Activation of the cardiac endothelin system in left ventricular hypertrophy before onset of heart failure in TG(mREN2)27 rats

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Abstract

Objective: To characterize the cardiac angiotensin and endothelin (ET) system in compensated left ventricular hypertrophy due to long standing arterial hypertension and to assess the role of angiotensin and ET converting enzymes in mediating the observed changes of angiotensin and ET levels, respectively. Methods: We studied the left ventricular renin–angiotensin system (RAS) and ET system in 20-week-old male transgenic hypertensive TG(mREN2)27 rats, a model of the monogenic renin-dependent form of severe hypertension. Age-matched Sprague–Dawley rats served as controls. Results: TG(mREN2)27 rats exhibited left ventricular hypertrophy without signs of congestion. Transgene overexpression led to an activation of the tissue RAS with increased angiotensin II levels in spite of unchanged angiotensin converting enzyme (ACE) activity and ACE mRNA levels. ET-1 production was markedly increased in TG(mREN2)27 rats indicating that the ET-system was activated. Cardiac ET-1 in TG(mREN2)27 originated most likely from increased preproET-1 production because preproET-1 mRNA levels were increased but ET converting enzyme gene expression and activity were unchanged. Furthermore, ET-1 binding sites were significantly increased in TG(mREN2)27 rats without changes in \( K_d \) values and ET\(_a\)/ET\(_n\) receptor ratios. ET\(_a\) receptor gene expression was not altered whereas ET\(_n\) receptor mRNA levels were up-regulated twofold in TG(mREN2)27 rats suggesting that ET\(_a\) and ET\(_n\) receptor expression may be regulated differentially. Conclusions: Cardiac ET and angiotensin systems are co-activated in compensated cardiac hypertrophy before onset of heart failure, and thus may be involved in the mechanism by which cardiac remodelling and progression of left ventricular dysfunction occur in TG(mREN2)27 rats. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Endothelins; Hypertension; Hypertrophy; Renin angiotensin system

1. Introduction

Myocardial hypertrophy in response to hemodynamic overload is an established risk factor for cardiovascular morbidity and mortality. Data from the Framingham study demonstrate that cardiac hypertrophy due to long standing hypertension is associated with an increased incidence of heart failure [1]. However, identifying the biochemical basis of the pathological process that promotes the progression of ventricular dysfunction remains an important unresolved problem in experimental and clinical cardiology. A large body of clinical and experimental evidence supports an important role for the renin–angiotensin system (RAS). Recent in vitro studies suggest that endothelin-1 (ET-1), a potent vasoconstrictor and growth factor for cardiomyocytes, is involved in the mechanism by which angiotensin II (Ang II) induces cardiac hypertrophy [2]. It has been shown that Ang II induces the expression of ET-1 in isolated cardiomyocytes involving autocrine regulation via ET\(_a\) receptors [3]. In a first effort to demonstrate the interaction between Ang II and ET-1 in

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vivo, the effect of increased circulating Ang II on cardiac ET-1 synthesis has been investigated in rats after chronic Ang II infusion [4,5]. However, these studies failed to detect increased cardiac ET-1 formation and it has been hypothesized that the cardiac ET system may require activation by the local rather than the circulating RAS [4,6].

The purpose of the present study was to investigate whether the cardiac RAS and ET system are co-activated in hypertensive cardiomyopathy before onset of heart failure. We used a well-characterized monogenic model of arterial hypertension, namely the TG(mREN2)27 rat. Previously, Rothermund et al. have shown that cardiac ET mRNA levels were not increased at a very early stage of cardiac hypertrophy in TG(mREN2)27 rats but were significantly elevated in failing hearts [7]. This finding did not answer the question whether activation of the ET-system precedes manifestation of heart failure and, therefore, may account at least in part for progressive cardiac dysfunction. In the present study, we investigated alterations of cardiac angiotensin II and ET-1 generation, ET receptor density, and steady state mRNA expression of preproET-1, ET receptors, and ET receptors in 20-week-old TG(mREN2)27 rats with compensated left ventricular hypertrophy. For the first time, activities of angiotensin and the endothelin converting enzymes, which have been considered to be rate limiting for Ang II and endothelin synthesis, respectively, have been determined in left ventricular myocardium from TG(mREN2)27 rats.

