Inducible nitric oxide synthase modulates hydronephrosis following partial or complete unilateral ureteral obstruction in the neonatal mouse

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Yoo KH, Thornhill BA, Forbes MS, Chevalier RL. Inducible nitric oxide synthase modulates hydronephrosis following partial or complete unilateral ureteral obstruction in the neonatal mouse. Am J Physiol Renal Physiol 298:F62–F71, 2010. First published November 4, 2009; doi:10.1152/ajprenal.00234.2009.—To investigate the role of endogenous inducible nitric oxide synthase (iNOS) in the response of the developing kidney to unilateral ureteral obstruction (UUO), neonatal iNOS null mutant (–/–) and wild-type (WT) mice were subjected to partial or complete UUO. At 7 and 21 days of age, apoptosis, renin, vascular endothelial growth factor (VEGF), fibroblasts (anti-fibroblast-specific peptide 1), myofibroblasts (α-smooth muscle actin), macrophages (F4/80), and collagen were measured in kidney tissue. Compared with WT, renal parenchymal thickness was increased, with preservation of the papilla, in –/– mice with partial UUO, but decreased in –/– mice with complete UUO. Ureteral peristalsis increased with severity of pelvic dilatation in WT, and increased further in –/– mice with partial UUO. Apoptosis, fibroblasts, and macrophages were increased in –/– mice with complete UUO, but there was no effect of iNOS on other histological parameters following complete UUO. Renin was decreased in –/– mice with partial UUO. There was no effect of iNOS genotype on renal collagen accumulation at either 7 or 21 days of age. These results are consistent with an injurious role for endogenous iNOS following partial UUO by inhibiting ureteral peristalsis and increasing renal renin although renal fibrosis is not affected. In contrast, in mice with complete UUO, iNOS attenuates apoptosis and enhances renal parenchymal thickness. Alterations in the severity of ureteral obstruction may therefore influence the effect of iNOS on long-term renal injury. Apoptosis; collagen; fibrosis; macrophages; ureteral peristalsis.

METHODS

Experimental animals and surgery. The development of animal models of congenital obstructive nephropathy involves careful attention to surgical technique (6). iNOS knockout mice (iNOS –/–) were bred from homozygous animals (Jackson Laboratory, Bar Harbor, ME) and were compared with control wild-type C57BL/6 mice. The protocol for this study was approved by the Institutional Animal Care and Use Committee of the University of Virginia. Under isoflurane and oxygen anesthesia, male and female mice were subjected within the first 48 h of life to complete UUO (n = 67) or 0.2-mm partial UUO (n = 61) as described previously (26). At the time of study, animals were killed with a lethal injection of pentobarbital sodium, the retroperitoneum was exposed through an abdominal incision, and the diameters of the renal pelvis and of the ureter proximal to the obstruction were measured with calipers. The kidneys were removed, weighed, and fixed in 10% phosphate-buffered formalin (pH 7.1) for 24–48 h before transfer to 70% ethanol.

Kidneys were dehydrated, embedded in paraffin, and sectioned (4 μm) on a RM2155 microtome (Leica). To delineate general morphology, sagittal sections of kidneys were stained with Masson’s trichrome (Fig. 1, A–F). Parenchymal thickness was measured at either pole and at the midsagittal point from the surface of the capsule to the junction of the outer and inner medulla as shown (Fig. 1, A and E). Following fixation in Bouin’s solution for 1 h at 60°C, sections were stained with Gill’s no. 2 hematoxylin for 2 min, Biebrich scarlet-acid fuchs in 5 s, phosphotungstic acid/phosphomolybdic acid for 5 min, and aniline blue for 15 min. Sections were destained for 30 s in 1% acetic acid and dehydrated. To determine the effects of endogenous iNOS on the renal cellular response to partial or complete UUO, quantitative immunohistochemical analysis was performed in kidneys of animals harvested at 7 days of age.

Additional animals were anesthetized at 7 days of age, placed on a heated table, and ureters were exposed through an abdominal incision. The ureters were exposed, and the vescera were covered with saline-soaked cotton. The exposed ureters were constantly bathed in saline heated to 37°C. Following a 15-min equilibration, the frequency of peristaltic waves along the obstructed ureter was counted by direct observation.
observation using a stopwatch for three 60-s intervals. Peristalsis in the contralateral kidney was <1 wave/min in all animals. At the end of each study, patency of the partial ureteral obstruction was documented by flow of India ink across the obstruction following injection of ink into the renal pelvis. Animals in which patency of the partial obstruction was not documented were not included in the study.

