**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

The three basic mechanisms regulating contractile force in vivo are the Frank-Starling mechanism, that is, increased contractile force in response to elevated end-diastolic volume, the force-frequency relation, and the sympathetic nervous system. In hypertrophied hearts, the force-frequency relation and the β-adrenoreceptor systems are impaired, whereas previous studies have suggested that the Frank-Starling mechanism is maintained in both hypertrophied and failing hearts. The stretch of cardiac muscle generates a biphasic force response. The immediately occurring rapid phase of the Frank-Starling mechanism is thought to be mediated by length-dependent activation of contractile element. This is mediated, at least partially, by increased Ca$^{2+}$ affinity of the Ca$^{2+}$-binding part of the contractile element, troponin C. During the following minutes, the slow phase develops, accounting for $\approx 20\%$ of the total contractile response to load in physiological temperatures. Previously, it has been shown that basal release of nitric oxide contributes to the Frank-Starling response by decreasing the diastolic stiffness, but otherwise little is known concerning the regulation of this response by paracrine/autocrine mediators.

In the long term, mechanical overload of the heart leads to compensatory left ventricular hypertrophy (LVH) and eventually to heart failure. Myocyte stretch has been suggested to induce the release of angiotensin II (Ang II) and endothelin-1 (ET-1), which then both contribute to the hypertrophic growth response. Elevated plasma levels of ET-1 have been demonstrated in chronic heart failure (CHF) in humans and experimental animal models, and studies with ET-1 receptor antagonist treatment in experimental models of CHF have shown improvement in cardiac function and survival. However, the usefulness of these drugs in chronic CHF in humans has not yet been confirmed by clinical trials. Surprisingly, a report from the REACH-1 trial suggested that initiation of high-dose treatment with the endothelin A/B (ET$_{AI/B}$) receptor antagonist bosentan is accompanied by an increased number of events leading to worsening clinical
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 double transgenic (dTG) rats harboring human renin and angiotensinogen genes by using the mixed ET\textsubscript{A/B} receptor antagonist bosentan. Furthermore, the angiotensin type 1 (AT\textsubscript{1}) 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 ascites or pleural effusion. The dTG and NTG rat strains were obtained from Mollegaard Breeding Center Deutschland GmbH (Schönwalde, 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 Chemical Industries Ltd (Osaka, Japan), and zoniporide by Dr Ross Tracey, Pfizer Inc (Groton, Conn). GF-109203X was purchased from Calbiochem.

Isolated Perfused Heart Preparation

The retrograde perfusion system and the Krebs-Henseleit bicarbonate buffer were similar, as previously described. Left ventricular contractility was assessed by measuring isovolumic left ventricular pressure with a fluid-filled balloon placed in the left ventricular chamber. For more detailed description of the preparation, please see the online data supplement at http://hyper.ahajournals.org.

Experimental Protocol and Analysis

The 50-minute stabilization period was followed by 10 minutes of pretreatment with the desired drug. Thereafter, the infusion was continued and the volume of the intraventricular balloon was increased in 10-μL steps. The control experiments were continued at a left ventricular end-diastolic pressure (LVEDP) of 3 mm Hg. Cardiac function was assessed within 1 minute of each volume increment, when a new steady state was reached. The whole assessment of the Frank-Starling response was completed within 15 minutes.

The concentrations of bosentan (1 μmol/L), CV-11974 (10 nmol/L), GF-109203X (90 nmol/L), and zoniporide (1 μmol/L) were chosen because these concentrations are known to effectively block ET\textsubscript{A/B} receptors and AT\textsubscript{1} receptors to suppress protein kinase C activity, and to inhibit Na\textsuperscript{+}–H\textsuperscript{−} exchanger isoform 1 (NHE-1), respectively.

Maximal balloon volume (V\textsubscript{max}) was defined as the value at which peak developed pressure (DP) was reached, and a further increase led to a decrease of DP. Due to the different size of left ventricles of the dTG and NTG rats, comparison between dTG and NTG ventricular function was done at an LVEDP of 3 mm Hg and also at 50% of the V\textsubscript{max}. For analysis of mRNA and peptide levels, left ventricles of unstretched, vehicle-treated hearts were used.

Radioimmunoassay

The ET-1 radioimmunoassay was performed as previously described. Tissue ET-1 is expressed per milligram of wet weight.

