Arginase inhibition slows the progression of renal failure in rats with renal ablation

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Sabbatini, Massimo, Antonio Pisani, Francesco Uccello, Giorgio Fuiano, Raffaele Alfieri, Antonio Cesaro, Bruno Cianciaruso, and Vittorio E. Andreucci. Arginase inhibition slows the progression of renal failure in rats with renal ablation. Am J Physiol Renal Physiol 284:F680–F687, 2003. First published December 10, 2002; 10.1152/ajprenal.00270.2002. Exogenous arginine slows the progression of chronic renal failure (CRF) in remnant rats through a nitric oxide (NO)-dependent mechanism. We tested whether the inhibition of arginase could induce similar results through the increased availability of endogenous arginine. Three groups of remnant rats were studied for 8 wk: 1) untreated rats (REM); 2) remnant rats treated with 1% L-arginine (ARG); and 3) remnant rats administered a Mn2+-free diet to inhibit arginase (MNF). Normal rats (NOR) were used as controls. Liver arginase activity was depressed in MNF rats (−35% vs. REM, P < 0.01). No difference in metabolic data was detected among the groups throughout the study; blood pressure was significantly lower in MNF vs. ARG and REM rats after 6 wk (P < 0.001). The glomerular filtration rate (GFR) was greatly depressed in REM rats (−47% vs. NOR, P < 0.03) but was higher in ARG and MNF rats (+40 and +43% vs. REM, respectively, P < 0.05), with comparable changes in renal hemodynamics. Despite the better GFR, proteinuria was decreased in both ARG and MNF rats (−42%, P < 0.05, and −57%, P < 0.01, respectively, vs. REM rats). Arginine plasma levels, significantly reduced in REM rats (−41% vs. NOR, P < 0.01), were partially restored in MNF rats (+38% vs. REM), and urinary nitrite excretion, greatly depressed in REM rats (−76% vs. NOR, P < 0.01), was significantly increased in MNF rats (+209% vs. REM, P < 0.05). At the renal level, arginase activity was only slightly depressed in MNF rats (−18% vs. REM), but intrarenal concentrations of arginine were lower in this latter group (P < 0.05 vs. other groups). Beyond the hemodynamic modifications, MNF rats showed a lower glomerular sclerosis index (P < 0.05 vs. REM and ARG). Inhibition of arginase slows the progression of CRF in remnant rats similarly to arginine-treated rats; the better histological protection in MNF rats, however, suggests that additional factors are involved in these modifications.

remnant rat; arginine; nitric oxide; chronic renal failure

THE METABOLISM OF ARGinine is particularly complex: this semiessential amino acid, in fact, is endogenously synthesized in renal proximal tubules from L-citrulline (Citr) and is then degraded, through distinct enzymatic routes, to several metabolites that may affect both renal function and morphology (25, 38). The first important pathway is represented by arginase, mostly present in the liver and to a much lesser extent in the kidney, which leads to formation of urea and ornithine (Orn), the precursor of polyamines and proline involved in cell-replication turnover and collagen synthesis (25). The second metabolic route is constituted by the arginine decarboxilase (ADC), active in the brain and in the kidney, which forms agmatine, a substance able to induce several positive effects on renal function (18). The last important pathway is nitric oxide (NO) synthase (NOS), with at least two different isoforms: endothelial (eNOS) and inducible (iNOS). The former synthesizes small amounts of NO, which modulate systemic hemodynamics through a paracrine action; conversely, the stimulation of iNOS, mostly represented in inflammatory migrating cells, produces huge amounts of NO, which enhance inflammation and generation of free radicals, potentially harmful to the cells (16).

In the last decade, particular attention has been given by nephrologists to arginine-derived NO because of its role in both regulating renal hemodynamics and modulating inflammatory and proliferating response to various stimuli. Several experimental studies, in fact, have unequivocally demonstrated that it is possible to increase the availability of NO by the administration of exogenous supplements of arginine, as witnessed by the increased plasma and urinary levels of nitrites (2, 11, 23), or to reduce its production by the use of specific inhibitors of NO synthesis (6, 10, 28). Promising results have been obtained in arginine-treated rats in nonimmunological models of chronic renal failure (CRF) (2, 11, 15, 27) and in other experimental conditions (6, 24, 26, 28), which seemed to open new, encouraging perspectives even in curing human renal failure.

