Supplementation with a low dose of \(\text{L-arginine}\) reduces blood pressure and endothelin-1 production in hypertensive uraemic rats

Yannick Dumont, Martin D’Amours, Marcel Lebel and Richard Larivière

Research Center and Division of Nephrology, CHUQ, L’Hôpital-Dieu de Québec, and Department of Medicine, Laval University, Quebec, Canada

Abstract

**Background.** We documented recently that increased endothelin-1 (ET-1) production in blood vessels and glomeruli of uraemic rats plays a crucial role in the development of hypertension and the progression of chronic renal failure. Normally, biological effects and local production of ET-1 are attenuated by the immediate release of nitric oxide (NO). Increasing evidence suggest, however, that NO release is impaired in chronic renal failure. We investigated whether supplementation with \(\text{L-arginine}\), the natural precursor of NO, improves NO synthesis in uraemic rats with reduced renal mass and modulates vascular and renal ET-1 production as well as blood pressure and renal failure progression.

**Methods.** One week after surgical renal mass reduction, the uraemic and sham-operated animals received either no treatment or 0.1% \(\text{L-arginine}\) in drinking water for 5 weeks. In another series of experiments, uraemic rats received 1% \(\text{L-arginine}\) for 5 weeks. Immunoreactive-ET-1 (ir-ET-1) levels in plasma, urine, and vascular and renal tissue preparations were measured by radioimmunoassay after sample extraction and purification.

**Results.** Before treatment, systolic blood pressure was significantly elevated in uraemic animals compared to sham-operated controls (156 ± 7 vs 111 ± 3 mmHg, respectively; \(P < 0.01\)). Thereafter, systolic blood pressure increased further in uraemic-untreated rats (systolic blood pressure at week 5: 199 ± 9 mmHg, \(P < 0.01\)), whereas it remained similar in uraemic rats supplemented with 0.1% \(\text{L-arginine}\) (171 ± 9 mmHg, NS). At the end of the study, serum creatinine and urea, proteinuria and ir-ET-1 excretion were significantly augmented, while creatinine clearance was reduced in uraemic animals compared to the controls. Ir-ET-1 level was also increased in glomeruli as well as in thoracic aorta, mesenteric arterial bed, and pre-glomerular arteries, and was associated with vascular hypertrophy as assessed by tissue weight. In contrast, ir-ET-1 level was diminished in the renal papilla of uraemic rats. Treatment with 0.1% \(\text{L-arginine}\) significantly reduced proteinuria and urinary ir-ET-1 excretion (\(P < 0.05\)) as well as ir-ET-1 level in glomeruli (\(P < 0.01\)) and in thoracic aorta (\(P < 0.05\)). These changes were associated with increased plasma NO metabolites NO\(_2\)/NO\(_3\) levels in \(\text{L-arginine}\)-treated animals (\(P < 0.01\)) and reduced aortic hypertrophy (\(P < 0.05\)). In contrast, supplementation with 1% \(\text{L-arginine}\) had no effect on systolic blood pressure in uraemic rats, but exacerbated proteinuria and urinary ir-ET-1 excretion and increased serum urea (\(P < 0.05\)) were observed.

**Conclusions.** These results indicate that improvement of NO release with a low dose but not with a high dose of \(\text{L-arginine}\) significantly attenuates the development of hypertension and the progression of renal insufficiency in rats with reduced renal mass. These protective effects may be mediated in part by the reduction of vascular and renal ET-1 production.

**Keywords:** chronic renal failure; endothelin-1; hypertension; \(\text{L-arginine}\); nitric oxide

Introduction

Endothelin-1 (ET-1) has been implicated in various renal diseases, such as chronic renal failure, and may play a crucial role in the pathogenesis of hypertension and renal damage [1,2]. Increased plasma ET-1 levels and urinary ET-1 excretion in progressive and end-stage renal failure patients have been documented to correlate with deterioration of renal function and elevated blood pressure [1–3]. Recently we and others have reported that in rat remnant kidney models of chronic renal failure, these changes are related to ET-1 overproduction in blood vessels and glomeruli [4–8]. Increased ET-1 levels in vascular and renal tissues may
lead to local vasoconstriction and vascular hypertrophy as well as glomerular sclerosis, renal fibrosis and hyperfiltration [4,9–11]. Treatment of uremic rats with ET-1 receptor antagonists blunt the rise of blood pressure and attenuate the development of glomerular damage [8,11], supporting a major role for ET-1 in the development of hypertension and the progression of renal insufficiency.