Isolation and Analysis of Cytoplasmic RNA

RNA isolation and atrial natriuretic peptide (ANP), c-fos, and 18S RNA Northern blot analysis were performed as described. Real-Time Quantitative Reverse Transcription–Polymerase Chain Reaction

ET\textsubscript{A}, receptor-ET-1, AT\textsubscript{1} receptor, ACE, phospholamban, NHE-1, and Na\textsuperscript{+}–Ca\textsuperscript{2+} exchanger (NCX) mRNA levels were measured by real-time quantitative reverse transcription–polymerase chain reaction analysis, using Taqman chemistry on an ABI Prism 7700 Sequence Detection System (Applied Biosystems) as described. For forward and reverse primers and probes for mRNA detection, please see the online data supplement at http://hyper.ahajournals.org.

Statistics

The results are expressed as mean±SEM. The Student t test was used for the comparison between 2 groups. The hemodynamic variables were analyzed with 1-way ANOVA followed by the Student-Newman-Keuls post hoc test. Repeated-measures ANOVA was used for multivariate analysis. Differences at the 95% level were considered statistically significant.

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±2 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 left 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\textsubscript{max}, the contractile function was increased in dTG rat hearts (Table and Figure 2). Because of the geometrical changes in hypertrophied dTG hearts, the V\textsubscript{max} was 186±18 μL compared with 234±22 μL in NTG hearts (P=0.05).
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 A/B antagonist) and CV-11974 (AT 1 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 max 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 min, 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, 2.7±2.8% and 3.1±2.8% during the 30-minute infusion with bosentan and CV-11974, respective-

Characteristics of dTG and NTG Rats

<table>
<thead>
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<th>Characteristics at LVEDP=3 mm Hg</th>
<th>NTG Rats</th>
<th>dTG Rats</th>
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<tr>
<td>Hemodynamics</td>
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<tr>
<td>LVEDP, mm Hg</td>
<td>29.6±1.8</td>
<td>44.5±1.9</td>
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<tr>
<td>DP, mm Hg</td>
<td>1216±61</td>
<td>1578±69</td>
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<tr>
<td>dP/dt max, mm Hg/s</td>
<td>-684±34</td>
<td>-899±34</td>
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<tr>
<td>dP/dt min, mm Hg/s</td>
<td>820±53</td>
<td>1105±70</td>
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<td>Data are mean±SEM. LVEDP indicates left ventricular end diastolic pressure; DP, developed pressure; dP/dt, derivative of pressure; V rae, left ventricular balloon volume producing the maximal DP.</td>
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Figure 1. Representative Northern blots and respective bar graphs show ANP and c-fos mRNA levels in dTG and NTG left ventricles measured by Northern blot analysis and related to respective 18S RNA levels. Note different scales in graphs. *P<0.05; †P<0.001.

Figure 2. Plots show DP, dP/dt max, LVEDP, and dP/dt min in NTG and dTG rat hearts during stepwise increment of intraventricular balloon volume. NTG and dTG differ significantly in all parameters (P<0.01).

Figure 3. Plots show changes in Frank-Starling responses as measured by DP and dP/dt max, in response to treatment with ETA/B antagonist bosentan (1 μmol/L), AT 1 antagonist CV-11974 (10 nmol/L), or vehicle in NTG and dTG rat hearts. *P<0.05 vs respective vehicle-infused group.
ly). The coronary flow rate of 5.8 mL/g per minute resulted in 30±1 and 54±2 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. As also previously reported, these drugs did not have any effect on perfusion pressure under these experimental conditions (perfusion pressure change: dTG, 0.8±0.1 and 0.3±0.0 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. 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.
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 \(\text{ET}_A\) 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 \(\text{ET}_A/\text{B}\) 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, \(\text{dP/dt}_{\text{max}}\), 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 \(\text{AT}_1\) 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 \(\text{ET}_{\text{A/B}}\) or \(\text{AT}_1\) 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. Furthermore, contribution of the endogenous ET-1 system to the Frank-Starling mechanism in the hypertrophied dTG rat hearts may be related to the ET-1–mediated improvement of the contractile efficacy.

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 \(\text{ET}_{\text{A/B}}\) antagonist bosentan, the \(\text{AT}_1\) 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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References

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