Unfortunately, these experimental results have not been confirmed in immunological models of renal diseases like glomerulonephritis (22), in which supplements of arginine were even harmful, nor in patients

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with chronic renal insufficiency. Indeed, from a unique prospective, randomized trials in which adequate doses of arginine were administered for a prolonged period of time to patients either with CRF or with renal transplantation failed to induce any beneficial effect on GFR, hypertension, and proteinuria (9, 39).

The confounding results of all these studies may be partially explained by the complex metabolism of arginine, conditioned by the presence of the different enzymatic pathways in organs and cells, and by the changes in such activities in response to diet, hormones, cytokines, and even the type of renal disease (38). This suggests the possibility of exogenously modulating the metabolism of arginine in an attempt to modify its impact on renal disease. Because activation of any of the pathways of arginine catabolism is strictly dependent on the concentration of both substrate and enzymes into the cells (21), it is reasonable to argue that the partial inhibition of arginase, the main system in degrading arginine, could offer higher amounts of arginine to the remaining routes.

Thus this study was undertaken to figure out whether the progression of CRF in rats was influenced by the inhibition of liver arginase. We tested this hypothesis in the “remnant” rat, a nonimmunological model of CRF, not only because it shares three critical aspects with human CRF (anemia, proteinuria, hypertension) but mostly because this model is also characterized by the spontaneous downregulation of iNOS (1). This should allow available arginine to be metabolized mostly by pathways “beneficial” to the kidney like eNOS or ADC. Arginase inhibition was obtained by the administration of a Mn\(^{2+}\)-free diet, because Mn\(^{2+}\) is a key cofactor for arginase activity; with respect to other inhibitors of arginase commonly used in vitro studies (like borate, valine), a dietary deficiency of Mn\(^{2+}\) can decrease arginase activity to a reasonable extent, with no side effects in the young rat (5). Today, new inhibitors are also available, like N\(^{5}\)-hydroxy-L-arginine, which is mostly used in cells because of its short half-life and yet has an agonist action on NO synthesis, and S-(2-boroethil)-L-cysteine, which can be used in vivo (36) but was not available to us when we started the study.

MATERIALS AND METHODS

The study was carried out in 84 male Sprague-Dawley rats (Charles River), with an initial body weight of \(\sim 300\) g. Chronic renal failure was induced by \% ablation of renal parenchyma, which involved a right nephrectomy and, 1 wk later, ligation of two or three branches of the left renal artery, both under light pentobarbital sodium anesthesia (Nembutal, 40 mg/kg). Immediately after completion of the surgery, the rats were randomly assigned to one of the following experimental groups: REM (untreated remnant rats used as controls \((n = 10)\); ARG (remnant rats treated with a supplement of L-arginine (1% wt/vol) in tap water throughout the study \((n = 13)\); and MNF (remnant rats treated with a Mn\(^{2+}\)-free diet throughout the study to inhibit arginase activity \((n = 10)\) (5). Nine unmanipulated littermates were used to assess the normal values of this strain of rats (NOR).

The Mn\(^{2+}\)-free diet was similar to the control diet in protein content (18% casein), the number of calories, vitamins, and minerals, and L-arginine concentration (0.96 g/100 g) (Altromin, Rieper, Italy). Recent studies have shown that a 1-mo administration of a similar diet results in a 26% decrease in arginase activity (5).

One week after randomization, the rats were placed in individual metabolic cages and, after a 3-day acclimatization period, a complete, timed 24-h collection of urine was performed for determination of urinary protein excretion; body weight and food and water intake were also recorded (time 0). The same metabolic study was repeated 4 and 8 wk later. The duration of our experimental study was 8 wk to avoid possible complications resulting from more prolonged Mn\(^{2+}\) deficiency and from the accumulation of ammonia after inhibition of arginase, which, in mammals, represents the main pathway of removal of this toxin from the body; preliminary 12-wk studies, however, were executed to evaluate the effects of a Mn\(^{2+}\)-free diet in normal rats \((n = 8)\) and to test the survival of remnant rats on such a diet \((n = 10)\). On the other hand, a peculiar resistance to retain Mn\(^{2+}\) and to detoxify ammonia via glutamine synthesis has been suggested in rats (5).