Immunohistochemistry. Fibroblasts [anti-fibroblast-specific peptide 1 (FSP-1)], myofibroblasts [α-smooth muscle actin (α-SMA)], mac-

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**Fig. 1.** A–F: median sagittal sections (trichrome stain) of completely and partially obstructed kidneys. +/+, Wild-type; −/−, inducible nitric oxide synthase (iNOS) knockout. A and B: 7-day-old mice with complete unilateral ureteral obstruction (UUO). C and D: 7-day-old mice with partial UUO. E and F: 21-day-old mice with partial unilateral ureteral obstruction. Regardless of the age or degree of obstruction, the wild-type kidneys exhibit severe hydronephrosis. Although complete obstruction results in similar hydronephrosis in iNOS knockout kidneys (B), after partial obstruction (D and F) much of the parenchymal thickness is retained. Yellow lines denote measurements of parenchymal thickness at either pole and at the mid-sagittal point, from capsule through outer medulla (A and E). G and H: terminal transferase-dUTP-nick-end labeling staining showing apoptotic cells in both tubular epithelial cells and interstitial cells of 7-day-old mice with complete UUO; apoptosis is far more pronounced in iNOS −/− (H) than wild-type mouse (G). Scale bar = 100 μm (G and H). I: peristaltic rate related to renal pelvic diameter (2-way ANOVA, *P* < 0.05).
rophages (F4/80), vascular endothelial growth factor (VEGF), and renin were detected by immunohistochemistry, using an avidin-biotin immunoperoxidase method (Vectastain ABC kit, Vector Laboratories, Burlingame, CA). Sections were deparaffinized with xylene, followed by rehydration in a descending series of ethanol. Sections of a pair of iNOS knockout and a pair of wild-type kidneys were placed on individual microscope slides, so that effects of slide-to-slide variation on experimental variables (strain and UUO) would be minimized. Endogenous peroxidase was quenched in 3% hydrogen peroxide in methanol for 30 min. Antigen retrieval procedures were performed for FSP-1 (heat retrieval for 10 min in 10% citrate buffer), F4/80 (1% pronase incubation, 5 min), and VEGF (heat retrieval in 10 mM Tris·HCl, pH 10.0). Non-specific biotin signal was blocked by use of an avidin/biotin blocking kit (Vector Laboratories). Sections were incubated for 30 min with normal serum and were then incubated overnight at 4°C with primary antibodies (Table 1). As negative controls, PBS was substituted for the primary antibody. After incubation, sections were washed twice for 5 min in PBS. Sections were then incubated for 30 min with biotinylated peroxidase-conjugated secondary antibodies, washed in PBS, and incubated for 30 min with Vectastain ABC reagent. Bound antibodies were detected using 3,3′-diaminobenzidine (Biogenex, San Ramon, CA) to produce a brown chromogen. The sections were counterstained with 0.5% methylene blue solution or 0.75% hematoxylin solution (Sigma, St. Louis, MO), dehydrated, and evaluated by light microscopy. All positive stains were quantified using image analysis software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD) by scanning 10 nonoverlapping fields for each kidney section (7). Positive area was expressed as a percentage of total area. Care was exercised in restricting the measurement of immunoreactive FSP-1, α-SMA, and F4/80 to the interstitial compartment (6).

The relative content of renin was assessed on the basis of examination of immunostained slides and calculating the percentage of glomerular profiles which had positive staining in their immediate proximity (juxtaglomerular cells). In general, this entailed a total of scoring 100 glomeruli/kidney section. In those sections containing <100 total glomeruli (notably, severely hydronephrotic kidneys in 21-day UUO animals), the percentage of renin association was based on the total glomerular count for each section.

