Endothelin-1 Contributes to the Frank-Starling Response in Hypertrophic Rat Hearts

Jarkko Piuhola, István Szokodi, Pietari Kinnunen, Mika Ilves, Rudolf deChâtel, Olli Vuolteenaho, Heikki Ruskoaho

Abstract—Endothelin-1 is involved in mechanical load–induced cardiac growth processes; it also has effects on contractility. The interaction of endothelin-1 and the Frank-Starling response is unknown. The present study aimed to characterize the role of endothelin-1 in the regulation of the Frank-Starling response, one of the major mechanisms regulating cardiac contractile force, in both normal and hypertrophied hearts. Nontransgenic rat hearts and hypertrophic hearts of hypertensive double transgenic rats harboring human angiotensinogen and renin genes were studied in a Langendorff isolated heart setup with a liquid-filled balloon inside the left ventricle used to measure contractile parameters. The rats were studied at compensated phase, before showing any signs of heart failure. Compensated hypertrophy in double transgenic rat hearts resulted in improved contractility at a given level of preload when compared with nontransgenic rat hearts. Hearts of both rat lines showed preserved Frank-Starling responses, that is, increased contractile function in response to increased end-diastolic pressure. The mixed endothelin A/B receptor antagonist bosentan attenuated the Frank-Starling response by 53% (P<0.01) in the double transgenic hearts but not in nontransgenic hearts. The diastolic parameters remained unaffected. The left ventricles of the double transgenic rat hearts showed an 82% higher level of endothelin type A receptor mRNA and a 25% higher level of immunoreactive endothelin-1 compared with nontransgenic rat hearts. The type 1 angiotensin II receptor antagonist CV-11974 had no significant effect on contractile function in response to load in either strain. These results show that endogenous endothelin-1 contributes to the Frank-Starling response in hypertrophied rat hearts by affecting systolic performance.

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Key Words: angiotensin II ■ endothelin ■ hypertrophy ■ rats, transgenic ■ stress, mechanical
status. This may reflect a role of ET-1 in the maintenance of cardiac contractile function in failing hearts, as suggested by a previous study with experimental CHF.

ET-1 has a direct positive inotropic effect on the heart in vitro, whereas the effects of Ang II are more complex. ET-1 also increases the contractile efficacy in isolated rat hearts. However, it has not been established whether ET-1 contributes to the Frank-Starling response. To test this hypothesis, we studied the regulation of the Frank-Starling mechanism in isolated perfused hearts of both normotensive, nontransgenic (NTG) Sprague-Dawley rats and hypertensive dTG rats harboring human renin and angiotensinogen genes by using the mixed ET<sub>1</sub> receptor antagonist bosentan. Furthermore, the angiotensin type I (AT<sub>1</sub>) receptor antagonist CV-11974 was used to study whether Ang II is involved in the regulation of the Frank-Starling response. The dTG rat model was chosen because both ET-1 and Ang II contribute to the end-organ damage in this rat strain.

Methods

Animals

Experiments were conducted in 7- to 8-week-old male dTG rats harboring both the human angiotensinogen and human renin genes (n=72) and in age-matched normotensive NTG rats (n=68). None of the dTG rats showed any clinical signs of heart failure, including asciates or pleural effusion. The dTG and NTG rat strains were obtained from Mollegaard Breeding Center Deutschland GmbH (Schingenwalde, Germany) and RCC Ltd (Itingen, Switzerland). The rats were kept in plastic cages with free access to tap water and normal rat chow. The Animal Use and Care Committee of the University of Oulu approved the experimental design.

Drugs

The following drugs were used: bosentan, CV-11974, GF-109203X, and zoniporide. Bosentan was generously supplied by Dr Martine Clozel, Hoffmann-La Roche Ltd (Basel, Switzerland) and Actelion Ltd (Basel, Switzerland), CV-11974 by Dr Hajime Toguchi, Takeda Pharmaceuticals (Osaka, Japan), and zoniporide by Dr Ross Tracey, Pfizer Inc (Groton, Conn). GF-109203X was purchased from Calbiochem. Et-1 also increases the contractile efficacy in isolated rat hearts, 17,18 whereas the effects of Ang II are more complex. 19 ET-1 and Ang II contribute to the end-organ damage in hypertrophied dTG hearts, the V<sub>max</sub> was increased in 10-fold higher in dTG than in NTG rat ventricles, respectively (P<0.001 and P<0.05, Figure 1).