Systolic blood pressure (SBP) was measured by the tail-cuff method 2 wk after completion of surgery and during week 6 of the observation period: the average of three measurements was used as a single value for each rat. At the end of the last clearance study (week 8), the animals were anesthetized with Inactin (80 mg/kg body wt ip) and prepared for a hemodynamic study, with determination of insulin clearance and renal inulin extraction (blood collection from the renal vein through a sharpened micropipette), as previously reported (28). At the end of the experiment, biopptic samples for renal histology were collected.

Six additional remnant rats for each group were anesthetized 8 wk after surgery to obtain, after adequate stabilization (60 min), a sterile specimen of urine under ice (4°C) for determination of urinary NO\(_X\)/NO\(_3\) urinary excretion. An arterial blood sample was then collected for determination of plasma concentration of arginine, Citr, and Orn. Finally, specimens of hepatic and renal tissue were also obtained under sterile conditions for determination of enzymatic activities. Six normal littersmates were also killed to obtain urine, plasma, and hepatic and renal tissue for determination of normal, control values of our rat strain (NOR).

Histology

For the evaluation of the effects of the different regimens on renal pathology, kidney biopsies were evaluated in a blind fashion. Plastic-embedded 3-μm sections were cut, and hematoxylin-eosin, periodic acid-Schiff (PAS), and Jones staining were performed. As recently suggested (12), histological analysis was made using the Banff criteria, grading (0–3) the following variables: glomerulosclerosis, arteriosclerosis, and interstitial fibrosis/tubular atrophy. In particular, glomerulosclerosis was defined as mild (score = 1), in the case of an increase in the mesangial matrix with broadening of mesangial areas up to the diameter of two mesangial cells; moderate (score = 2), in the case of broadening of mesangial areas by more than the diameter of two mesangial cells; and severe (score = 3), in the case of mesangial sclerosis corresponding to at least 25% of the glomerular area. The score for arteriosclerosis was assigned on the basis of severity of the fibrointimal thickening of arteries. A combined score was given to interstitial fibrosis/tubular atrophy, defined and graded according to the criteria defined by the Banff group.
(22a). To increase the sensitivity of the method, the score of the lesions found in each glomerulus and artery within each biopsy was recorded and the sum of the scores was added, giving a ratio as score/number of glomeruli or arteries in each biopsy (12). A similar approach was used for the evaluation of tubular atrophy/interstitial fibrosis: in this case 10 different high-power (×400) microscopic fields within each biopsy were examined.

Analytic Determinations

Urinary volume was measured gravimetrically in preweighed vials. The concentrations of inulin were measured by the diphenylamine method (35); urinary protein concentrations were analyzed by the Lowry method, using bovine serum albumin as a standard. Urinary protein concentrations were measured at the end of the experimental period. 

Histological data were analyzed by the Ablation of 5 of renal parenchyma resulted in a 37% reduction in GFR values in REM rats compared with NOR rats; this modification was associated with a rise in mean blood pressure, a decrease in hematocrit values, and an obvious reduction in total kidney weight (Table 1). Proteinuria, measured in 24-h samples, was significantly higher in remnant rats at the end of the experimental period (Table 1). Arginase activity was similar between REM and NOR rats, but arginine plasma levels were significantly depressed in remnant rats (−41%, P < 0.01), whereas plasma concentrations of Orn and Citr were significantly higher, as commonly described in CRF. Finally, renal arginase activity was reduced to a significant extent in REM rats (−53%, P < 0.01 vs. NOR), but only slight changes were observed in arginine and Orn tissue levels (Table 1). Urinary excretion of nitrates was dramatically reduced in the REM group (2.28 ± 0.59 vs. 0.67 ± 0.37 nmol·min⁻¹·g kidney wt⁻¹, P < 0.001).