The factors modulating ET-1 production in chronic renal failure remain unidentified. Under normal conditions, however, a slight increase of ET-1 secretion within the vessel wall induces the immediate release of the endothelium-derived relaxing factor nitric oxide (NO), which attenuates the biological effects of ET-1 and suppresses its local production to maintain blood pressure homeostasis [12–14]. Inhibition of NO synthase (NOS) in intact animals potentiates the systemic and renal vasconstrictor response to ET-1 [15]. In addition, as in uremia, chronic NOS inhibition causes hypertension and renal damage associated with increased plasma and urine ET-1 levels [16–18]. In chronic renal failure, NO release appears to be impaired [19]. This has been attributed in part to the accumulation in plasma of endogenous inhibitors of NOS and enhanced production of oxygen free radicals, which rapidly inactivate NO [20–22].

The present study was designed to investigate the role of NO in the modulation of ET-1 production in chronic renal failure. The effects of dietary supplementation with l-arginine, the natural precursor of NO, at a low dose (0.1%) and a high dose (1%), on ET-1 production in vascular and renal tissues as well as blood pressure and renal function were examined in uremic rats with reduced renal mass.

Subjects and methods

Animal experiments

Animal experiments, approved by the Animal Care Committee of Laval University, were performed on 250 g male Sprague-Dawley rats (Charles River Laboratories, St Constant, Quebec, Canada). The animals were allowed free access to tap water and standard laboratory rat chow containing 18% protein (Agribrands Canada, Woodstock, Ontario, Canada), and housed under controlled humidity, temperature and a 12-h light-dark cycle. Renal mass was reduced as described previously [6,7], by ligating 2–3 branches of the left renal artery, followed 1 week later by right nephrectomy under pentobarbital anaesthesia (Somnotol, 50 mg/kg, i.p., MTC Pharmaceuticals, Cambridge, Ontario, Canada). In a series of experiments, 1 week after surgery, uremic and sham-operated control animals were divided into two groups with similar systolic blood pressure (Figure 1) (systolic blood pressure increase was previously shown to be closely related to the degree of renal failure [6]), and received either no treatment (n = 8 and 7 respectively) or were given 0.1% l-arginine (Sigma Chemical Co., St Louis, MO, USA) (n = 9 and 8 respectively) in drinking water for 5 weeks. The amount of l-arginine administered during the study was approximately 0.2 g/kg/day. In another series of experiments, uremic rats received either no treatment (n = 9) or 1% l-arginine (approximately 2.3 g/kg/day) (n = 9). Both doses of l-arginine were selected based on previous reports documenting renal protection in uremic rats [23–26]. 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). The blood pressure readings were analysed by a computerized data acquisition system (Model MP100, Biopac Systems, Goleta, CA, USA), and the average of three separate measurements was recorded. At week 6, the rats were placed in metabolic cages for an adaptation period of 24 h followed by collection of 24-h urine samples that were then stored at −20°C. The animals were anaesthetised with pentobarbital (50 mg/kg i.p.) and exsanguinated by abdominal aortic puncture. The thoracic aorta, segment from the 1st to the 8th caudal rib, and the complete mesenteric vascular bed, from the 1st cranial artery to the intestinal border, were removed, cleaned of blood and adipose tissue, frozen quickly and stored at −80°C. The heart was removed, cleaned of blood and weighed. The remnant kidney of uremic rats and the left kidney of the controls were removed, weighed, and dissected longitudinally. The papilla was removed and frozen quickly, as above. The renal cortex was immersed in Hank’s balanced salt solution (HBSS) (137 mmol/l NaCl, 5.4 mmol/l KCl, 0.04 mmol/l MgSO4, 0.5 mmol/l MgCl2, 1.25 mmol/l CaCl2, 0.44 mmol/l KH2PO4, 0.33 mmol/l Na2HPO4, and 4 mmol/l NaHCO3) for the preparation of preglomerular arteries and glomeruli.