**Identification of cellular apoptosis.** Apoptotic cells were identified with the terminal transferase-dUTP nick-end labeling technique, using the ApopTag peroxidase in situ Apoptosis Detection Kit (Chemicon International, Temecula, CA). Positive nuclei were visualized by applying peroxidase-conjugated anti-digoxigenin antibody for 30 min, followed by a 0.05% solution of 3,3′-diaminobenzidine tetrahydrochloride for 4 min. For negative controls, the TdT enzyme was omitted. Slides were counterstained with methylene blue. The number of positive-staining tubular and interstitial nuclei was counted at ×400 magnification in each of 10 nonoverlapping microscopic fields evenly distributed across the section.

**Identification of collagen.** The fractional distribution of collagen in tissue sections was determined for kidneys of mice harvested at both 7 and 21 days of age, by performing digital morphometry of Picro-sirius red-stained material (Polysciences, Warrington, PA). Following rehydration, sections were placed into Picro-sirius red solution for 10 min. After that, slides were washed with 0.5% aqueous acetic acid and dehydrated. Image-analysis software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD) was used to quantitate the distribution of collagen by scanning 10 nonoverlapping fields at ×400 magnification for each kidney section.

**Statistical analysis.** Data are presented as means ± SE and were evaluated for statistical significance with Sigmastat 3.0 (Jandel Scientific, San Rafael, CA). Two-way ANOVA, followed by a Holm-Sidak pairwise multiple comparison, was used to compare the effects of severity of obstruction (partial vs. complete UUO) and iNOS gene expression on each parameter in 7- and 21-day treatment groups. Comparisons between left and right kidneys were performed using Student’s t-test for paired data. Two-way ANOVA was also used to compare the effects of severity of pelvic dilatation and iNOS gene expression on ureteral peristaltic frequency. Statistical significance was defined as P < 0.05.

**RESULTS**

**Effects of iNOS and UUO on kidneys and ureters.** As shown in Table 2, body weight was not affected by severity of UUO (partial vs. complete UUO) or by the presence of functional iNOS at either 7 or 21 days of age. Body weight more than doubled between 7 and 21 days. Kidney weight was not affected by genotype, but weight of the completely obstructed kidney was slightly greater than that of the partially obstructed kidney at 7 days, and markedly less at 21 days. At 21 days, weight of the obstructed kidney was lower than that of the contralateral kidney in each group, with a greater decrease following complete than partial UUO. Growth of the contralateral kidney was greater following complete than partial UUO at 21 days.

At 7 days, compared with wild-type mice, renal parenchymal thickness was increased in iNOS−/− mice following partial UUO, but decreased following complete UUO; ureteral diameter was less in partially obstructed iNOS−/− than wild-type mice (Fig. 1, Table 2). At 21 days, renal parenchymal thickness was decreased following complete UUO regardless of genotype. The greater parenchymal thickness of partially obstructed iNOS−/− kidneys was associated with superior preservation of the renal papilla (Fig. 1, D and F) and narrower ureteral diameter compared with wild-type kidneys (Fig. 1, C and E, Table 2). At 21 days, ureteral diameter in iNOS−/− mice was 80% greater following complete than partial UUO. As shown in Fig. 1, the rate of ureteral peristalsis proximal to the partial obstruction increased with the severity of pelvic dilatation in 7-day-old wild-type mice and was markedly increased in iNOS−/− compared with wild-type mice.

**Seven-day UUO.** As shown in Fig. 1, G and H, and Tables 3 and 4, there was minimal effect of genotype on tubular or interstitial apoptosis following 7 days’ partial UUO, but with complete UUO tubular and interstitial apoptosis were increased in iNOS−/− compared with wild-type mice. Immunoreactive renin was decreased in iNOS−/− compared with wild-type mice subjected to partial UUO, while there were no differences in those subjected to complete UUO (Fig. 2, A and B, Tables 3 and 4). Similar changes were observed for immunoreactive VEGF, which was increased in complete vs. the partially obstructed kidney (Fig. 2, C and D, Tables 3 and 4).

