA and mechanical load. These findings, which have not been previously reported, suggest that stretch alone is not a major stimulus for myocardial IGF-1 expression after myocardial infarction in rats.

The pattern of IGF-1 expression found in the present study is very similar to those of TNF-α, IL-1β, IL-6, and TGF-β found in cardiac nonmyocytes in an identical model (42), suggesting similar regulatory mechanisms for IGF-1 and cytokines participating in the inflammatory processes. A role of IGF-1 in inflammation and repair agrees well with the transient increase in myocardial IGF-1 expression, the higher expression in cardiac nonmyocytes, and the lack of association to mechanical load after myocardial infarction.

Patterns of gene expression. The differential patterns of myocardial ET-1, ANP, and IGF-1 expression demonstrated in the present study reveal differing regulatory responses to mechanical forces and chemical stimuli. ANP expression was closest related to mechanical forces, and IGF-1 expression increased simultaneously with the highest chemical influences, whereas ET-1 expression was associated with both stimuli.

The results also confirm distinct differences in gene expression between the border zone and remote area. These differences might be caused by regional differences in wall stress. Using a finite element model, Bogen et al. (2) estimated 3.7 times higher systolic wall stress in the border zone than in the remote area of the acute infarcted canine left ventricle. However, border zone wall stress was reduced as infarct stiffness increased with time, suggesting time-dependent influences of mechanical load on border zone gene expression. Gene expression of isolated cardiomyocytes support this assumption, because ANP expression was highest in the border zone after 7 days but highest in the remote area after 42 days.

The observed patterns of gene expression agree well with the concepts of an early and a late phase in postinfarction remodeling. The general high expression of all genes after 7 days is consistent with high levels of several inducing factors, including mechanical forces, hormones, and paracrine factors induced by inflammation and repair in infarcted areas. After 42 days, gene expression had decreased in all regions except for ANP in hearts with large infarctions, consistent with abating inflammation but sustained high mechanical load. There was, however, still high expression of ET-1, ANP, and IGF-1 in the infarcted area and border zone after 42 days. The infarct and adjacent myocardium might therefore exert paracrine and humoral activity even in chronic postinfarction heart failure.

Hemodynamic measurements. The study results demonstrate a consistent association between size of myocardial infarction and degree of cardiac remodeling, reduced cardiac function, and increased LV mechanical load. Most prominent was the marked increase in end-diastolic wall stress after large infarctions. Diastolic wall stress estimates the diastolic stretching forces working per area of myocardium. Consequently, in postinfarction failure increased stretching forces act on the noninfarcted myocardium during diastole when contracting myofibrils do not protect the tissue. The correlation between diastolic wall stress and gene expression of ET-1 and ANP suggests that diastolic myocardial stretch is an important stimulus to postinfarction remodeling.

In conclusion, the present study documents that ET-1, ANP, and IGF-1 are expressed in markedly different patterns in the infarcted heart in relation to time, functional regions, cellular distribution, and me-
mechanical load. Diastolic wall stress is associated with a substantial increase in ANP expression and a moderate increase in ET-1 expression in the viable myocardium during acute and chronic postinfarction failure, suggesting that stretching in diastole induces these genes in vivo.

This work was supported by grants from the Norwegian Research Council, Sintef Unimed, the Langfeldt Fund for Research in Physiology and Medical Biochemistry, the Blix Fund for the Promotion of Medical Science, and the Funds for Cardiovascular and Medical Research at Trondheim University Hospital. J. P. Lønnechen was the recipient of a research fellowship from the National Council on Cardiovascular Diseases.

REFERENCES

31. Sakai S, Miyauchi T, Sakurai T, Kasuya Y, Ibara M, Yamaguchi I, Goto K, and Sugishita Y. Endogenous endothelin-1 participates in the maintenance of cardiac function in rats
with congestive heart failure. Marked increase in endothelin-1 production in the failing heart (See comments). Circulation 93: 1214–1222, 1996.