Preparation of preglomerular arteries and glomeruli

Preglomerular arteries and glomeruli from renal cortex of the left kidney of control rats and the remnant kidney of
uraemic rats were prepared by a sieving method, as described previously [6,7]. Briefly, renal cortex were homogenized by passage through a 0.4-mm stainless steel grid. Preglomerular interlobar, arcuate, and interlobular arteries retained on the grid were washed with HBSS, minced with scissors and transferred onto a 150-μm mesh nylon sieve (NiteX, B & SH Thompson Co., Montreal, Quebec, Canada). To detach the remaining glomeruli, the tissues were pressed against the nylon sieve with a spatula and rinsed with HBSS. The renal arteries were transferred to a centrifugation tube, while filtrated tissues were combined with the first homogenate. Glomeruli in the homogenate were isolated by filtration through 150-, 50- and 100-μm mesh nylon sieves and washed with HBSS. Glomeruli retained on the last sieve were transferred to a centrifugation tube, and aliquots were used to determine the number of glomeruli under light microscopy. Preparations from the different groups of rats contained less than 5% contamination with connective tissues and tubules. Preglomerular arteries and glomeruli were 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.

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

One thoracic aorta segment, mesenteric arterial bed, or preparation of pregglomerular arteries and glomeruli was utilized per extraction tube and assayed individually [6,7]. Frozen tissues were weighed, homogenized twice with a Tissue-Tearor (Biospec Products, Bartlesville, OK, USA) in 2 ml ice-cold extraction solution containing 1 N HCl, 1% acetic acid, 1% trifluoroacetic acid (TFA) and 1% NaCl and 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 [3]. 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).

Measurement of NO$_2$//NO$_3$ in plasma and urine

Plasma and urine samples were diluted with one volume potassium phosphate buffer pH 7.4, deproteinized by ultrafiltration in Centrisart tubes with a molecular weight cut-off of 20 000 Dalton (Sartorius, Gottinger, Germany), and centrifuged at 2000 g for 45 min at 4°C. The ultrafiltrate (250 μl) was incubated in the presence of 0.4 U/ml nitrate reductase, 150 μg/ml NADPH, and 3 μg/ml FAD (Roche Diagnostics, Laval, Quebec, Canada) for 30 min at 37°C to convert nitrate to nitrite. A 150-μl aliquot of the reaction mixture was added to an equal volume of Greiss reagent (prepared by mixing 1 part of 1% sulphanilamide to 1 part of 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride in 5 N HCl) and incubated for 5 min at room temperature. Absorbance at 550 nm was measured, and nitrite concentration was determined using potassium nitrate standard which was also reduced to nitrate with nitrate reductase. The reaction was linear from 0.25 to 80 μmol/l of nitrate.

Other biochemical analyses

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 and urea as well as urinary protein, creatinine and urea were measured with an auto-analyser system (Ilab 1800, Lexington, MA, USA). Hematocrit was assessed in Pre-Cal micro-hematocrit heparinized tubes (Becton Dickinson, Parsippany, NJ, USA) after 2 min of centrifugation in a bench top micro-centrifuge. Plasma renin activity was measured using a radioimmunoassay kit for angiotensin I purchased from DuPont NEN.

Analysis of data

The results are expressed as means ± SEM. Mean values were compared by ANOVA, followed by Student–Newman–Keul test for multiple comparisons. Differences were considered significant at a value of P < 0.05. Simple correlations were obtained by Pearson regression analysis using the Inplot program (GraphPad Software, San Diego, CA, USA).

Results

Effects of 0.1% l-arginine

One week after renal mass reduction, systolic blood pressure was already significantly higher in uraemic rats compared to sham-operated controls (156 ± 7 vs 111 ± 3 mmHg, P < 0.01). Thereafter, systolic blood pressure increased further in untreated uraemic rats (P < 0.01), whereas supplementation with 0.1% l-arginine prevented the aggravation of hypertension (Figure 1). In contrast, l-arginine had no effect on systolic blood pressure in control animals.