Metabolic Studies

There was no difference in body weight and food intake (and consequently of protein, phosphorus, and other nutrients) among the three groups of remnant

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Table 1. Main hemodynamic and biochemical data of normal rats and remnant untreated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>BP, mmHg</th>
<th>GFR, ml/min</th>
<th>RVR, mmHg·g⁻¹·min⁻¹</th>
<th>RPF, ml/min</th>
<th>Ht, %</th>
<th>FF, %</th>
<th>KW, g</th>
<th>Prot, mg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOR</td>
<td>118.1</td>
<td>1.32</td>
<td>22.1</td>
<td>2.96</td>
<td>47.8</td>
<td>26.8</td>
<td>2.971</td>
<td>14.7</td>
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<tr>
<td></td>
<td>(n = 9)</td>
<td>±6.8</td>
<td>±0.21</td>
<td>±4.54</td>
<td>±0.57</td>
<td>±2.8</td>
<td>±2.6</td>
<td>±0.33</td>
</tr>
<tr>
<td>REM</td>
<td>146.7</td>
<td>0.83</td>
<td>29.6</td>
<td>2.98</td>
<td>42.2</td>
<td>28.2</td>
<td>2.935</td>
<td>298.2</td>
</tr>
<tr>
<td></td>
<td>(n = 10)</td>
<td>±24.9*</td>
<td>±0.17*</td>
<td>±8.91*</td>
<td>±0.40</td>
<td>±5.4*</td>
<td>±5.8</td>
<td>±0.470*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver, mol/g tissue</th>
<th>Arg-act</th>
<th>Orn</th>
<th>Arg</th>
<th>Orn</th>
<th>Citr</th>
<th>Arg-act</th>
<th>Arg</th>
<th>Orn</th>
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<td>NOR</td>
<td>689.5</td>
<td>0.77</td>
<td></td>
<td>120.2</td>
<td>53.3</td>
<td>52.3</td>
<td>44.3</td>
<td>1.24</td>
<td>0.83</td>
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<td></td>
<td>(n = 6)</td>
<td>±170.2</td>
<td>±0.22</td>
<td>±27.9</td>
<td>±12.8</td>
<td>±21.5</td>
<td>±8.5</td>
<td>±0.31</td>
<td>±0.29</td>
</tr>
<tr>
<td>REM</td>
<td>614.9</td>
<td>0.74</td>
<td></td>
<td>70.8</td>
<td>97.2</td>
<td>116.5</td>
<td>20.5</td>
<td>0.81</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>(n = 6)</td>
<td>±100.7</td>
<td>±0.26</td>
<td>±16.1*</td>
<td>±18.2*</td>
<td>±42.1†</td>
<td>±3.8§</td>
<td>±0.32</td>
<td>±0.16</td>
</tr>
</tbody>
</table>

Values are means ± SD. n, No. of rats; BP, mean blood pressure; Ht, hematocrit; GFR, glomerular filtration rate; ml/min; RVR, renal vascular resistance; RPF, renal plasma flow; FF, filtration fraction; KW, residual kidney wt; Prot, 24-h urinary protein excretion after 8 wk; NOR, normal rats; REM, untreated rats; Arg, l-arginine; Orn, Ornithine; Citr, citrulline; Arg-act, arginase activity. *P < 0.03, †P < 0.01 (minimum value). REM vs. NOR (Student’s t-test for unpaired data).
rats and NOR rats at the end of the 8-wk experimental period (Table 2). Twenty-four-hour proteinuria, comparable among the groups after 1 wk, was significantly lower in both ARG and MNF rats compared with untreated REM rats (−42%, ARG vs. REM, P < 0.05, and −57%, MNF vs. REM, P < 0.01) at the end of the experimental period (Fig. 1). SBP, which was similar in the three groups of rats after the first 2 wk of observation, was significantly lower in MNF rats during week 6 (P < 0.001 vs. both REM and ARG).

### Hemodynamic Studies

Mean blood pressure was similar in the three groups of remnant rats during the experiments, despite a tendency to be slightly higher in MNF rats (+13% vs. REM, not significant (NS)) (Table 3). GFR, measured as inulin clearance, averaged 0.832 ± 0.17 ml/min in the REM group. Arginine treatment determined a slower progression of renal failure, with a GFR significantly higher than in the REM group (+40%, P < 0.05); a similar result was also observed in the MNF group (+43% vs. REM, P < 0.05). This similarity was explained by comparable hemodynamic changes: in fact, in both groups we observed a reduction in renal vascular resistance (RVR) compared with REM (ARG −36%, P < 0.05, and MNF −25%, NS), leading to a marked increase in renal plasma flow (ARG +58% and MNF +53% vs. REM, respectively, both P < 0.01). Filtration fraction was numerically decreased in ARG and MNF rats (−10 and −9%, respectively, vs. REM, NS).

