Tissue-dependent expression of matrix proteins in human endothelin-1 transgenic mice

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ABSTRACT

Endothelin-1 (ET-1) is a potent pro-fibrotic growth factor. However, little is known about its specific effects on the synthesis of matrix proteins in vivo. We used male 12-month-old ET-1 transgenic mice characterized by transgene expression in the kidney and (to a lesser extent) in the heart. Global cardiac and renal matrix protein synthesis was analysed after Sirius Red and periodate–Schiff staining. Specific expression of collagen types I, III and IV, laminin and fibronectin was examined using immunohistochemistry followed by computer-aided image analysis. Analysis of blood pressure revealed that mean arterial blood pressure was similar in ET-1 transgenic mice and controls. The total cardiac matrix protein content was increased in the myocardium of ET-1 transgenic mice. Analysis of specific cardiac matrix proteins showed increased cardiac expression of collagen type III (+211%; P < 0.001) and laminin (+128%; P < 0.01) in transgenic mice. The expression of collagen types I and IV and fibronectin was not altered. Global analysis of renal matrix proteins confirmed earlier studies showing pronounced interstitial fibrosis and glomerulosclerosis. Laminin expression was markedly increased in the glomerula (+152%; P < 0.01) and even more so in the interstitium (+211%; P < 0.001), whereas expression of collagen type III was reduced in glomerula (−48%; P < 0.01) and interstitial tissue (−55%; P < 0.01) of ET-1 transgenic mice. In conclusion, a primary overexpression of ET-1 does not cause uniformly enhanced synthesis of matrix proteins. In contrast, the effects of ET-1 on the matrix protein pattern is tissue-specific. The major renal and cardiac alterations in matrix proteins induced by ET-1 is a marked enhancement of laminin expression.

INTRODUCTION

In addition to vasoconstriction, the endothelins (ETs) have been shown to cause a variety of biological activities in non-vascular tissues. The paracrine ET system is involved in the regulation of renal blood flow, glomerular filtration rate, and tubular water and sodium reabsorption [1–3]. Furthermore, ET-1 is able to stimulate mesangial cell proliferation, and seems to be involved in the biosynthesis of matrix proteins in the kidney [4]. These findings evoke the possibility of an involvement of the paracrine ET system in the pathogenesis of kidney fibrosis. Several reports [5–8] demonstrate a correlation between kidney fibrosis and an activated renal ET system. It is still unknown whether endogenous activation of the paracrine ET system is a cause or a consequence...
of kidney injury. A powerful method to answer this question is an animal model with a primary activated paracrine renal ET system. We have recently generated human ET-1 transgenic mice. The transgene was expressed predominantly in the brain, lung and kidney. Renal transgene expression was associated with a pathological phenotype manifested by signs such as age-dependent development of interstitial fibrosis and glomerulosclerosis, leading to a progressive decrease in the glomerular filtration rate in a blood-pressure-independent manner. The aim of the present study was to analyse which matrix proteins are affected by the primary overexpression of ET-1 in the kidney and heart.

MATERIALS AND METHODS

Materials

Unless otherwise stated, all reagents were of analytical grade and were purchased from Merck (Darmstadt, Germany), Boehringer Mannheim (Mannheim, Germany), or Sigma Chemical (Munich, Germany).

Animals

Male 12-month-old heterozygous human ET-1 transgenic mice (line 856/+−) were used for this study. The animals were fed a commercial diet (Altromin; Altromin G.m.b.H.) and given water ad libitum. All animal experiments were conducted in accordance with local institutional guidelines for the care and use of laboratory animals.

Measurement of blood pressure and glomerular filtration rate

The mice were anaesthetized with thiobutabarbitral (20 mg/100 g) and ketamine (20 mg/100 g). Animals were placed on a heating table and a tracheotomy was performed. A Teflon catheter connected to a polyethylene catheter was inserted into the right femoral artery, with the tip of the Teflon catheter positioned at the aortic bifurcation for measurement of arterial blood pressure and heart rate. A second catheter was inserted into the right femoral vein. The arterial catheter was connected to an electromagnetic pressure transducer. Blood pressure signals were transmitted to a blood pressure amplifier that allowed measurement of the mean arterial blood pressure. For urine collection, a polyethylene catheter was inserted through a suprapubic incision into the urinary bladder. The endogenous creatinine clearance was calculated using the equation 

\[ C = \frac{U_c \times U_{vol}}{S_c}, \]

where \( C \) is creatinine clearance, \( U_c \) is urinary creatinine concentration, \( U_{vol} \) is urine volume and \( S_c \) is serum creatinine concentration. To replace fluid loss, a continuous infusion of isotonic saline (180 µl) was begun.

Blood pressure and heart rate were recorded continuously and analysed after a stable baseline of the above-mentioned parameters had been achieved. Only those animals that had blood pressure variations of < 8% and heart rate variations of < 15% during the whole collecting period were included in the study. Samples of urine were collected over a period of 3 h after equilibration of haemodynamic parameters. Blood samples (100 µl) were taken at the end of that period.

Histological evaluation

For pathohistological evaluation, all samples were submitted to Masson/Trichrome. The severity of matrix deposition was evaluated using the Image 1.61 program (NIH). We measured the relationship of the green-stained area to the total area of the whole section, as described previously [9].