2. Methods

2.1. Animals

Heterozygous transgenic TG(mREN2)27 animals and Sprague-Dawley (SD) control rats were obtained from Mollegard (Denmark). The animals were handled in conformity with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). TG(mREN2)27 and SD rats were kept in a room at a temperature of 25°C with a 12 h light-dark cycle. The animals were kept on a standard laboratory animal diet and tap water ad libitum. SD rats were the animals into which the transgene was originally introduced. Systolic and diastolic blood pressures were measured by the tail-cuff method according to Pfeffer et al. [8]. Animals were weighed and killed by cervical dislocation at the age of 20 weeks. The hearts were rapidly removed, washed with saline, blotted dry with filter paper, weighed and immediately frozen in liquid nitrogen. Samples were stored at −80°C. Lung water content was determined by calculating the difference between lung wet weight and dry weight after lyophilization.

2.2. Radioligand binding studies

ET-receptor density was determined as described previously [9]. Non-specific binding was defined as binding not displaced by 10 μmol/l bosentan and was lower than 10% of maximum total binding. ET-1 and BQ-123 competition curves were analyzed by the iterative curve fitting program Graph Pad Prism (GraphPad software). From the ET-1 competition curves, Bmax and KD values were calculated.

2.3. Myocardial angiotensin II content

For determination of myocardial Ang II content, left ventricular tissue samples were homogenized with a glass-teflon homogenizer in ice-cold homogenization buffer (10 mmol/l Tris–HCl, 1 mmol/l EDTA, 1 mmol/l dithiothreitol, pH 7.4) including the protease inhibitors leupeptin (0.5 μg/ml), aprotinin (0.5 μg/ml) and PMSF (1 mmol/l). After centrifugation (100 000×g, 15 min), the resulting supernatant was purified on Sep-Pak C18 columns. The eluates were lyophilized and the dry residues were resolved in 1.2 ml Tris–HCl reconstitution buffer (pH 7.5). Ang II was determined with a commercially available radioimmunoassay according to the instructions of the manufacturer (Biermann, Bad Nauheim, Germany). The sensitivity of the radioimmunoassay was 0.7 pg/ml and the cross reactivity of the Ang II antibody was 0.14% for Ang I.

2.4. Immunohistochemistry

Left ventricular tissue specimens obtained from TG(mREN2)27 rats were embedded in OCT (Miles Lab, USA), snap-frozen in liquid nitrogen, and stored at −70°C until used. Four-μm sections were cut, mounted on poly-L-lysine-coated slides and dried at room temperature for 3 h. The sections were fixed in acetone/methanol (1:1, −20°C) for 5 min, washed with PBS three times and incubated in 10% normal sheep serum for 30 min at room temperature followed by incubation with monoclonal mouse anti-rat ET-1 antibody (dilution 1:50) overnight at 4°C. After three more washes in PBS, the sections were stained using a Cy3 conjugated secondary sheep anti-mouse antibody (Sigma, dilution 1:1000). For negative controls, sections were incubated with normal mouse IgG (Sigma) instead of the primary antibody.

2.5. Collagen quantification

The hearts fixed in paraformaldehyde solution (4% w/v) were processed for paraffin embedding. Coronal sections (5 μm) of the median part of the left ventricle were obtained. Tissue sections were dewaxed with ethanol and stained with Sirius red F3BA (0.5% in saturated aqueous picric acid, Aldrich). Collagen density was evaluated throughout the inner third (subendocardial myocardium), the middle third (midmyocardium), and the outer third
(subepicardial myocardium) of the circumference of the left ventricle. From each of two nonconsecutive serial sections, five fields in each region of the heart (magnification ×20) were recorded by a digital camera (Nikon Corporation, Japan). The severity of interstitial fibrosis was evaluated after Sirius red staining with the use of the Scion Image software (Scion Corporation, USA). Perivascular fibrosis was estimated from cross sections of coronary arteries. A single investigator blinded to the experimental groups performed the analysis.

2.6. Tissue levels of immunoreactive ET-1

Approximately 200 mg left ventricular tissue was homogenized in five volumes of a phosphate homogenization buffer (0.14 mol/l NaCl, 2.6 mmol/l KCl, 8 mmol/l Na₂HPO₄, 1.4 mmol/l KH₂PO₄, 1% Triton-X, pH 7.4). The homogenates were then centrifuged at 100 000×g for 60 min at 4°C. Aliquots (200 μl; 2 mg protein) were assayed in duplicate using an ET-1 ELISA kit (Biomedica). This kit exhibits cross reactivity with other ET peptides as follows: ET-2 (100%), endothelin-3 (<5%), big ET-1–38 (<1%), big ET-2–38 (<1%).