<table>
<thead>
<tr>
<th>Primary Antibody</th>
<th>Type</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
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<tr>
<td>α-SMA</td>
<td>Monoclonal</td>
<td>1:300</td>
<td>Sigma</td>
</tr>
<tr>
<td>F4/80</td>
<td>Polyclonal</td>
<td>1:25</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>FSP-1</td>
<td>Polyclonal</td>
<td>1:600</td>
<td>Neomarkers/Lab Vision</td>
</tr>
<tr>
<td>Renin</td>
<td>Polyclonal</td>
<td>1:10,000</td>
<td>T. Inagami, Vanderbilt University</td>
</tr>
<tr>
<td>VEGF</td>
<td>Monoclonal</td>
<td>1:100</td>
<td>Oncogene</td>
</tr>
</tbody>
</table>

α-SMA, α-smooth muscle actin (marker for myofibroblasts); F4/80, a member of the epidermal growth factor (EGF)-transmembrane 7 (TM7) family (marker for macrophages); FSP-1, fibroblast-specific protein-1, a member of the calmodulin S100 troponin C superfamily (marker for fibroblasts); VEGF, vascular endothelial growth factor.
Immunoreactive FSP-1 and \( \alpha \)-smooth muscle actin tended to be decreased in the partially obstructed iNOS \(-/-\) compared with the wild-type kidney and increased in iNOS \(-/-\) mice following complete obstruction (Fig. 2, E–H, Tables 3 and 4). Macrophage infiltration (F4/80 immunoreactivity) increased with severity of obstruction, but was not significantly affected by genotype (Fig. 3, A and B, Tables 3 and 4). There was no effect of genotype on renal collagen accumulation in either partially or completely obstructed kidneys at 7 days, although collagen accumulation increased with severity of obstruction (Fig. 3, C and D, Tables 3 and 4).

As shown in Tables 3 and 4, there was no effect of genotype or severity of UUO on tubular apoptosis in the intact contralateral kidney, but interstitial apoptosis was greater in iNOS \(-/-\) than wild-type mice and increased with severity of obstruction. In contrast, contralateral kidney immunostaining renin was decreased in iNOS \(-/-\) vs. wild-type mice. Compared with partial UUO, renin, VEGF, fibroblasts, and macrophage immunostaining were increased in contralateral kidneys of iNOS \(-/-\) mice (Tables 5 and 6). There was no effect of iNOS genotype or severity of obstruction on contralateral kidney \( \alpha \)-SMA or collagen staining (Tables 3 and 4).

21-Day UUO. After 21 days of UUO, changes in most of the parameters of renal injury in the obstructed kidney were greater for complete than partial UUO: apoptosis, fibroblasts, myofibroblasts, macrophages, and collagen (Fig. 3, E and F; Table 5). This is consistent with a marked loss of renal mass and parenchymal thickness after 21 days of complete UUO (Table 2). The effects of iNOS genotype on apoptosis, renin, and fibroblasts observed at 7 days (Tables 3 and 4) did not persist to 21 days of UUO (Tables 5 and 6). An exception to this trend was a reduction in macrophage infiltration in partially obstructed kidneys of iNOS \(-/-\) vs. wild-type mice and an increase in macrophages in completely obstructed mutant kidneys (Tables 5 and 6). By 21 days of obstruction, there were no effects of genotype on indices of injury in the contralateral kidney, but renin immunostaining was markedly suppressed in mice subjected to complete compared with partial UUO (Tables 5 and 6).

DISCUSSION

The results of this study demonstrate complex interactions between a number of factors known to mediate or modulate the renal response to UUO. In addition, comparison of the effects of partial or complete UUO on the rapidly growing neonatal kidney reveals important differences in the renal response to these two forms of injury and the contribution of endogenous iNOS. Most dramatic is the contrasting effect of iNOS on renal parenchymal thickness following partial vs. complete UUO: renal parenchymal thickness is enhanced by deletion of iNOS after 7 or 21 days of partial UUO, but decreased after 7 days of complete UUO in iNOS \(-/-\) mice. The lack of differences in kidney weight following 7 days of UUO is likely due to edema, followed by reduced kidney weight reflecting impaired growth of the obstructed kidney by 21 days (proportional to severity of obstruction).