### Biochemical Studies

**Liver homogenates.** The Mn^{2+}-free diet decreased arginase activity in MNF rats with respect to the other groups (−35% vs. REM and ARG, both P < 0.01); this influenced the tissue concentrations of Orn, which was lower in MNF rats (−32% vs. REM, NS, and −45% vs. ARG, P < 0.05) (Table 4). It is noteworthy that measurable arginine levels could not be detected in liver parenchyma in any of the groups under study.

**Plasma and urine.** The administration of exogenous arginine (ARG rats) significantly increased its plasma level (+71% vs. REM, P < 0.05), leading to a higher production of Orn (+34% vs. REM, NS). In MNF rats, the inhibition of arginase determined a rise in plasma arginine by 38% compared with REM rats (NS). Orn concentrations in MNF rats was significantly lower than in the ARG group (−32%, P < 0.05), reflecting both the different baseline plasma levels of arginine and the partial inhibition of arginase.

Arginase inhibition also determined a marked increase in urinary nitrates in MNF rats (2.05 ± 0.9 nmol·min⁻¹·g kidney wt⁻¹, P < 0.05 vs. REM); this excretion was even higher after in ARG rats after arginine administration (2.99 ± 0.9 nmol·min⁻¹·g kidney wt⁻¹, P < 0.001, vs. REM). These differences and their relationships with the respective plasma levels of arginine are shown in Fig. 2.

**Kidney homogenates.** Renal arginase activity was greatly decreased in all the groups of remnant rats. Arginine administration slightly increased, and a Mn^{2+}-free diet further decreased this enzymatic activity, with an average difference of 25% between the ARG and MNF groups (P < 0.05) (Table 4). Intrarenal arginine concentration was dramatically lower in MNF rats (−55% vs. REM, NS; and −71% vs. ARG, P < 0.01); nevertheless, Orn concentration in the MNF group was similar to that in the REM group.

### Histological Results

Table 5 depicts the biopsy findings in the four groups of rats. NOR rats had no sign of histological damage. The morphological changes in the three experimental groups of rats were of low grade. The score for glomerulosclerosis, however, was significantly lower in the MNF group compared with the other two experimental groups (both P < 0.05). Tubulointerstitial and arteriolar changes were significantly more severe in all three groups of rats with remnant kidneys, without differences among them. The degree of arteriolar sclerosis

### Table 2. Body weight and systolic blood pressure in the different groups under study

<table>
<thead>
<tr>
<th></th>
<th>NOR (n = 9)</th>
<th>REM (n = 10)</th>
<th>ARG (n = 11)</th>
<th>MNF (n = 11)</th>
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<tbody>
<tr>
<td>BW, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>342 ± 59</td>
<td>312 ± 53</td>
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<td>Week 4</td>
<td>449 ± 53</td>
<td>409 ± 62</td>
<td>415 ± 38</td>
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<td>Week 8</td>
<td>544 ± 51</td>
<td>495 ± 51</td>
<td>489 ± 38</td>
<td>479 ± 31</td>
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<tr>
<td>Food, g/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>22.7 ± 3.9</td>
<td>21.6 ± 4.7</td>
<td>20.2 ± 3.1</td>
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</tr>
<tr>
<td>Week 4</td>
<td>25.2 ± 3.9</td>
<td>22.0 ± 6.9</td>
<td>22.3 ± 4.2</td>
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<tr>
<td>Week 8</td>
<td>21.8 ± 2.6</td>
<td>24.8 ± 5.2</td>
<td>21.8 ± 6.6</td>
<td>22.7 ± 4.3</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Week 2</td>
<td>168 ± 17</td>
<td>160 ± 24</td>
<td>151 ± 21</td>
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<tr>
<td>Week 6</td>
<td>151 ± 13*</td>
<td>191 ± 10</td>
<td>188 ± 12</td>
<td>159 ± 11*</td>
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</tbody>
</table>

Values are means ± SD. ARG, remnant rats treated with L-arginine; MNF, remnant rats administered a Mn^{2+}-free diet; BW, body wt; SBP, systolic blood pressure. *P < 0.001 vs. REM and ARG.