2.7. Tissue ACE activity assay

Assays were performed according to the method of Cushman and Cheung [10]. Aliquots of resuspended membranes were incubated with 10 mmol/l Hip-His-Leu for 30 min at 37°C with and without 1 μmol/l enalapril. Enzyme activity was quantified by fluorometric measurement of hippurate cleavage from the substrate. ACE activities are expressed as moles (captopril inhibitable) hippuryl acid formed per gram of protein per minute.

2.8. Tissue ECE activity assay

Left ventricular membrane preparations (25 μg protein) were incubated in the presence of big ET-1. The standard enzyme reaction was carried out in 50 μl of assay buffer (50 mmol/l Tris–HCl, pH 7.0, 100 μmol/l NaCl, 5 mg/ml BSA) containing 10 μmol/l big ET-1 at 37°C in the absence and presence of the ECE-inhibitor phosphoramidon (100 μmol/l). After an incubation period of 3 h the reaction was stopped by adding 50 μl of 10 mmol/l EDTA. The amount of formed ET-1 was determined using an ET-1 ELISA (Amersham) which exhibits cross reactivity to big ET-1 (<1%). Samples were diluted 1:1000 and the ELISA-assay was performed according to the manufacture's protocol. Venticular membrane conversion of exogenous big ET-1 proved to be time- and protein-dependent (linear up to 4 h and 60 μg membrane protein, data not shown).

2.9. Blot hybridization experiments

Northern blots were prepared from 10 μg total RNA as described previously [9]. A [32P] labelled 411 bp fragment of rat ANP cDNA and a [32P] labelled 571 bp fragment of rat preproET-1 cDNA, respectively, were used as a specific probe.

2.10. Quantitative RT-PCR analysis of ET-receptor and ECE mRNA expression

Internal standard RNA with a deletion of 10–20% were generated as described previously [9]. Two μg of the total RNA and 10 pg of mut ET₆, mut ET₇, mut ppET-1, or mut ECE standard mRNA, respectively, were mixed and reverse transcribed with random primers. The single stranded cDNA was amplified by polymerase chain reaction (for primer sequences see Ref. [9]). PCR-products were quantified by Southern blot technique and laser densitometry (Image Quant). PCR-amplification has been shown to be linear within a range of 28–40 cycles. Non-competitive amplification of GAPDH was used to demonstrate equivalence of RNA-loading in RT-PCR reactions.

3. Results

3.1. Characteristics of TG(mREN2)27 rats

Cardiac morphological and functional parameters are summarized in Table 1. Blood pressure was significantly higher in TG(mREN2)27 rats than in SD controls. Hearts of TG(mREN2)27 exhibited concentric hypertrophy but no dilatation. No signs of congestion were observed in lungs or any other organ. Especially lung water was unchanged in TG(mREN2)27 and SD. The ratio of left ventricular weight to body weight was significantly elevated by about 40% in TG(mREN2)27 compared to SD. No differences in relative mass were observed in atria and right ventricles. Heart rates were significantly increased in TG(mREN2)27 compared to SD-controls. Left ventricular ANP mRNA expression, which has been related to the degree of left ventricular impairment, was significantly higher in TG(mREN2)27 rats than in SD controls. Interstitial and perivascular collagen content in left ventricles increased 2.3-fold and 2.4-fold, respectively, in TG(mREN2)27 rats compared to age-matched SD rats (Fig. 1). Perivascular fibrosis was accompanied by an increase in the thickness of the tunica media of coronary arteries (Fig. 1A, media.
Table 1
Characteristics of 20-week-old TG(mREN2)27 rats versus Sprague–Dawley (SD) control rats

<table>
<thead>
<tr>
<th></th>
<th>SD</th>
<th>TG(mREN2)27</th>
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<tbody>
<tr>
<td></td>
<td>n=12</td>
<td>n=12</td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>483±9</td>
<td>492±33</td>
<td>N.S.</td>
</tr>
<tr>
<td>LV weight/body weight, mg/g</td>
<td>2.91±0.02</td>
<td>3.76±0.10</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>RV weight/body weight, mg/g</td>
<td>0.91±0.02</td>
<td>1.04±0.08</td>
<td>N.S.</td>
</tr>
<tr>
<td>Lung water, g</td>
<td>1.15±0.03</td>
<td>1.34±0.10</td>
<td>N.S.</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>361±6</td>
<td>405±7</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>137±4</td>
<td>192±11</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>ANP mRNA/GAPDH mRNA</td>
<td>0.74±0.14</td>
<td>3.81±0.21</td>
<td>P&lt;0.0001</td>
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</tbody>
</table>

SBP, systolic blood pressure; N.S., not significant.

thickness/lumen diameter: SD 0.39±0.05, n=10; TG(mREN2)27 0.73±0.04, n=10, P<0.05).

