Endothelin ET\textsubscript{A} receptor blockade prevents the progression of renal failure and hypertension in uraemic rats

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Abstract

Background. Elevated plasma and urine endothelin-1 (ET-1) levels have been reported in renal failure and may be involved in renal disease progression. We investigated whether these changes are related to increased vascular and renal ET-1 production in the pole resection remnant kidney model of chronic renal failure in the rat.

Methods. Uraemic Wistar rats were prepared by surgical renal mass 5/6 ablation and compared with sham-operated controls (protocol 1). Immunoreactive-ET-1 (ir-ET-1) concentration was measured by radioimmunoassay after sample extraction and purification. To investigate the functional role of ET-1 during the progression of chronic renal failure, uraemic rats (protocol 2) were treated with either the vehicle or the ET-1 type A (ET\textsubscript{A}) receptor antagonist LU135252 (LU).

Results. Systolic blood pressure and serum creatinine, as well as urinary volume and proteinuria, were significantly higher, whereas creatinine clearance was reduced in uraemic rats compared with sham-operated controls. As expected, plasma and urine ir-ET-1 concentrations were increased in uraemic rats (\(P<0.01\)) and were related to the increased ir-ET-1 levels in blood vessels and glomeruli (\(P<0.01\)). Positive correlation was found between plasma, thoracic aorta and mesenteric arterial bed ir-ET-1 levels and systolic blood pressure, as well as blood vessel hypertrophy. In addition, increased urinary ir-ET-1 excretion correlated with the rise in serum creatinine and proteinuria. In protocol 2, a 3-week treatment period with LU was initiated once uraemia and hypertension were established. In untreated uraemic rats, systolic blood pressure increased further (\(P<0.05\)), but this was not the case in LU-treated uraemic rats. At the end of treatment, serum creatinine and proteinuria were significantly lower (\(P<0.05\)) and creatinine clearance was higher (\(P<0.01\)) in LU-treated rats compared with uraemic-untreated animals. While plasma ir-ET-1 concentration was similar in the two groups, ir-ET-1 concentration in thoracic aorta, mesenteric arterial bed, renal cortex and urine was significantly lower in LU-treated animals (\(P<0.01\)). In addition, heart, thoracic aorta and mesenteric arterial wet weight to body weight ratios were also significantly reduced in LU-treated uraemic rats (\(P<0.05\)).

Conclusions. Elevated plasma ET-1 concentration and urinary ET-1 excretion in rats with renal mass ablation are related to enhanced ET-1 production in vascular and renal tissues, thus suggesting an important role for ET-1 in the aggravation of hypertension and vascular hypertrophy as well as in the progression of renal insufficiency. These pathophysiological effects are prevented by treatment with selective ET\textsubscript{A} receptor blockade.

Key words: chronic renal failure; endothelin-1; ET\textsubscript{A} receptor; hypertension; LU135252; remnant kidney

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Introduction

Endothelin-1 (ET-1) appears to be involved in the pathogenesis and progression of various renal diseases. Increased urinary ET-1 excretion and plasma ET-1 levels have been reported in progressive and end-stage renal failure patients, and correlate with the deterioration of renal function and the elevation of blood pressure [1–3]. These changes were recently attributed to ET-1 overproduction in vascular and renal tissues [4–7]. ET-1 is mainly a local mediator, produced in vascular endothelial and glomerular mesangial cells, as well as in tubular epithelial cells, and acts in an autocrine and paracrine fashion to modulate vascular tone and extracellular volume [8,9]. However, increased circulating ET-1 levels produce potent vasoconstriction leading to systemic and renal haemodynamic changes and hypertension [8,10,11]. In addition, ET-1 induces vascular smooth muscle and mesangial cell growth as well as extracellular matrix protein accumulation [12–15], which are associated...
with vascular remodelling, renal fibrosis and glomerulosclerosis.