Serum creatinine and urea were also significantly higher in uraemic rats compared to the controls (P < 0.01), while haematocrit was reduced (P < 0.01;

<table>
<thead>
<tr>
<th>Group</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Serum creatinine (μmol/l)</th>
<th>Haematocrit (%)</th>
<th>Plasma ir-ET-1 (pg/ml)</th>
<th>Plasma NO$_2$/NO$_3$ (μmol/l)</th>
<th>Serum urea (mmol/l)</th>
<th>Plasma renin activity (ng Ang I/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>113 ± 2</td>
<td>49 ± 2</td>
<td>40.0 ± 0.4</td>
<td>2.5 ± 0.6</td>
<td>6.6 ± 3.1</td>
<td>5.6 ± 0.2</td>
<td>24.9 ± 5.7</td>
</tr>
<tr>
<td>Control + l-arg</td>
<td>111 ± 2</td>
<td>39 ± 2</td>
<td>41.1 ± 0.5</td>
<td>4.7 ± 0.7</td>
<td>16.0 ± 3.1*</td>
<td>6.1 ± 0.4</td>
<td>27.5 ± 2.7</td>
</tr>
<tr>
<td>Uraemic</td>
<td>199 ± 9**</td>
<td>122 ± 18**</td>
<td>32.8 ± 2.0**</td>
<td>4.1 ± 0.6</td>
<td>25.3 ± 2.3**</td>
<td>21.1 ± 4.5**</td>
<td>8.4 ± 1.4**</td>
</tr>
<tr>
<td>Uraemic + l-arg</td>
<td>171 ± 9***</td>
<td>109 ± 7***</td>
<td>37.2 ± 1.9**</td>
<td>3.5 ± 0.4</td>
<td>36.2 ± 2.8***</td>
<td>20.1 ± 2.4**</td>
<td>2.0 ± 0.4**</td>
</tr>
</tbody>
</table>

Values are means ± SEM obtained from 7-9 rats per group, l-arg, l-arginine; ir-ET-1, immunoreactive endothelin-1; NO$_2$/NO$_3$, nitrate and nitrite; Ang I, angiotensin I. *P < 0.05 vs control animals; **P < 0.01 vs control groups; †P < 0.05; ‡P < 0.01 vs uraemic animals.
Table 1. l-arginine supplementation had no effect on serum creatinine and urea, but attenuated the anaemia in uremic animals (P < 0.05). Plasma ir-ET-1 concentrations were similar in the four groups of rats (Table 1). Plasma NO$_2$/NO$_3$, however, was significantly higher in uremic animals compared to the controls (P < 0.01; Table 1). As expected, treatment with l-arginine increased plasma NO$_2$/NO$_3$ in both control and uremic rats. In contrast, plasma renin activity was suppressed in uremic rats and was not affected by l-arginine (Table 1).

Urinary volume, protein, ir-ET-1 (P < 0.01) and urea (P < 0.05) were also greater in uremic rats than in the controls, whereas NO$_2$/NO$_3$ and creatinine clearance were lower (P < 0.01; Table 2). l-arginine attenuated the proteinuria and urinary ir-ET-1 excretion in uremic rats (P < 0.05), but had no effect on urinary volume, urea, NO$_2$/NO$_3$ and creatinine clearance (Table 2).

Ir-ET-1 concentration in thoracic aorta and preglomerular arteries was higher in uremic rats compared to the controls (P < 0.01), but was similar in the mesenteric arterial bed of the two groups (Figure 2). However, total ir-ET-1 content in thoracic aorta, mesenteric arterial bed and preglomerular arteries was significantly greater in uremic rats than in the controls (14.7 ± 3.7 vs 3.5 ± 0.5; 68.9 ± 9.0 vs 45 ± 3, and 185 ± 36 vs 51 ± 1 pg respectively; P < 0.01). These differences may be attributed to hypertrophy of vessels of uremic animals. Indeed, the wet wt of the thoracic aorta, the mesenteric arterial bed, and preglomerular arteries as well as the heart wt to body weight ratio were significantly greater in uremic rats compared to the controls, indicating cardiovascular hypertrophy (Table 3). Although treatment with l-arginine had no effect on ir-ET-1 concentrations in any blood vessel preparation (Figure 2), it significantly reduced the ir-ET-1 content in thoracic aorta of uremic animals (8.9 ± 1.2 pg; P < 0.05), and tended to lower ir-ET-1 content in preglomerular arteries (151 ± 18 pg, NS). This difference may be related to reduced thoracic aorta wet weight in uremic rats treated with l-arginine (Table 3). However, l-arginine did not affect ir-ET-1 content in the mesenteric arterial bed of uremic animals (63.4 ± 6.5 pg).