3.2. LV renin–angiotensin system

Tissue Ang II concentrations were significantly increased (Fig. 2) in left ventricles of TG(mREN2)27 rats indicating a chronic activation of the RAS in the hypertrophied myocardium. Enhanced cardiac conversion of Ang I seems not to be responsible for increased tissue Ang II levels since ACE mRNA expression and ACE activity (Fig. 2) was unchanged in the hypertrophied myocardium of TG(mREN2)27 rats compared to SD controls.

3.3. LV endothelin system

To localize ET-1 and to elucidate cell type-specific distribution patterns, immunohistochemical studies were

![Fig. 1. Collagen content in left ventricular myocardium of TG(mREN2)27 rats and Sprague–Dawley (SD) control rats. (A) Representative coronal sections of left ventricles of 20-week-old TG(mREN2)27 (I, II) and SD rats (III) stained with Sirius red (magnification I, III: ×200; II: ×100). Fibrotic tissue appears black. (B) Analysis of interstitial and perivascular fibrosis. *P<0.05 vs. SD (n=30).]
0.8±0.1 ECE mRNA/ECE mut RNA, n=5) in the left ventricle in TG(mREN2)27 rats did not differ from that in SD. Conversion of big ET-1 to mature ET-1 in membrane fractions of left ventricular myocardium was determined in the absence and in the presence of the ECE-inhibitor phosphoramidon. The difference of both values, used as a measure of ECE-activity, did not significantly differ between TG(mREN2)27 (0.9±0.2 ng ET-1/h/mg, n=8) and SD (0.8±0.1 vs. ng ET-1/h/mg, n=8) (Fig. 3C). These findings indicate that the remarkably increased expression of myocardial ET-1 originates from an increase in preproET-1 production rather than from an increase in ECE expression or activity.

The characteristics of ET-receptors were assessed by competition binding experiments in left ventricular membrane preparations (Fig. 4). To discriminate between ETₐ and ETₐ receptors, the ETₐ receptor antagonist BQ-123BA was used. Total ET receptor binding sites were significantly increased 1.6-fold in left ventricular membranes from TG(mREN2)27 rats compared to controls. This increase was due to an up-regulation in ETₐ and ETₐ receptor density (Table 2). $K_d$ values for ET-1 (96.7 pmol/l vs. 96.9 pmol/l) and ETₐ/ETₐ receptor ratios (5.3 vs. 5.5) were not significantly different in TG(mREN2)27 rats from those in SD controls. ETₐ receptor gene expression, as assessed by competitive RT-PCR studies, was unchanged (1.2±0.1 vs. 1.2±0.1 ET mRNA/ET mut RNA, n=5). In contrast, ETₐ receptor mRNA levels were up-regulated twofold in TG(mREN2)27 rats (0.7±0.2 vs. 1.6±0.2 ETₐ mRNA/ETₐ mut RNA, n=5; shown in Fig. 4A). These results indicate that ETₐ and ETₐ receptor expression in transgenic rats is regulated by different mechanisms.

4. Discussion

We have used the TG(mREN2)27 rat, an established model of the monogenic pathogenesis of hypertension, which allows investigation of phenotypic changes in various organs caused by the perturbation of one system, namely the RAS. In TG(mREN2)27 rats overexpression of the mouse renin Ren2d gene led to an activation of the tissue RAS [11] with increased Ang II formation in the left ventricular myocardium. This occurred despite unchanged cardiac ACE activity and ACE mRNA expression. In contrast, plasma renin activities, plasma Ang I and plasma Ang II concentrations were unchanged which indicates that circulating RAS was not activated in TG(mREN2)27 rats [11]. We have previously demonstrated that among ACE-inhibitors, AT1-receptor antagonists, β-adrenoceptor blockers, and vasodilators, blockade of RAS was most effective to reduce left ventricular hypertrophy in TG(mREN2)27 rats despite comparable effects on blood pressure [12]. Cardiac hypertrophy was positively correlated with cardiac angiotensinogen expression [13].
Fig. 3. Protein and gene expression of main components of the cardiac endothelin system in TG(mREN2)27 rats and Sprague–Dawley (SD) control rats. (A) Cellular localization of ET-1 expression. Cryostat sections were probed with the specific ET-1 antiserum or with control IgG. Bar: 100 μm. (B) ET-1 tissue concentrations (*P<0.001 vs. SD, n=6). (C) Left ventricular ECE activity in membrane preparations (n=8). (D) Steady state mRNA concentrations of preproET-1 (ppET-1) and ECE-1 in TG(mREN2)27 rats. Values are expressed relative to mRNA expression in SD control animals (*P<0.05 vs. SD, n=5).