The effects of ET-1 are mediated by two receptor subtypes, ET\(_A\) and ET\(_B\), which have distinct pharmacological and molecular characteristics as well as tissue distribution [16–18]. ET\(_A\) and ET\(_B\) are members of the G-protein-coupled, seven-transmembrane-domain receptor family, both coupled to the phospholipase C signalling pathway. The ET\(_B\) receptor is responsible for the vasocostrictor and mitogenic effects of ET-1 on vascular smooth muscle and mesangial cells. In contrast, the ET\(_A\) receptor is the sole receptor subtype expressed in endothelial cells and mediates the release of the endothelium-derived relaxing factor nitric oxide (NO) and prostacyclin [19,20], which play an important role in the normal compensatory vasorelaxation in response to ET-1. In the kidney, the ET\(_A\) receptor predominantly controls the haemodynamic effects of ET-1, while the ET\(_B\) receptor is involved in the tubular regulation of salt and water reabsorption [21].

Recently, we [6,7] and others [4,5] reported increased urinary ET-1 excretion due to overproduction in preglomerular arteries and in glomeruli, in the renal artery branches ligation rat remnant kidney model of chronic renal failure. Although plasma ET-1 level was normal in uraemic animals, ET-1 production was significantly enhanced in blood vessels [6]. The rapid progression of renal failure in this remnant kidney model has been attributed to the activation of the renin–angiotensin system [22]. In fact, treatment with an angiotensin type 1 receptor antagonist normalized blood pressure as well as proteinuria due in part to the reduction in vascular and renal ET-1 production [7]. In the present study, we investigated ET-1 production in another rat remnant kidney model of chronic renal failure with pole resection, in which the renin–angiotensin system appears to be suppressed [22]. To confirm the role of ET-1 in the aggravation of hypertension and the progression of renal insufficiency in this model, one group of uraemic animals was treated with the selective ET\(_A\) receptor antagonist LU1325252 (LU).

**Methods**

**Animal experiments**

Animal experiments, approved by the Laval University Animal Care Committee, were performed on 250 g male Wistar rats (Charles River Canada Inc., St-Constant, Quebec, Canada). The animals were allowed free access to standard laboratory rat chow and tap water and were housed under controlled humidity, temperature and a 12 h light:12 h dark cycle. Renal mass reduction (5/6 nephrectomy) was performed by renal pole resection as described previously [23]. Briefly, the animals were anaesthetized with sodium pentobarbital (Somnotol, 50 mg/kg i.p.; MTC Pharmaceuticals, Cambridge, Ontario, Canada) and, via a flank incision, approximately two-thirds of the left kidney was removed by excision of the upper and lower poles. Blood loss was minimized by the application of gelatin sponges (Gelfoam, Upjohn, Don Mills, Ontario, Canada). One week later, the right kidney was removed. In protocol 1, a group of nephrectomized rats was compared with sham-operated animals used as control. The animals were studied at week 8, i.e. 3 weeks after hypertension was established (systolic blood pressure > 150 mmHg). In protocol 2, all animals underwent subtotal nephrectomy. Similar to in protocol 1, once uraemia and hypertension were established, at week 3, they were divided into two groups with similar systolic blood pressure (180 ± 11 and 172 ± 7 mmHg, n.s.). The first group received the ET\(_A\) receptor antagonist, LU 50 mg/kg/day (kindly provided by Michael Kirchengast, Knoll AG, Ludwigsshelfen, Germany) for 3 weeks, mixed with powdered standard rat chow. The other group received the same food mixture without LU. The dose of LU utilized in this study was previously found to effectively blunt the rise in blood pressure in the angiotensin-II-induced hypertension model [24] as well as the erythropoietin-induced hypertension in this rat remnant kidney model of chronic renal failure [25]. Systolic blood pressure was measured by the tail-cuff method after warming and with slight restriction using an IITC blood pressure system fitted with a Model 29 pulse sensor (IITC Life Science, Woodland Hills, CA, USA). Blood pressure readings were recorded and analysed using a computerized acquisition system (MP100, Biopac System, Goleta, CA, USA). The average of three separate blood pressure readings was used in the data analysis. At the end of each protocol, the animals were placed in metabolic cages and 24-h urine samples were collected and stored at −20 °C for the assessment of protein, sodium, creatinine and immunoreactive ET-1 (ir-ET-1) excretion. The animals were then anaesthetized with pentobarbital (50 mg/kg i.p.) and exsanguinated by abdominal aortic puncture. Blood samples were collected for the measurement of haematocrit, serum creatinine and plasma ir-ET-1. The thoracic aorta, segmented from the first to the eighth caudal ribs, and the complete mesenteric arterial bed, from the first cranial artery to the intestinal border, were removed and cleaned of adipose tissue. Tissues were quickly frozen and stored at −80 °C for ir-ET-1 measurements. The heart was removed, cleaned of blood and weighed. Finally, the remnant kidney of uraemic rats and the kidneys of control animals were removed and dissected longitudinally. The papilla was discarded and the cortex was either immersed in 0.9% saline for immediate preparation of glomeruli (protocol 1) or cut into pieces of 100–50 mg, quickly frozen and stored at −80 °C (protocol 2).