![Fig. 1. Twenty-four-hour urinary protein excretion (U_protein) in the 3 groups of remnant rats under study: (untreated rats (REM; n = 10; open bars); remnant rats treated with L-arginine (ARG; n = 13; gray bars); remnant rats administered a Mn^{2+}-free diet (MNF; n = 10; filled bars).](http://ajprenal.physiology.org/)

*P < 0.05 vs. REM. **P < 0.01 vs. REM.
Table 3. Hemodynamic data in the groups under study

<table>
<thead>
<tr>
<th>Group</th>
<th>BP, mmHg</th>
<th>GFR, ml/min</th>
<th>RVR, mmHg·ml⁻¹·min⁻¹</th>
<th>RPF, ml/min</th>
<th>Ht, %</th>
<th>FF, %</th>
<th>KW, g</th>
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<tr>
<td>REM</td>
<td>146.7</td>
<td>0.832</td>
<td>29.56</td>
<td>2.98</td>
<td>42.2</td>
<td>28.2</td>
<td>2.235</td>
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<td>(n = 10)</td>
<td>±24.9</td>
<td>±0.17</td>
<td>±8.91</td>
<td>±0.40</td>
<td>±5.4</td>
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<td>±0.470</td>
</tr>
<tr>
<td>ARG</td>
<td>149.7</td>
<td>1.158</td>
<td>18.87</td>
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<td>45.3</td>
<td>25.4</td>
<td>1.995</td>
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<td>(n = 13)</td>
<td>±37.3</td>
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<td>±1.13†</td>
<td>±4.2</td>
<td>±2.7</td>
<td>±0.273</td>
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<td>MNF</td>
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<td>1.183</td>
<td>22.08</td>
<td>4.58</td>
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<td>25.7</td>
<td>2.026</td>
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<td>(n = 10)</td>
<td>±26.2</td>
<td>±0.30*</td>
<td>±7.37</td>
<td>±1.09†</td>
<td>±4.7</td>
<td>±2.3</td>
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Values are means ± SD. *P < 0.05 vs. REM. †P < 0.01 vs. REM.

was numerically lower in MNF rats with respect to the other groups.

DISCUSSION

Our data demonstrate that the partial inhibition of arginase activity in remnant rats is able to slow the progression of CRF, preserving GFR to the same extent as exogenous arginine administration. Inhibition of arginase activity, however, confers an additional histological protection to the kidney, witnessed by the lower degree of glomerular sclerosis compared with REM and ARG rats.

As observed in previous works using the same experimental model (2, 11, 25, 24), our rats treated with arginine show beneficial effects on renal function (GFR +40% vs. REM). In all of these studies, the effects of arginine on GFR, proteinuria, and renal histology were quite variable, mostly depending on the different rat strain, length of the observation period, and entity of residual renal function; one circumstance, however, seems constantly confirmed: rats administered low doses of arginine (0.1–0.125% wt/vol of tap water) have significantly lower values of blood pressure and proteinuria than their untreated remnant controls (2, 11, 31), whereas higher doses (1%) do not affect blood pressure. This distinction seems very important because inhibition of arginase in our study mimics the condition of "low" arginine supplementation: improved GFR, lower blood pressure, and reduced proteinuria. Moreover, the great increase in urinary nitrite excretion in MNF rats (+206% vs. REM), despite incompletely restored plasma levels of arginine, strongly suggests that our experimental model enhances the production and the action of NO. A further confirmation of the role of NO as mediator of these modifications is that the changes in renal hemodynamics after arginase inhibition overlap those observed after arginine administration, i.e., a similar improvement in GFR and renal plasma flow and a slight increase in filtration fraction, factors denoting glomerular vasodilatation.