together, these observations strongly suggest that cardiac hypertrophy in TG(mREN2)27 rats is mediated, at least in part, by an activation of the local RAS.

Until now, the role of the cardiac endothelin system for the progression of left ventricular hypertrophy into heart failure rats has not been well characterized. It has been reported previously that the cardiac ET-system was not activated in 10-week-old TG(mREN2)27 rats although cardiac hypertrophy and compensated LV dysfunction appears established by 10 weeks [7]. This finding argues against a role for the cardiac ET-system but it does not answer this question definitely. The present study, designed to investigate alterations of the ET system immediately before onset of congestive heart failure, clearly demonstrates that the cardiac endothelin system is indeed activated in compensated LV hypertrophy in TG(mREN2)27 rats at an age of 20 weeks. PreproET-1 mRNA expression and ET-1 concentrations were increased, whereas both endothelin converting enzyme-1 (ECE-1) mRNA expression and functional ECE activity were unchanged in TG(mREN2)27 rats. Thus, elevated tissue ET-1 is most likely due to increased preproET-1 production rather than increased conversion of big ET-1. Immunohistochemical staining showed that increased tissue ET-1 originates from both cardiac myocytes and vascular cells. Interestingly, alterations of the cardiac ET-system found in TG(mREN2)27 rats resemble those generally found in failing myocardium [14]. Like TG(mREN2)27 rats, in experimental animal models and in patients with congestive heart failure ECE-1 expression
was found to be unchanged, whereas preproET-1 gene expression was increased which led to increased tissue ET-1 [9,15]. Rothermund et al. have demonstrated that cardiac ET system activity is depressed in the very early state of cardiac hypertrophy in TG(mREN2)27 rats [7]. In the decompensated state, the cardiac ET system was activated with increased ECE mRNA expression and ET<sub>A</sub> receptor density in the failing left ventricles from TG(mREN2)27 rats [7]. The present study shows that an activation of the cardiac ET system precedes the development of congestive heart failure. These data are in line with the interpretation that ET-1 plays an important role in the progression of left ventricular dysfunction.

Although mechanical stretch has been shown to directly increase the release of ET-1 from myocardial cells, in vivo studies demonstrate that pressure overload is not generally associated with an activated cardiac ET system. Unlike TG(mREN2)27 rats, several experimental animal models

Table 2
Characterization of ET-1 binding sites in left ventricular membrane preparations from TG(mREN2)27 and SD control rats

<table>
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<tr>
<th></th>
<th>SD</th>
<th>TG(mREN2)27</th>
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<tbody>
<tr>
<td></td>
<td>&lt;i&gt;n&lt;/i&gt;=5</td>
<td>&lt;i&gt;n&lt;/i&gt;=5</td>
</tr>
<tr>
<td>Total ET-1 binding sites</td>
<td>172±22</td>
<td>271±26</td>
</tr>
<tr>
<td></td>
<td>10.3±0.08</td>
<td>10.3±0.05</td>
</tr>
<tr>
<td>ET&lt;sub&gt;A&lt;/sub&gt; binding sites</td>
<td>143±13</td>
<td>227±22</td>
</tr>
<tr>
<td></td>
<td>84.7±3.1</td>
<td>84.1±3.4</td>
</tr>
<tr>
<td>ET&lt;sub&gt;B&lt;/sub&gt; binding sites</td>
<td>20±3</td>
<td>33±4</td>
</tr>
<tr>
<td></td>
<td>15.3±3.1</td>
<td>15.9±3.4</td>
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N.S., not significant.
of pressure overload have shown normal ET-1 levels in the hypertrophied myocardium in the compensated state [16,17]. In aortic banded rats cardiac ET-1 expression was transiently increased and returned to basal levels at day 4 in spite of persistent pressure overload [18,19]. Overall the information available would suggest that other factors than pressure overload per se may prove to be essential in ET system activation. One of these factors may be the RAS. In cultured neonatal cardiomyocytes and cardiac fibroblasts Ang II has been shown to stimulate autocrine secretion of ET-1 which in turn may contribute to the growth promoting effects of Ang II [3,20]. The present study together with these in vitro findings provide evidence for a role of tissue Ang II to stimulate cardiac ET-1 synthesis.