**Preparation of glomeruli**

Glomeruli were prepared as described previously [6,7]. To obtain a sufficient amount of tissue for the assessment of ir-ET-1 concentration, kidneys harvested from two animals were utilized per group. Renal cortex was homogenized by passage through a 0.4-mm stainless-steel grid. Tissues retained on the grid were washed with 0.9% saline solution, minced with scissors and transferred onto a 150-μm mesh nylon sieve (Nixet, B & S.H. Thompson Co., Montmel, Quebec, Canada). Tissues were pressed against the nylon sieve with a spatula to detach the remaining glomeruli used rinsed with saline solution. Glomeruli contained in the homogenate were isolated by filtration through 150, 50 and 100-μm mesh nylon sieves and washed with saline solution. Glomeruli retained on the last sieve were transferred to a centrifugation tube and the average of three aliquots was used to determine the number of glomeruli under a light-microscope. Preparations from control and uraemic rats
contained <5% contamination with connective tissue and tubes. Glomeruli were then collected by centrifugation at 3000 g for 15 min at 4 °C. Supernatants were removed, and the pellets were quickly frozen and stored at −80 °C for the assessment of tissue ir-ET-1.

Measurement of ir-ET-1 in tissue, plasma and urine

One thoracic aorta, mesenteric arterial bed or preparation of glomeruli was utilized per extraction tube and assayed individually as described previously [6,7]. Frozen tissues were weighed, homogenized with a Tissue-Tearor (Biospec Products, Bartlesville, OK, USA) three times for 15 s in 2 ml ice-cold extraction solution containing 1 N HCl, 1% acetic acid, 1% trichloroacetic acid (TFA) and 1% NaCl. The homogenate was centrifuged at 3000 g for 30 min at 4 °C. The supernatant was collected and extracted on a C18 Sep-Pak column (Waters, Milford, MA, USA). Similarly, plasma and urine samples (2 ml) were acidified with 0.2% TFA, and extracted on a C18 Sep-Pak column as reported previously [3,6]. Ir-ET-1 in the sample extracts was measured by a specific radioimmunoassay, and the concentrations were corrected for losses in the extraction and purification steps using small amounts of 125I-ET-1 (~1000 c.p.m.; DuPont NEN, Boston, MA, USA).

Other biochemical analysis

Serum was obtained from 1-ml blood samples incubated for 1 h at room temperature and centrifuged for 2 min in a bench-top microcentrifuge. Serum creatinine, as well as urinary creatinine and protein, were measured with an autoanalyzer system (Ilab 1800, Lexington, MA, USA). Haematocrit was assessed in heparinized Pre-Cal micro-haematocrit tubes (Becton-Dickinson Co., Parsippany, NJ, USA) after 2 min of centrifugation in a bench-top microcentrifuge.