Adequate Frank-Starling responses were noted in both NTG and dTG hearts when the intraventricular balloon volume was increased. The dTG rat hearts showed augmented contractile function in comparison with NTG rat hearts at a given level of preload (LVEDP=3 mm Hg, Table). Also at corresponding operating points of the Frank-Starling curve, 50% of the V<sub>max</sub>, the contractile function was increased in dTG rat hearts (Table and Figure 2). Because of the geometrical changes in hypertrophied dTG hearts, the V<sub>max</sub> was 186±18 μL compared with 234±22 μL in NTG hearts (P=0.05).

Results

Cardiac Function in NTG and dTG Rat Lines

The dTG rat line is characterized by high blood pressure and remarkable LVH. The body weights of NTG and dTG rats were 219±4 and 204±3 g, respectively (P<0.001). The left ventricles of NTG and dTG rats weighed 618±10 and 891±17 mg, respectively, resulting in a left ventricle/body weight ratio of 2.83±0.03 and 4.37±0.06 mg/g (P<0.0001). In addition, the alterations in the cardiac gene expression were analyzed by measuring the expression levels of ANP, a hallmark of LVH, and c-fos, a proto-oncogene implicated in alteration of gene expression in response to mechanical stimulus. The ANP and c-fos mRNA levels were 9.2- and 1.6-fold higher in dTG than in NTG rat ventricles, respectively (P<0.001 and P<0.05, Figure 1).
Role of ET-1 and Ang II in the Frank-Starling Response

To determine the role of ET-1 and Ang II in regulation of the Frank-Starling response, bosentan (mixed ET<sub>A/B</sub> antagonist) and CV-11974 (AT<sub>1</sub> receptor antagonist) were used in Langendorff-perfused NTG and dTG rat hearts during the increment of intraventricular balloon volume. In NTG rat hearts, the antagonists had no effect on the Frank-Starling response (n = 8 and n = 5, respectively, Figure 3). In contrast, the increase in DP and dP/dt<sub>max</sub> was attenuated by 53% and 61% (P < 0.01 for both parameters, n = 8), respectively, in the presence of bosentan in dTG rat hearts (Figure 3). CV-11974 did not have a significant effect (n = 7, Figure 3). Bosentan and CV-11974 did not influence the diastolic stiffness, as shown by a similar increase in LVEDP with increasing balloon volumes. Also, minimal negative derivatives of intraventricular pressure, dP/dt<sub>min</sub>, were unaffected by the antagonists (Figure 4), suggesting that alterations seen in the Frank-Starling response are related to systolic rather than diastolic function. Bosentan or CV-11974 had no effect on contractile parameters in control-perfused hearts (LVEDP = 3 mm Hg) (DP change: dTG, -3.7 ± 3.8% and 0 ± 2.7%; NTG, -1.6 ± 2.0% and -3.1 ± 2.8% during the 30-minute infusion with bosentan and CV-11974, respective-

### Characteristics of dTG and NTG Rats

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<th>Characteristics of dTG and NTG Rats</th>
<th>NTG Rats</th>
<th>dTG Rats</th>
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<tr>
<td><strong>Hemodynamics at LVEDP=3 mm Hg</strong></td>
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<tr>
<td>DP, mm Hg</td>
<td>29.6 ± 1.8</td>
<td>44.5 ± 1.9§</td>
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<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt;, mm Hg/s</td>
<td>1216 ± 61</td>
<td>1578 ± 69‡</td>
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<tr>
<td>dP/dt&lt;sub&gt;min&lt;/sub&gt;, mm Hg/s</td>
<td>-684 ± 34</td>
<td>-899 ± 34§</td>
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<td><strong>Hemodynamics at V&lt;sub&gt;b&lt;/sub&gt;=50% V&lt;sub&gt;max&lt;/sub&gt;</strong></td>
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<tr>
<td>DP, mm Hg</td>
<td>38.8 ± 2.8</td>
<td>59.8 ± 4.1‡</td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt;, mm Hg/s</td>
<td>1450 ± 102</td>
<td>1934 ± 151*</td>
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<tr>
<td>dP/dt&lt;sub&gt;min&lt;/sub&gt;, mm Hg/s</td>
<td>820 ± 53</td>
<td>1105 ± 79*</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>11.9 ± 1.8</td>
<td>23.7 ± 4.4*</td>
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Data are mean ± SEM. LVEDP indicates left ventricular end diastolic pressure; DP, developed pressure; dP/dt, derivative of pressure; V<sub>b</sub>, left ventricular balloon volume producing the maximal DP.