Radioligand binding studies demonstrated that both left ventricular ET₄ and ET₆ receptors were significantly up-regulated in TG(mREN2)27 rats although ET₄ receptor mRNA concentrations were unchanged. The reason for this discrepancy is not known. In the present study, total ET₄ mRNA and ET₄ receptor binding sites in the left ventricular myocardium have been determined and it has not been differentiated between the cell types, like cardiac myocytes, fibroblasts, or vascular smooth muscle cells, which are known to express ET₄ receptors. It is likely that the ET₄ receptor expression is regulated differentially in different cell types and we hypothesize that this may account for the obvious discrepancy of increased ET₄ receptor number in spite of unchanged ET₄ receptor mRNA levels.

Several findings suggest that RAS activation may influence ET receptor expression. Indeed, Ang II has been reported to induce a heterologous up-regulation of ET₆ receptors in rat cardiomyocytes [21] and ET₄ receptors in rat vascular smooth muscle cells [22]. Most ET-1 effects on cardiomyocytes are initiated through the ET₄ receptor subtype including induction of proto-oncogene expression [23]. Moreover, ET-1 modulates fibroblast function, such as collagen turnover, via ET₄ and ET₆ receptor stimulation [24]. Therefore, activation of the cardiac ET-system with increased local ET-1 production and a significant up-regulation of ET-receptors, as revealed in the present study, may contribute to the left ventricular remodelling process present in TG(mREN2)27 rats leading to cardiac hypertrophy and fibrosis [25].

Cardiac function in TG(mREN2)27 rats has been studied before by our group and by others. Together, these studies clearly demonstrate that (i) left ventricular dysfunction occurs as soon as arterial hypertension is established and (ii) left ventricular dysfunction is progressive in TG(mREN2)27 rats. Peak rates of contraction and relaxation were significantly decreased in electrically driven papillary muscle strip preparations from 12–14-week-old TG(mREN2)27 rats compared to SD controls [26]. Similar results were obtained when cardiac function was assessed in vivo or in explanted Langendorff-perfused hearts from transgenic animals [7,27]. These data indicate an impairment of both systolic and diastolic contractile function which occurs already at an age of 10 weeks. We have considered several mechanisms leading to contractile dysfunction, including altered MHC isoform expression, altered SERCA/PLB expression and cardiac fibrosis [26]. Endothelin has been shown to act as a mitogen inducing hypertrophy of cardiac myocytes [23]; ET-1 impairs sarcoplasmic reticulum Ca²⁺ handling [28] and modulates fibroblast function and matrix protein synthesis [24]. From these observations one could argue that activation of the cardiac ET system may promote disease progression from cardiac hypertrophy to failure. In an attempt to answer this question, the TG(mREN2)27 model has been used previously to compare the role of the cardiac endothelin system in compensated left ventricular hypertrophy and in heart failure [7]. It has been demonstrated that the cardiac ET-system was activated in the failing hearts from 30-week-old transgenic rats but was not activated or even depressed (with respect to the ET₄ receptor expression) at an earlier age of 10 weeks [7]. Thus, activation of the cardiac endothelin system obviously occurs not before arterial hypertension, left ventricular hypertrophy, and systolic/diastolic dysfunction are established in TG(mREN2)27 rats. The present study clearly shows that activation of the cardiac endothelin system precedes overt heart failure and is not just associated with the end-stage heart failure phenotype. The mechanisms which trigger activation of the cardiac endothelin system during disease progression in the TG(mREN2)27 model remain to be defined. One of these mechanisms may be the cross talk between the activated RAS and the ET system. In addition, increased wall stress may be involved. Stretch induces ET-1 in isolated cardiomyocytes and cultured endothelial cells [29,30], but the role of mechanical load on myocardial ET-1 expression in vivo is still unclear.

In summary, the cardiac endothelin and angiotensin systems are co-activated in compensated cardiac hypertrophy before onset of heart failure, and thus may be involved in the mechanism by which cardiac remodelling and progression of left ventricular dysfunction occur in TG(mREN2)27 rats. These findings provide evidence for a potentially useful therapeutic approach by the use of ET antagonists to prevent long-term cardiac complications.

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References