Analysis of data

The results are expressed as means ± SEM. Since protocols 1 and 2 were not performed simultaneously, mean values of the two groups of animals within each protocol were compared using the Student’s t-test and were considered significant at a value of P < 0.05. Simple correlations were obtained by Pearson regression analysis using the Inplot program (GaphPad Software, San Diego, CA, USA).

Results

Systemic and renal parameters in uraemic rats

Body weight was slightly, but significantly, lower in uraemic rats compared with sham-operated controls (P < 0.05; Table 1, protocol 1). In contrast, systolic blood pressure, serum creatinine and plasma ir-ET-1 concentration were significantly higher (P < 0.01), whereas haematocrit was lower in uraemic rats (P < 0.01). In addition, urinary volume, protein and ir-ET-1 excretion were significantly greater in uraemic rats compared with controls (P < 0.01), whereas creatinine clearance was lower (P < 0.01; Table 2, protocol 1). Changes in systolic blood pressure correlated positively with serum creatinine (r = 0.709, P < 0.01) as well as with plasma ir-ET-1 levels (Figure 1). Moreover, the increased urinary ir-ET-1 excretion correlated with the rise in serum creatinine and proteinuria (Figure 2). This suggests that plasma and urinary ET-1 levels are associated with the aggravation of hypertension and the progression of renal insufficiency in this rat remnant kidney model. In fact, uraemic animals in protocol 2, which had a higher degree of renal failure and hypertension compared with uraemic rats in protocol 1 (due to variability in surgical renal mass ablation), were associated with greater plasma and urinary ET-1 levels (Figures 1 and 2).

Tissue ir-ET-1 concentration in uraemic rats

Compared with the controls, ir-ET-1 concentration in the thoracic aorta (P < 0.05) and glomeruli (P < 0.01) was significantly higher in uraemic rats, whereas it was similar in the mesenteric arterial bed (Figure 3). However, the total ir-ET-1 content in both the thoracic aorta and in the mesenteric arterial bed of uraemic rats was significantly higher (9.5 ± 1.8 and 32 ± 3 pg/vessel, respectively) compared with the controls (3.6 ± 0.1 and 22 ± 3 pg/vessel, respectively; P < 0.01). This may be attributed to hypertrophy of vessels of uraemic rats since the thoracic aorta and mesenteric arterial bed wet weight to body weight ratios were significantly greater in uraemic rats compared with controls (Table 3, protocol 1); likewise, the heart wet weight to body weight ratio was also significantly greater in uraemic animals. Similar to plasma ir-ET-1, changes in ir-ET-1 content in the thoracic aorta and the mesenteric arterial bed were correlated with the rise in systolic blood pressure (r = 0.820 and 0.681, respectively; P < 0.01) and, interestingly, with vessel wet weight to body weight ratios (r = 0.507; P < 0.05 and r = 0.766; P < 0.01, respectively).

Effect of the ET_A receptor antagonist LU135252 in uraemic rats

The time course of systolic blood pressure in LU-treated uraemic rats is depicted in Figure 4. At week 3, prior to treatment, systolic blood pressure was significantly higher in uraemic rats (178 ± 7 mmHg) compared with systolic blood pressure before renal mass ablation (120 ± 5 mmHg; P < 0.01). Systolic blood pressure in uraemic–untreated rats increased significantly with time (P < 0.05), whereas it did not change in LU-treated uraemic rats (Figure 4).