*P < 0.05, †P < 0.01, §P < 0.001, ¶P < 0.0001 vs NTG.
ly). The coronary flow rate of 5.8 mL/g per minute resulted in 30/110 mm Hg perfusion pressure in NTG and dTG rat hearts, respectively (P<0.001). The increased perfusion pressure in dTG rats probably is due to alterations in vascular morphology caused by hypertension.31 As also previously reported,25–27 these drugs did not have any effect on perfusion pressure under these experimental conditions (perfusion pressure change: dTG, 0.8±0.4% and 0.3±0.5 mm Hg during the 30-minute infusion with bosentan and CV-11974, respectively), indicating that the observed changes in contractility cannot be attributed to alterations in coronary vascular tone.

PKC and NHE have been suggested to mediate the positive inotropic effect of exogenous ET-1 in cardiac myocytes.18 To assess the role of PKC and NHE-1 as downstream mediators of ET-1 signaling in the Frank-Starling response in dTG hearts, the effect of GF-109203X, a selective PKC inhibitor, and zoniporide, a potent and selective inhibitor of NHE-1, were tested. The dP/dt max increased to 143±4% and 135±3% of baseline in vehicle-infused and GF-109203X–infused hearts, respectively (P=0.08). Moreover, during the stepwise increase in the intraventricular balloon volume, the increase in dP/dt max was significantly attenuated in the presence of GF-109203X in comparison with vehicle infusion (P<0.05, Figure 5). In contrast, zoniporide had no effect on the Frank-Starling response, with dP/dt max increasing to 148±4% (P=NS versus vehicle). The drugs had no effect on baseline (LVEDP=3 mm Hg) contractile force or the perfusion pressure. In NTG rats, the Frank-Starling response remained unaltered during the infusion with GF-109203X and zoniporide (data not shown).

Characterization of the ET-1 System in NTG and dTG Rat Hearts

Because a significant contribution of ET-1 to the Frank-Starling response in dTG but not in NTG rat hearts was observed, we investigated the activity of the endothelin system in left ventricles of both strains. Immunoreactive ET-1 peptide levels were 25% higher in the left ventricles of control-perfused dTG than NTG hearts (P<0.05, Figure 6). Furthermore, the ET A receptor mRNA level was 82% higher in dTG left ventricles (P<0.05, Figure 6). There was no difference between NTG and dTG rat left ventricles in ET-1 mRNA levels. A 29% decrease in AT1 receptor mRNA was noted in dTG left ventricles (P<0.005, Figure 5). The ACE mRNA levels were similar in the left ventricles of both rat lines (data not shown). Further analysis to investigate possible mechanisms by which the ET-1 stimulation might influence contractility showed no differences between the rat lines in the NHE-1 (dTG, 0.71±0.15 versus NTG, 0.73±0.10 arbitrary units), or phospholamban (dTG, 1.00±0.05 versus NTG, 0.98±0.09 arbitrary units) mRNA levels. The NCX mRNA levels in dTG rat left ventricles were 28±8% higher.

Figure 5. Plot shows effect of PKC inhibitor GF-109203X and NHE-1 inhibitor zoniporide on the Frank-Starling response as measured by change in dP/dt max in dTG rat hearts. *P≤0.05.

Figure 6. Bar graphs show ET-1 mRNA, immunoreactive ET-1 (irET-1), ET A receptor mRNA, and AT1 receptor mRNA levels in left ventricles of NTG and dTG rats. mRNA levels were measured with quantitative reverse transcription–polymerase chain reaction and related to respective 18S RNA levels. Data are shown relative to NTG levels. Note different scales in graphs. *P<0.05; †P<0.005.
than those in NTG rat left ventricles (dTG, 0.89 ± 0.05 versus NTG, 0.69 ± 0.02 arbitrary units, \( P < 0.05 \)).

Regulation of the Frank-Starling Response in SHR Hearts
To test whether involvement of ET-1 in the Frank-Starling response is limited to severe LVH as in dTG rats, the effect of bosentan and CV-11974 were tested in 8-week-old spontaneously hypertensive rat (SHR) hearts, and age-matched Wistar-Kyoto (WKY) control rats (left ventricle/body weight ratio of 2.90 ± 0.16 and 2.60 ± 0.07 mg/g, respectively, \( P < 0.05 \)). Our results showed that bosentan and CV-11974 had no effect on the Frank-Starling response in SHR hearts (maximal DP compared with the level at LVEDP = 3 mm Hg: 153 ± 8, 163 ± 8% and 164 ± 7%, vehicle, bosentan, and CV-11974 treated hearts, respectively, \( P = \text{NS} \)). In contrast to the dTG rat hearts, mRNA levels of ET\(_A\) receptor were similar in SHR and WKY rat hearts (1.50 ± 0.40 versus 1.63 ± 0.37 arbitrary units, SHR versus WKY, \( P = \text{NS} \)).