Immunohistochemistry

The detection of matrix proteins in the kidney and heart was performed as described by Schäfer et al. [10], with minor modifications, using polyclonal rabbit antibodies against collagen types I, III and IV, laminin and fibronectin. Briefly, for antibody incubations, 5-µm-thick cryostat sections were mounted on poly(L-lysine)-coated glass slides. Polyclonal rabbit antibodies against collagen types I-IV, laminin and fibronectin (generously donated by Professor Dr D. Schuppan, Erlangen, Germany) were applied at dilutions of 1:200 in PBS containing 1% (w/v) BSA. Detection of the bound antibodies was performed using a biotinylated second antibody and streptavidin–Texas Red (Amersham Buchler, Braunschweig, Germany), according to the manufacturer’s instructions. Control experiments were performed by omitting the first antibody and using PBS instead. Texas Red-stained sections were analysed by fluorescence microscopy and by a computer-aided image analysis system. We measured the relationship of the Texas Red-stained area (connective tissue) to the total area of the whole section using a computer-aided image analysis system.

Analysis of data

Differences between groups of data were compared using Student’s \( t \) test. Results were considered significantly different at a value of \( P < 0.05 \).

RESULTS AND DISCUSSION

Blood pressure was similar in 12-month-old transgenic mice and in age-matched controls (Table 1). Although transgene expression in blood vessels caused vascular hypertrophy [9], blood pressure was unaffected in the ET-1 transgenic mice. These observations are consistent with data from Schiffrin et al. [11], which showed a
Table 1  Blood pressure, glomerular filtration rate, kidney and heart weight in 12-month-old heterozygous human ET-1 transgenic mice and age-matched controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ET-1 transgenic mice</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>93 ± 9</td>
<td>98 ± 8</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml min⁻¹ · 100 g⁻¹ body weight)</td>
<td>0.20 ± 0.08</td>
<td>0.49 ± 0.12*</td>
</tr>
<tr>
<td>Kidney/body weight (g/100 g body)</td>
<td>8.8 ± 1.0</td>
<td>7.5 ± 0.8</td>
</tr>
<tr>
<td>Heart/body weight (g/100 g body)</td>
<td>6.7 ± 0.5</td>
<td>5.0 ± 0.5</td>
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*P < 0.05 compared with age-matched controls.

Figure 1  Renal and cardiac matrix protein expression in human ET-1 transgenic mice

Sections show typical staining of glomerula and interstitium with an anti-laminin antibody in ET-1 transgenic mice (A, C) and in wild-type littermates (B, D), and with an anti-(collagen type III) antibody in the myocardium of ET-1 transgenic mice (E) in comparison with age-matched controls (F). Magnification: (A), (B), (E) and (F), × 400; (C) and (D), × 200.

Overexpression of the human ET-1 gene influenced the composition of matrix proteins in a tissue-dependent manner. Expression analysis of extracellular matrix proteins in the kidneys and heart of heterozygous ET-1 transgenic mice showed significant changes compared to age-matched controls. A correlation of vascular ET-1 gene expression in deoxycorticosterone acetate- and salt-treated spontaneously hypertensive rats with hypertension, but not with hyper-tension.

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transgenic mice was done using specific antibodies. The main finding was a significant increase in the basement membrane protein laminin in the glomerula and renal interstitium in comparison with non-transgenic littermates, whereas expression of collagen type III was significantly reduced in the glomerula and renal interstitium (Figure 1, Table 2). By contrast, analysis of the composition of the cardiac matrix showed increased expression of collagen type III. Laminin expression was also enhanced (Figure 1, Table 3).

Our finding of increased laminin expression is in agreement with data from Nakamura et al. [12], which showed increased levels of laminin mRNA in streptozocin-induced diabetic rats. Recent studies showed that the ET system is up-regulated in rats with diabetes; moreover, application of ET-receptor antagonists had an anti-fibrotic effect and reduced proteinuria [13,14], indicating that ET receptor antagonists might be a new approach in the treatment of diabetic nephropathy.

The impact of ET-1 on matrix expression in in vitro models is well known. ET-1 acts as a potent mitogen, but little is known about the individual expression of matrix proteins. The expression of fibronectin and collagen type IV mRNAs is increased in mesangial cells by ET-1 through activation of early response genes [15]. ET-1 also increases the mRNA expression of collagen types I, II and IV, and laminin in mesangial cells. The synthesis of fibronectin in smooth muscle cells is likewise stimulated [16]. In contrast with these in vitro studies, our present study indicates that ET-1 induces in vivo matrix protein synthesis not in general, but rather in a specific way (e.g. up-regulation of laminin and type I collagen, and down-regulation of collagen type III, in the renal interstitial tissue).

The enhanced expression of matrix proteins in the kidneys of ET-1 transgenic mice may be due to a direct stimulation of matrix protein synthesis by ET-1, as well as increased ET-1-induced expression of growth factors such as transforming growth factor-β, which would result in a synergic stimulation of matrix protein synthesis.

The most important finding of our present study (markedly increased laminin expression in ET-1 transgenic mice) is in good agreement with studies analysing abnormal matrix protein expression in animal models, as well as in humans with chronic renal failure such as the Alport syndrome, membranous glomerulonephritis, and in experimental lupus nephritis [17–19].

In summary, the present study indicates that a primary overexpression of ET-1 in vivo results in a tissue-specific matrix protein pattern. The major renal and cardiac alteration of ET-1-induced matrix proteins is markedly enhanced laminin expression.

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