Body weight, haematocrit and plasma ir-ET-1 concentration were similar in untreated and LU-treated uraemic rats (Table 1, protocol 2), whereas serum creatinine was significantly lower in LU-treated rats (P < 0.01). In addition, proteinuria was lower (P < 0.05) and creatinine clearance was higher (P < 0.01) in LU-treated rats compared with uraemic–untreated rats (Table 2, protocol 2). Surprisingly, urinary volume was increased in LU-treated rats (P < 0.01). Although urinary ir-ET-1 excretion tended to be lower in LU-treated rats, the difference did not reach statistical
Table 1. Body weight, systolic blood pressure, serum creatinine, haematocrit and plasma ir-ET-1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body weight (g)</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Serum creatinine (µM)</th>
<th>Haematocrit (%)</th>
<th>Plasma ir-ET-1 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>483 ± 17</td>
<td>124 ± 4</td>
<td>44 ± 3</td>
<td>41 ± 1</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>Uraemic</td>
<td>8</td>
<td>432 ± 6³</td>
<td>180 ± 8³</td>
<td>106 ± 10b</td>
<td>33 ± 2b</td>
<td>5.8 ± 0.5b</td>
</tr>
<tr>
<td>Protocol 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uraemic</td>
<td>10</td>
<td>319 ± 25</td>
<td>222 ± 7</td>
<td>277 ± 40</td>
<td>25 ± 2</td>
<td>7.3 ± 0.8</td>
</tr>
<tr>
<td>Uraemic + LU</td>
<td>8</td>
<td>369 ± 14</td>
<td>164 ± 6c</td>
<td>128 ± 13b</td>
<td>28 ± 2</td>
<td>8.6 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SEM. n, number of animals; LU, LU135252; ir-ET-1, immunoreactive-endothelin-1. *P < 0.05 and bP < 0.01 vs control rats; cP < 0.01 vs uraemic untreated rats in protocol 2.

Table 2. Urinary volume, proteinuria, creatinine clearance and ir-ET-1 excretion

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Urinary volume (ml/day)</th>
<th>Urinary protein (mg/day)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Urinary ir-ET-1 (pg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>14 ± 2</td>
<td>8 ± 1</td>
<td>2.13 ± 0.22</td>
<td>52 ± 12</td>
</tr>
<tr>
<td>Uraemic</td>
<td>8</td>
<td>40 ± 5*a</td>
<td>212 ± 29*a</td>
<td>0.97 ± 0.09a</td>
<td>147 ± 32a</td>
</tr>
<tr>
<td>Protocol 2</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uraemic</td>
<td>10</td>
<td>33 ± 4</td>
<td>252 ± 31</td>
<td>0.20 ± 0.05</td>
<td>434 ± 79</td>
</tr>
<tr>
<td>Uraemic + LU</td>
<td>8</td>
<td>51 ± 4*c</td>
<td>156 ± 35b</td>
<td>0.79 ± 0.11c</td>
<td>256 ± 80</td>
</tr>
</tbody>
</table>

Values are means ± SEM. LU, LU135252; ir-ET-1, immunoreactive-ir-ET-1. aP < 0.01 vs control rats; *P < 0.05 and bP < 0.01 vs uraemic untreated rats in protocol 2.

Discussion

In the present study, we reported that ir-ET-1 concentration is significantly increased in the blood vessels and glomeruli of rats with surgical renal mass ablation. These changes are similar to those observed in the renal artery branches ligation remnant kidney model [6,7]. In addition, the increased urinary ET-1 excretion correlated with renal insufficiency, which was associated with greater ET-1 production in renal tissues, particularly in glomeruli. Furthermore, uraemic rats with pole resection have a higher plasma ir-ET-1 concentration. The latter observation contrasts to the former remnant kidney model, as well as chronic non-dialysed uraemic patients, where plasma ir-ET-1 is unchanged [2,4,6,7]. Although one might attribute the higher plasma ET-1 levels to increased ET-1 production in blood vessels, tissue ir-ET-1 concentration in both remnant kidney models are similar [6,7]. Thus, it may be postulated that plasma ET-1 clearance is altered in this remnant kidney model. Normally, locally produced ET-1 in vascular endothelial cells is released on the abluminal side towards smooth muscle cells and only small amounts of ET-1 reach the systemic circulation [26]. ET-1 released into the blood stream is rapidly cleared from the circulation after passage through the lungs [19]. However, it has been reported recently that pulmonary ET-1 clearance is reduced in patients with pulmonary hypertension, leading to a rise in plasma ET-1 [27]. Pulmonary hypertension also occurs in the pole resection rat remnant kidney model and may be a contributing factor for the elevation of circulating ET-1 [28].
The present findings that increased ET-1 levels in vascular and renal tissues, as well as in the plasma of uraemic rats, correlated positively with blood pressure and renal insufficiency support a role for ET-1 in the aggravation of hypertension and the progression of chronic renal failure. Since ET-1 is predominantly a local mediator, the elevated tissue ET-1 levels can cause powerful vasoconstriction leading to hypertension and renal haemodynamic changes [8,10]. These pathophysiological changes may also be related to the increased circulating ET-1 level. Indeed, Wilkins et al. [11] have shown that a long-term, 2-fold rise in circulating ET-1 produces a significant elevation of blood pressure associated with increased total peripheral and renal vascular resistance, and reduced glomerular filtration rate. In the kidney, afferent and efferent arteriolar constriction in response to ET-1 reduces renal blood flow and increases glomerular capillary pressure [10], which may, in the long-term, contribute to glomerular injury.