The ir-ET-1 level was also significantly higher in glomeruli of uremic rats compared to the controls (P < 0.01; Figure 3), whereas it was reduced in the renal papilla (P < 0.05). Treatment with l-arginine normalized the ir-ET-1 concentration in glomeruli of uremic rats, but had no effect on renal papillary ir-ET-1 concentration. Interestingly, urinary ir-ET-1 excretion correlated positively with glomerular ir-ET-1 concentration (Figure 4A) as well as with proteinuria (Figure 4B), suggesting that changes in urinary ET-1 in chronic renal failure reflect its overproduction in the glomeruli and renal damage progression.

Fig. 2. Immunoreactive endothelin-1 (ir-ET-1) concentration in thoracic aorta (A), mesenteric arterial bed (B), and preglomerular arteries (C) of control and uremic rats untreated or treated with 0.1% l-arginine (l-arg). Values are means ± SEM obtained from 7–9 rats per group. *P < 0.01 vs control groups.

Table 2. Renal parameters at week 6 in control and uremic rats untreated or treated with 0.1% l-arginine

<table>
<thead>
<tr>
<th>Group</th>
<th>Urinary volume (ml/24 h)</th>
<th>Urinary protein (mg/24 h)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Urinary ir-ET-1 (pg/24 h)</th>
<th>Urinary NO$_2$/NO$_3$ (μmol/24 h)</th>
<th>Urinary urea (mol/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18 ± 2</td>
<td>23 ± 2</td>
<td>2.2 ± 0.1</td>
<td>39 ± 4</td>
<td>15.5 ± 1.4</td>
<td>9.5 ± 0.4</td>
</tr>
<tr>
<td>Control + l-arg</td>
<td>15 ± 2</td>
<td>24 ± 4</td>
<td>2.6 ± 0.5</td>
<td>37 ± 4</td>
<td>13.2 ± 1.6</td>
<td>8.5 ± 0.8</td>
</tr>
<tr>
<td>Uremic</td>
<td>33 ± 3***</td>
<td>138 ± 41***</td>
<td>0.7 ± 0.1***</td>
<td>158 ± 48***</td>
<td>7.2 ± 1.1***</td>
<td>11.9 ± 0.8*</td>
</tr>
<tr>
<td>Uremic + l-arg</td>
<td>33 ± 2***</td>
<td>62 ± 14</td>
<td>0.7 ± 0.1**</td>
<td>72 ± 12**</td>
<td>6.5 ± 1.5**</td>
<td>11.5 ± 0.9*</td>
</tr>
</tbody>
</table>

Values are means ± SEM obtained from 7–9 rats per group. l-arg, l-arginine; ir-ET-1, immunoreactive endothelin-1. *P < 0.05 and **P < 0.01 vs control groups; †P < 0.05 vs uremic animals.
Effects of 1% L-arginine

Uraemic rats were also supplemented with a higher dose of L-arginine (1%) to determine whether additional protective effects can be achieved. In contrast, supplementation with 1% L-arginine had no effect on systolic blood pressure in uraemic rats compared to untreated uraemic rats (systolic blood pressure at end of the study; 181 ± 10 vs 175 ± 11 mmHg respectively; NS). Serum creatinine (88 ± 10 vs 91 ± 6 μmol/l) and creatinine clearance (1.2 ± 0.1 vs 1.1 ± 0.1 ml/min) were also similar in untreated and L-arginine-treated uraemic rats. In contrast, we observed increased urinary volume (49 ± 2 vs 31 ± 2 ml/24 h; \( P < 0.01 \)), ir-ET-1 excretion (116 ± 35 vs 45 ± 11 pg/24 h; \( P < 0.05 \)), and proteinuria (81 ± 25 vs 47 ± 12 mg/24 h) in uraemic animals receiving 1% L-arginine, although the changes in proteinuria was not statistically significant. Moreover, serum urea (21 ± 3 vs 14 ± 2 mmol/l; \( P < 0.05 \)) and urinary urea (12.5 ± 0.9 vs 9.2 ± 0.6 mol/24 h; \( P < 0.01 \)) were significantly greater in uraemic animals treated with 1% L-arginine compared to untreated uraemic rats.