In contrast to other studies reporting near-normal plasma concentrations of arginine in rats with CRF (25, 27), our REM rats show significantly lower plasma levels of this amino acid compared with normal rats (Table 1). This finding, however, is not surprising; in the presence of high concentrations of Citr, the synthesis of arginine by renal tubular cells is conditioned by the number of functioning nephrons (4); its reduction cannot allow a normal cumulative renal release of the amino acid, and, indeed, a 60% reduction in Citr uptake has been observed in the kidneys of remnant rats (25). Therefore, normal plasma arginine concentration in CRF must depend on its extrarenal synthesis and release, as demonstrated in patients with CRF with a high protein catabolic rate (34). This probably occurred in the study by Reyes et al. (27), in which plasma arginine levels were normal or were increased by a high dose (1%) of the amino acid. These rats, in fact, had very extensive renal ablation (7/8) and a very low body weight gain throughout the study (~0.7 g/day). Our rats, on the contrary, have greater residual kidneys, better renal function, and a near-normal weight gain (2.7 g/day), all factors that seem to exclude a hypercatabolic state. Furthermore, arginine administration to rats in the present study normalizes its plasma values, unmasking a real deficit in its renal synthesis not compensated for by extrarenal sources.

The variability of plasma arginine concentration in the different groups in our study is particularly intriguing. In fact, despite the fact that intracellular levels of arginine are 10-fold higher than the $K_m$ of eNOS (21), the intracellular pool of the amino acid is poorly accessible to the enzyme, which is thus presently activated by extracellular (plasmatic) arginine.

Table 4. Biochemical parameters in the groups of rats under study

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver, mol/g tissue</th>
<th>Arg-act</th>
<th>Orn</th>
<th>Arg</th>
<th>Orn</th>
<th>Citr</th>
<th>Kidney, mol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>REM</td>
<td>614.9 ± 0.74</td>
<td>70.8</td>
<td>97.2</td>
<td>116.5</td>
<td>20.5</td>
<td>0.81</td>
<td>0.69</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>±100.7 ± 0.26</td>
<td>±16.1*</td>
<td>±18.2</td>
<td>±42.1</td>
<td>±3.8</td>
<td>±0.32</td>
<td>±0.16*</td>
</tr>
<tr>
<td>ARG</td>
<td>623.7 ± 0.91</td>
<td>121.2</td>
<td>134.4</td>
<td>123.6</td>
<td>22.5</td>
<td>1.19</td>
<td>1.18</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>±96.7 ± 0.24</td>
<td>±40.9</td>
<td>±21.5</td>
<td>±22.9</td>
<td>±3.4</td>
<td>±0.33</td>
<td>±0.34</td>
</tr>
<tr>
<td>MNF</td>
<td>397.7 ± 0.50</td>
<td>98.1</td>
<td>90.7</td>
<td>123.3</td>
<td>16.8</td>
<td>0.36</td>
<td>0.78</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>±88.6* ± 0.17*</td>
<td>±17.3</td>
<td>±31.3*</td>
<td>±29.9</td>
<td>±1.7*</td>
<td>±0.11†</td>
<td>±0.18*</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 vs. ARG. †P < 0.01 vs. REM and ARG.

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As a consequence, when the plasma concentration of arginine is somehow raised, this may play a relevant role in enhancing the synthesis of NO ("arginine-eNOS paradox"), as well as other metabolites whose enzymatic pathways are not inhibited (agmatine?).

A striking finding of this study is the significant preservation of the kidneys from glomerular damage in MNF compared with REM, and even with ARG, rats despite the fact that this latter group has a hemodynamic pattern comparable to that of MNF rats. These results can be ascribed, to a great extent, to the lower SBP observed in MNF rats up to 6 wk of observation (Table 1). The importance of reducing blood pressure to slow the progression of CRF, in fact, is widely recognized in many experimental and clinical studies, with the best results obtained with angiotensin-converting enzyme inhibitors, which increase the availability of NO (13, 14, 17, 20). All of these studies have clearly demonstrated the direct correlation between the levels of SBP and both the functional and histological damage in the kidney; this study further strengthens how lower levels of SBP may positively influence the course of CRF, mostly when associated to a greater availability of NO, as in the case of MNF rats.