The direct involvement of ET-1 in the present uraemic animal model was further confirmed by the fact that treatment with an ET_A receptor antagonist prevented the aggravation of hypertension and the progression of renal insufficiency. However, blood pressure and renal parameters such as proteinuria were not reduced to normal values. This may be attributed, in part, to the unblocked ET_B receptor, which can also mediate the vasoconstrictor response to ET-1 [29]. However, blockade of both receptor subtypes with the ET_A/ET_B antagonist bosentan causes a similar blood pressure lowering effect and attenuation of the progression of renal insufficiency as with ET_A receptor blockade [25,30]. Thus, other mechanisms, not affected by ET-1 receptor blockade, must be involved in the maintenance of hypertension and renal disease progression. These may include impaired vasorelaxing effect of the endothelium-derived relaxing factor NO. In fact, NO release appears to be reduced in uraemia, due in part to the accumulation in plasma of asymmetrical dimethylarginine, an endogenous inhibitor of NO synthase [31]. This is consistent with the fact that L-arginine supplementation in uraemic animals attenuates the rise in blood pressure and the progression of renal failure [32]. With respect to the renin–angiotensin system, although plasma renin activity is suppressed in this rat remnant kidney model [22,30], angiotensin-converting enzyme inhibition normalizes blood pressure and significantly attenuates proteinuria [30], suggesting that angiotensin II is also implicated in the pole resection model, similar to in the renal artery branches ligation model [7].

In this study, we also showed that increased vascular ET-1 levels in uraemic rats were associated with greater wet weight to body weight ratio of blood vessels. In addition, blood vessel wet weight to body weight ratios were significantly reduced in LU-treated rats to levels comparable with those in sham-operated animals, suggesting a role for ET-1 in vascular hypertrophy in uraemia. This is consistent with our previous studies in hypertensive rats where increased ET-1 production in blood vessels was associated with increased tissue wet weight and vascular media hypertrophy, which were both significantly reduced by ET-1 receptor blockade [33–35]. ET-1 may contribute to vascular hypertrophy in uraemia through its potent effect on smooth muscle cell growth as well as its effect on extracellular matrix protein accumulation [12,14]. A similar phenomenon probably occurs in the glomerulus to promote glomerulosclerosis [15,36]. This is supported by the study of Orisio et al. [5] documenting the relationship between increased renal ET-1 production and glomerulosclerosis in rats with reduced renal mass. In addition, ET-1 receptor blockade has been shown to prevent glomerulosclerosis in remnant kidneys [30,37]. Thus, the ET_A receptor antagonism with LU may also cause protective effects by attenuating vascular hypertrophy and remodelling, and glomerular damage.

The mechanisms leading to enhanced ET-1 production in uraemic animals are not completely understood. Recently, we reported that angiotensin II is an important modulator of ET-1 production in blood vessels.
Effects of the endothelin type A (ET\(_A\)) receptor antagonist LU135252 (LU) on systolic blood pressure in uraemic rats. Treatment with LU (50 mg/kg/day) was initiated once uraemia and hypertension were established, at week 3, for 3 weeks. Nx, time point of subtotal (5/6) nephrectomy. *\(P<0.05\) systolic blood pressure in untreated uraemic rats at weeks 3 and 6; **\(P<0.01\) vs LU-treated uraemic rats at the same time point.