Discussion

In this study, we show that supplementation with a low dose of L-arginine (0.1%) blunted the rise in systolic blood pressure and significantly decreased proteinuria in rats with reduced renal mass. These changes are associated with diminished ET-1 levels in blood vessels and more significantly in glomeruli, suggesting that alteration of the L-arginine/NO pathway in rats with chronic renal failure plays an important role in the modulation of ET-1 production as well as the development of hypertension and the progression of renal insufficiency.

The major finding of the present study is that the antihypertensive and renal protective effects of L-arginine supplementation in rats with reduced renal mass may be related in part to reduction of vascular and renal ET-1 production. Using different remnant kidney models of chronic renal failure, we and others have shown previously that ET-1 plays an important role in the development of hypertension and the progression of renal insufficiency [4–6,8]. Indeed, increased ET-1 levels in blood vessels and glomeruli of rats with reduced renal mass was associated with elevated blood pressure and the progression of renal damage and glomerulosclerosis. In this regard, increased tissue ET-1 content was previously shown to be related to increased ET-1 gene expression in blood vessels of hypertensive rats [10,27]. This is similar to what we and others recently observed in

![Image](image_url)

**Fig. 3.** Immunoreactive endothelin-1 (ir-ET-1) concentration in renal papilla (A), and glomeruli (B) of control and uraemic rats untreated or treated with 0.1% L-arginine (L-arg). Values are means ± SEM obtained from 7–9 rats per group. * \( P < 0.05 \) vs control groups; ** \( P < 0.05 \) vs other groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood vessel wet weight (mg)</th>
<th>Heart wet weight/body weight ratio (10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (g)</td>
<td>Thoracic aorta</td>
</tr>
<tr>
<td>Control</td>
<td>479 ± 22</td>
<td>49 ± 3</td>
</tr>
<tr>
<td>Control + L-arg</td>
<td>471 ± 11</td>
<td>51 ± 2</td>
</tr>
<tr>
<td>Uraemic</td>
<td>390 ± 16**</td>
<td>72 ± 7*</td>
</tr>
<tr>
<td>Uraemic + L-arg</td>
<td>380 ± 15**</td>
<td>55 ± 5†</td>
</tr>
</tbody>
</table>

Values are means ± SEM obtained from 7–9 rats per group. L-arg, L-arginine. * \( P < 0.05 \) and ** \( P < 0.01 \) vs control groups; † \( P < 0.05 \) vs uraemic animals.
blood vessels and the remnant kidney of uremic rats [5,28]. This confirms that changes in vascular and renal tissue ET-1 levels reflect its local production. Increased tissue ET-1 levels may cause a potent vasoconstriction, leading to increased blood pressure and significant renal haemodynamic changes [9,29]. In addition, ET-1 can induce vascular smooth-muscle and glomerular mesangial cell proliferation [30,31], as well as expression of extracellular matrix proteins [32,33] which are involved in vascular hypertrophy and glomerulosclerosis.

With regard to vascular hypertrophy, we show that increased vascular ET-1 levels in uremic rats were associated with greater blood vessel wet weight. This is consistent with previous studies in hypertensive rats, showing positive correlation between vessel weight and media thickness in blood vessels with increased ET-1 production [10,34,35]. Recently, similar changes in structural parameter of vessels of uremic rats were observed [28]. The implication of ET-1 in these pathological processes in uremic rats is also supported by the fact that ET receptor blockade prevents the development of hypertension and the vascular hypertrophy as well as the development of glomerular sclerosis [8,11]. Normally, the vasopressor and hypertrophic effects of ET-1 as well as its local endothelial production are attenuated by NO [12,13]. The impaired NO compensatory effects in uremic rats may unmask the pathological actions of ET-1 and modulate its production. Consistent with this hypothesis is the fact that improvement of NO release with l-arginine supplementation reduced ET-1 levels in blood vessels and in glomeruli, which resulted in attenuated rise in blood pressure and vessel weight as well as the rate of progression of renal insufficiency. Moreover, Ingram et al. [23] recently documented that the reduced early cell proliferation in the remnant glomerulus following short-term (2 weeks) supplementation with l-arginine is mediated in part by the attenuation of local ET-1 production. The involvement of NO in the modulation of ET-1 production is further supported by the recent observation that treatment of uremic animals with a NO donor reduces ET-1 excretion [36].