The rise in mean blood pressure observed in MNF rats during the hemodynamic studies is surprising; unexpectedly, these rats after anesthesia have blood pressure values higher than those observed in the awake state, and even higher than in REM and ARG rats. The lower values of proteinuria and the best results obtained with angiotensin-converting enzyme inhibitors, which increase the availability of NO (13, 14, 17, 20). All of these studies have clearly demonstrated the direct correlation between the levels of SBP and both the functional and histological damage in the kidney; this study further strengthens how lower levels of SBP may positively influence the course of CRF, mostly when associated to a greater availability of NO, as in the case of MNF rats.

Finally, it is interesting to observe the different behavior of hepatic and renal arginase: the condition of CRF determines a sharp decrease in renal arginase activity in all the groups of remnant rats, barely affected by the high arginine tissue levels in the ARG group; the use of the Mn$^{2+}$-free diet, then, does not depress renal arginase activity to the same extent as hepatic arginase. Hepatic and renal arginase, indeed, represent two different isoforms of the enzyme, encoded by separate genes, with different intracellular locations (cytosolic in the liver and mitochondrial in the kidney), and a well-described isoform selectivity for the binding of both substrates or inhibitors (7). Despite both arginases requiring Mn$^{2+}$ for their activity, it remains unclear why MNF rats failed to show a greater decrease in the renal isoform. Several possibilities may be taken into account: 1) a lower affinity for this isoform for Mn$^{2+}$; 2) a greater ability to retain the ion because of the segregation of the renal enzyme inside the mitochondria; and 3) an increased compensatory synthesis of this enzyme secondary to the sharp decrease in liver activity, as demonstrated in inherited defects of liver arginase (33). It is certain, however, that the deficiency of Mn$^{2+}$ induces some peculiar changes in the kidneys of MNF rats, as shown by the very low concentrations of arginine in their renal tissue. The disappearance of arginine from renal tissue, however, is only "virtual" because both the high renal levels of Orn (similar to NOR rats despite 70% lower levels of arginine) and the consistent excretion of urinary nitrites suggest that, conversely, a consistent amount of the amino acid is present in the kidney. It is possible that arginine is metabolized very quickly, as occurs in the liver where the high metabolic rate of the urea cycle makes arginine undetectable (38), which was also observed in this study. It may only be hypothesized that the partial inhibition of renal arginase (or Mn$^{2+}$ deficiency) has somehow switched on a tight coupling of enzymatic activities responsible for a more rapid degradation of arginine, possibly through the ADC pathway, and also because this enzyme is located in mitochondria and the action of agmatine on renal dynamics is partially mediated by NO. Agmatine, in fact, leads to vasodilatation through stimulation of eNOS (30) and inhibits the activation of iNOS after cytokine stimulation (3); moreover, agmatine can reduce cellular proliferation by inhibition of Orn decarboxylase (and, indeed, renal Orn concentration was higher in our MNF rats despite reduced levels of argi-
nase and of arginine) and can suppress polyamine transport inside the cells (29). This is in agreement with the lower index of glomerular sclerosis in our MNF rats. Although Mn2+ is a key cofactor in many other enzymatic activities (like superoxide dismutase, catalases, or xanthine oxidase), it seems unlikely that our model of Mn2+ deficiency may have greatly influenced these pathways, which play a relevant role in protecting the cells from oxidative injury. In fact, as a final result their inhibition should result in worsening of both renal function and morphology, rather than in the observed beneficial effects.

Further studies, however, are necessary to determine ADC and eNOS activities in renal tissue, as well as agmatine concentration, to evaluate whether different inhibitors of arginase are able to elicit similar responses in the kidney and to exclude undesired effects due to Mn2+ depletion. These efforts could lead to a definition of a pharmacological approach also potentially useful in humans.

In conclusion, our in vivo data demonstrate that inhibition of arginase is capable of producing several beneficial effects in remnant rats with CRF, like preservation of GFR, reduction in proteinuria, decrease in blood pressure, and lowering of histological damage at the glomerular level. These changes seem to be linked to a greater availability of NO from endothelial cells. With respect to previous models in which arginine was administered exogenously, our experimental approach suggests that inhibition of arginase could be a more effective target to enhance NO production and slow the progression of CRF. This could open new perspectives into the treatment of patients with CRF, who have a reduced basal production of NO (37) but showed no benefit from exogenous arginine administration. New studies are in progress to clarify the molecular mechanisms of these interesting, although theoretical, data in experimental animals.

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REFERENCES