Table 3. Cardiovascular tissue wet weight to body weight ratio

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Wet weight/body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mesenteric arterial bed (10(^{-6}))</td>
</tr>
<tr>
<td>Protocol 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>82 ± 15</td>
</tr>
<tr>
<td>Uraemic</td>
<td>8</td>
<td>130 ± 13(^a)</td>
</tr>
<tr>
<td>Protocol 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uraemic</td>
<td>10</td>
<td>225 ± 31</td>
</tr>
<tr>
<td>Uraemic + LU</td>
<td>8</td>
<td>107 ± 11(^d)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. LU, LU135252.

\(^aP<0.05\) and \(^dP<0.01\) vs control rats; \(^dP<0.05\) and \(^dP<0.01\) vs uraemic untreated rats in protocol 2.
ET<sub>A</sub> receptor blockade in uraemic rats

ET<sub>A</sub> receptor blockade in uraemic rats induces NO release from endothelial cells, which inhibits ET-1 production [19,20].

In this study, we documented that ET<sub>A</sub> receptor blockade significantly reduced both ET-1 levels in blood vessels and renal tissues, as well as ET-1 excretion in uraemic animals. The mechanisms underlying this phenomenon remain unknown. However, it can be postulated that the reduction in blood pressure and, possibly, the haemodynamic shear stress may attenuate ET-1 production. This may also be related to ET<sub>B</sub>-induced NO release from the endothelial cells, which inhibit both the vasopressor effect and the local production of ET-1 [19,20]. In addition, it has been reported that the ET<sub>B</sub> receptor subtype is also involved in the clearance of ET-1 [43]. Thus, unblocked ET<sub>B</sub> receptor can contribute to reduce the tissue ET-1 level in uraemic animals. In contrast, it is unlikely that ET<sub>A</sub> receptor blockade directly affects ET-1 level. In fact, ET-1 can stimulate its own production in endothelial cells as well as in mesangial cells via the ET<sub>B</sub> receptor activation, but not the ET<sub>A</sub> receptor [44,45]. However, ET<sub>A</sub> receptor blockade may reduce ET-1 levels through the attenuation of ET-1-induced production of growth factors such as transforming growth factor (TGF)−β [46], a potent stimulator of ET-1 production in endothelial and mesangial cells [38,47].

In summary, increased ET-1 levels in the plasma and urine of uraemic rats with surgical renal mass ablation are related to enhanced ET-1 production in blood vessels and glomeruli. The protective effects of treatment with the selective ET<sub>A</sub> receptor antagonist LU135252 confirms the role of ET-1 in the aggravation of hypertension and the progression of renal insufficiency in this rat remnant kidney model of chronic renal failure.

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References


Fig. 5. Immunoreactive endothelin-1 (ir-ET-1) concentration in thoracic aorta (A), mesenteric arterial bed (B) and renal cortex (C) of uraemic rats untreated (Nx) and treated with LU135252 (Nx + LU). *P<0.01 vs untreated uraemic rats.

It has been reported that increased pressure causes a dose-dependent rise in ET-1 release in endothelial cells [39]. Increased blood pressure may also affect the haemodynamic shear stress, which as been shown to induce ET-1 production [40]. This remains, however, controversial since low shear stress stimulates [40], whereas high shear stress inhibits, ET-1 production [41,42]. The haemodynamic shear stress may vary in different vascular beds such as in conduit arteries (aorta) and in resistance arteries (mesenteric), thus affecting ET-1 production differently. The impaired NO release in uraemic animals could also influence ET-1 production in blood vessels and glomeruli. Under normal conditions, a slight change in ET-1 concentration in the vessel wall, and possibly in the glomerulus,