This study is also consistent with those indicating that urinary ET-1 excretion reflects its renal production and is a marker of renal glomerular damage [1,4,5,11]. Indeed, we have reported previously that urinary ET-1 excretion is related predominantly to its overproduction within the remaining glomeruli, but not in the papilla [6,7]. Similarly, we show here that the glomerular protection of l-arginine is associated with lower ET-1 excretion, which is related to a significant reduction of glomerular ET-1 production.

Although improvement of NO release with l-arginine supplementation significantly decreased ET-1 level in the thoracic aorta, it had moderate or no effect on renal and mesenteric artery ET-1 level. As reported previously, ET-1 level in thoracic aorta may be closely related to changes in blood pressure [6–8]. Therefore, variations in pressure and haemodynamic shear stress can affect ET-1 production in aortic endothelial cells [37,38]. In other vascular beds, however, pressure and shear stress may be differentially affected, thus modulating ET-1 production differently. In contrast, reduction of ET-1 production in glomeruli may be independent of systemic blood pressure reduction, but modulated by improvement of local NO release. In fact, we have recently documented that although treatment with the angiotensin AT1 receptor antagonist losartan, the angiotensin-converting enzyme inhibition captopril, and the conventional triple

---

**Fig. 4.** Correlation between changes in urinary immunoreactive endothelin-1 (ir-ET-1) excretion and glomerular ir-ET-1 concentration (A), and proteinuria (B) in control and uremic rats untreated or treated with 0.1% l-arginine (l-arg).
therapy with reserpine, hydralazine, and hydrochlorothiazide normalized blood pressure in rats with reduced renal mass, only losartan significantly prevented glomerular ET-1 overproduction and ET-1 excretion [7,39]. This indicates that the effects of angiotensin II receptor blockade on renal ET-1 production are independent on blood pressure changes, similarly as in this study with the improvement of renal NO release with L-arginine.

Our finding that supplementation with 0.1% L-arginine attenuates the rise in blood pressure in uraemic rats is consistent with studies documenting that NO release is impaired in chronic renal failure, and may also be involved in the pathogenesis of hypertension [19,24,40]. It is well recognized that basal release of the endothelium-derived relaxing factor NO is crucial in the control of blood pressure. Indeed, mice with deficient endothelial constitutive NOS have elevated blood pressure [41]. In addition, long-term NOS inhibition with the L-arginine analogue Nω-nitro-L-arginine methyl ester induces severe hypertension in normal animals [18]. The improvement of NO release in both control and uraemic rats supplemented with L-arginine is supported by significant elevation of the inactive NO metabolites NO2 NO3 in plasma. This increase in plasma NO2 NO3 was not related to reduction of renal clearance, since serum creatinine and urea as well as creatinine clearance and urinary urea excretion were unaffected. However, in uraemic rats whose blood pressure was significantly higher, plasma NO2 NO3 concentrations were significantly increased compared to the controls. Similar findings have been reported by other groups using the same rat remnant kidney model [42], as well as in patients with chronic progressive renal failure [19]. The heightened plasma NO2 NO3 level in rats with reduced renal mass has been attributed to increased vascular NOS activity [42]. However, Vaziri et al. [43] recently reported no changes in plasma NO2 NO3 and reduced aorta NOS activity in rats with subtotal 5/6 nephrectomy (renal mass resection model), indicating differential regulation of the vascular NOS system in different rat remnant kidney models. It may be postulated that in this remnant kidney model as well as in human chronic renal failure, although vascular NO release may be enhanced, it remains insufficient to prevent the development of hypertension. This notion is supported by the fact that NO inhibition aggravates hypertension in rats with reduced renal mass [44]. The inappropriate NO level in chronic renal failure has been attributed in part to the accumulation in plasma of endogenous NOS inhibitors such as asymmetrical dimethyl-arginine and methylguanidine [20,22]. In addition, the effects of NO may be limited by its rapid inactivation by oxygen free radicals, which are also increased in uraemic animals [21]. Based on the results of the present study, supplementation with L-arginine at a low dose may significantly enhance NO bioavailability, and consequently improve compensatory vasodilatation effect.