Discussion
The key finding of the present study is that endogenous ET-1 contributes to the Frank-Starling response in dTG rat hearts with compensated LVH but not in normal NTG rat hearts. The observation that ET\(_{A/B}\) receptor antagonist impaired the Frank-Starling response in dTG rat hearts raises the question of whether this was due to the alteration of diastolic or systolic function. Basal release of ET-1 is known to delay left ventricular relaxation in normal guinea pig hearts, and the mechanism behind augmentation of Frank-Starling response by basal release of NO appears to be mediated by improved diastolic distensibility. As shown in Figure 4, similar increase in LVEDP was observed when bosentan or vehicle was infused in dTG rat hearts, suggesting that the diastolic stiffness was not affected by endogenous ET-1. In addition, there was no change in maximal rate of isovolumic relaxation, \( \Delta P/\Delta t_{\text{max}} \), in response to bosentan. Thus, the inhibition of the Frank-Starling response in dTG rat hearts by ET-1 receptor blockade appears to be mediated by alteration of the systolic function.

In contrast with the results obtained with bosentan, the AT\(_1 \) receptor blocker CV-11974 did not have any significant effect on the Frank-Starling response in dTG rat hearts, even though increased Ang II release has been suggested to induce ET-1 action in response to myocyte stretch in the heart. The renin-angiotensin system components are found also in normal rat hearts, and they are induced during cardiac hypertrophy and failure. A role for locally generated Ang II in the Frank-Starling mechanisms in vivo cannot be excluded, since it is possible that local Ang II production would need blood-derived renin to be completely active, but in this model Ang II does not appear to be as significant a regulator of contractile function as ET-1.

Previous studies suggest that stretch-induced release of Ang II and ET-1 stimulates the NHE and thereby possibly activates the NCX in its reverse mode (\( \text{Ca}^{2+} \) in–\( \text{Na}^+ \) out). This has been suggested to be the mechanism for the slow phase of the Frank-Starling response in a cat papillary muscle preparation. In the whole-organ model used in our study, it is difficult to dissect between the slow and rapid phases, and therefore the peak contractile values are a result of combination of both slow and rapid phases of the Frank-Starling response. Our results are at variance with those of Pérez et al., since the Frank-Starling response was not affected by ET\(_{A/B}\) or AT\(_1\) antagonism or inhibition of NHE-1 in normal rat hearts.

Previously, PKC has been implicated in ET-1–induced responses in cardiomyocytes. The present results suggest that PKC is involved in the regulation of the Frank-Starling response in dTG rat hearts. Since the effect of GF-109203X was less prominent than that of bosentan, apparently other signaling mechanisms can contribute to this response. Endogenous ET-1 may also affect the properties of the more downstream elements of the contractile machinery, such as the \( \text{Ca}^{2+} \) affinity of the troponin C or the actomyosin cycling rate in hypertrophied hearts.

Based on our present results and those of previous studies, the Frank-Starling mechanism is preserved in hypertrophied and even in failing hearts. In contrast, the force-frequency relation and the \( \beta \)-adrenoceptor system are impaired in various models of LVH and CHF. Therefore, in these pathological conditions, the adaptation of the heart to varying levels of load is more dependent on the Frank-Starling response. Our findings imply that endogenous ET-1 plays a significant role in the maintenance of cardiac function during acute increases in hemodynamic load in severely hypertrophied hearts with an upregulated ET-1 system by contributing to the Frank-Starling response.

Perspectives
ET-1 contributes to the pathophysiology of cardiac hypertrophy and failure. Treatment with endothelin antagonists appears to be an attractive alternative to improve the poor prognosis of these patients by decreasing peripheral resistance and interrupting the direct growth-promoting effect of ET-1. However, previous reports showed that initiation of high-dose bosentan treatment worsened the clinical status in patients with CHF. Our results demonstrate that ET-1 contributes significantly to the Frank-Starling response in severely hypertrophied dTG rat hearts, providing a possible mechanism for the adverse effects observed in patients. Together with the previous findings, our results suggest that special care should be taken with endothelin receptor antagonist treatment in severe LVH because of the effects on the Frank-Starling response. In contrast to ET\(_{A/B}\) antagonist bosentan, the AT\(_1 \) receptor antagonist CV-11974 does not appear to affect the Frank-Starling mechanism, which agrees with the findings that pharmacological blockade of the renin-angiotensin system is well tolerated and improves survival in patients with CHF.

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