This study also indicates that improvement of renal NO release with L-arginine supplementation may have important beneficial consequences in uraemic rats. In contrast to peripheral vessels, NO formation appears to be suppressed in the remnant kidney as revealed by reduced NO2 NO3 excretion in uraemic rats. This is in line with a decline in renal NOS activity and the expression reported in different rat remnant kidney models [42,43,45]. Interestingly, reduced renal NOS activity has been recently associated with salt-sensitive hypertension [46]. In normal animals, however, salt loading markedly increased renal NOS activity, which may contribute to maintenance of normal blood pressure. This compensatory mechanism is probably related to the renal haemodynamic and the natriergic effects of NO [47]. It is therefore conceivable that reduction of blood pressure in uraemic rats supplemented with L-arginine was due in part to the improvement of renal NO formation. Furthermore, Katoh et al. [25] showed that supplementation with 1% L-arginine significantly attenuates glomerular capillary pressure in rats with reduced renal mass. NO exerts basal control on renal blood flow by reducing the potent vasoconstrictor effects of ET-1 and angiotensin II on afferent arterioles [14,48]. Improvement of renal NO release may also prevent the development of glomerulosclerosis and the resultant glomerular hyperfiltration and proteinuria, which may be due in part to reduction of glomerular ET-1 production. In fact, NO inhibits the mitogenic response and the expression of extracellular matrix proteins induced by various agonists in mesangial cells, including ET-1 [49,50]. This is consistent with the study of Reyes et al. [26] reporting that L-arginine-treated uraemic rats develop less glomerular injury than untreated uraemic rats.

Although one may anticipate that a higher dose of L-arginine would cause additional beneficial effects in uraemic rats, we show that supplementation with 1% L-arginine did not prevent the rise of blood pressure and the progression of renal insufficiency. This is consistent, however, with studies from other groups showing that 1% L-arginine has limited influence on blood pressure and proteinuria, although some degree of renal protection was observed, including reductions in glomerular injury and glomerular capillary pressure [23,25,26]. Similarly to the present study, Ashab et al. [24] reported effective antihypertensive and renal protective effects with a low dose of L-arginine (0.125%) in uraemic rats with subtotal 5/6 nephrectomy. Hence, supplementation with a high dose of L-arginine could have detrimental action in chronic renal failure. This may be related to the fact that L-arginine is the precursor of other products involved in tissue repair. L-arginine is metabolized to L-ornithine by arginase, which releases urea, and subsequently to polyamines and proline, and is essential for the synthesis of agmatine by arginine decarboxylase. Although agmatine may mediate some protective effects of L-arginine [51,52], polyamines and proline have no vasoactive properties, but induce mesangial cell proliferation and collagen deposit, leading to glomerulosclerosis and renal fibrosis [53]. In this study, 1% L-arginine treatment significantly increased serum and
urine urea levels in uremic rats, whereas 0.1% L-arginine had no effect on these parameters, but significantly increased plasma NO₂⁻NO₃ concentrations. This suggest that at low doses L-arginine is converted mainly to NO by NO synthases, whereas at high doses L-arginine is converted to L-ornithine by arginase, thus limiting the cardiovascular and renal protective effects mediated by L-arginine/NO pathway.

In summary, supplementation with a low dose but not with a high dose of L-arginine prevents the aggravation of hypertension and attenuates the progression of renal insufficiency in rats with reduced renal mass. The effects of L-arginine are related in part to decreased ET-1 production in vascular and renal tissues, indicating that impaired NO release in uremic rats is an important factor modulating ET-1 production.

Acknowledgements. The authors thank Danielle Lizotte and Claude Villeneuve for their technical assistance. This study was supported by a grant from The Kidney Foundation of Canada to RL. YD and MD are recipients of studentships from the Natural Sciences and Engineering Research Council of Canada.

References

34. Li JS, Larivière R, Schiffrin EL. Effect of a non selective endothelin antagonist on vascular remodeling in deoxycorticosterone acetate-salt hypertensive rats. *Hypertension* 1994; 24: 183–188

Received for publication: 23.5.00
Accepted in revised form: 11.9.00