### Table 2. Characteristics of mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>UUO Kidney Weight, mg</th>
<th>CL Kidney Weight, mg</th>
<th>UUO Kidney Parenchymal Thickness, mm</th>
<th>UUO Kidney Ureteral Diameter, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>3.6±0.1</td>
<td>21.6±0.9</td>
<td>21.8±1.1</td>
<td>0.7±0.1†</td>
<td>1.0±0.1‡</td>
</tr>
<tr>
<td>iNOS (-/-)</td>
<td>3.4±0.1</td>
<td>20.5±0.8‡</td>
<td>20.3±0.6</td>
<td>1.1±0.1‡</td>
<td>0.8±0.1‡</td>
</tr>
<tr>
<td>21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>8.6±0.4</td>
<td>58.8±3.8*</td>
<td>67.9±2.9</td>
<td>1.0±0.4‡</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>iNOS (-/-)</td>
<td>8.4±0.5</td>
<td>50.2±4.2*‡</td>
<td>57.9±2.9‡</td>
<td>1.6±0.2‡</td>
<td>1.1±0.2‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 5 \) mice/group. UUO, unilateral ureteral obstructed kidney; CL, contralateral kidney; WT, wild-type; NS, not significant. iNOS \(-/-\), inducible nitric oxide synthase knockout. *\( P < 0.05 \), obstructed kidney vs. contralateral kidney. †\( P < 0.05 \), WT vs. iNOS \(-/-\). ‡\( P < 0.05 \), partial vs. complete UUO. \( \# P < 0.05 \).
Table 3. Fractional distribution (%) of renal immunostaining, partial UUO, 7 days of age

<table>
<thead>
<tr>
<th>Group</th>
<th>Tubular Apoptosis, count/10 fields</th>
<th>Interstitial Apoptosis, count/10 fields</th>
<th>Renin, % positive glomeruli</th>
<th>VEGF</th>
<th>FSP-1</th>
<th>α-SMA</th>
<th>F4/80</th>
<th>Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>12.8±2.3</td>
<td>74±1.8</td>
<td>2.3±0.6*</td>
<td>9.2±1.1</td>
<td>8.2±1.3*</td>
<td>0.8±0.1</td>
<td>0.5±0.1</td>
<td>13.2±1.2*</td>
</tr>
<tr>
<td>iNOS−/−</td>
<td>14.2±4.7‡</td>
<td>78±1.2</td>
<td>3.2±1.0‡</td>
<td>9.2±1.3‡</td>
<td>5.4±1.2‡</td>
<td>2.4±0.5‡</td>
<td>0.5±0.0‡</td>
<td>0.3±0.1‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Abbreviations and symbols are defined as in Table 2.

Table 4. Fractional distribution (%) of renal immunostaining, complete UUO, 7 days of age

<table>
<thead>
<tr>
<th>Group</th>
<th>Tubular Apoptosis, count/10 fields</th>
<th>Interstitial Apoptosis, count/10 fields</th>
<th>Renin, % positive glomeruli</th>
<th>VEGF</th>
<th>FSP-1</th>
<th>α-SMA</th>
<th>F4/80</th>
<th>Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>15.8±3.0*</td>
<td>3.8±1.1</td>
<td>6.4±2.3†</td>
<td>25.7±3.4*</td>
<td>9.8±1.7</td>
<td>1.2±0.1**</td>
<td>168±1.6*</td>
<td>4.7±0.9*</td>
</tr>
<tr>
<td>iNOS−/−</td>
<td>37.4±7.9*</td>
<td>9.4±2.2</td>
<td>16.4±4.1*</td>
<td>25.3±2.2*</td>
<td>11.0±1.3</td>
<td>1.9±0.4</td>
<td>17.7±3.1*</td>
<td>5.8±1.1*</td>
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</table>

Abbreviations and symbols are defined as in Table 2.
In contrast to humans, in which the formation of renal pelvic smooth muscle begins at 12 wk gestation, pelvic smooth muscle development in the rodent begins after birth, and continues postnatally (22). The reduction in renal pelvic dilatation and preservation of the renal papilla in iNOS−/− animals (Fig. 1, D and F) compared with wild-type mice (Fig. 1, C and E) may be related to stimulation of ureteral peristalsis in the partially obstructed kidney of iNOS−/− mice (Fig. 1I). Following complete UUO in the adult rat, iNOS appears in ureteral smooth muscle before the development of ureteral fibrosis, increasing over 21 days and decreasing thereafter (8). There was an increase in ureteral peristaltic frequency with increasing pelvic diameter in wild-type mice, a phenomenon observed also with increasing urinary flow rates in rats subjected to partial UUO (28). The increase in peristaltic frequency with pelvic dilatation presumably results from stimulation of renal pelvic pacemaker cells in response to UUO (10).

The additional increase in peristaltic frequency in the iNOS knockout mice is consistent with reduced inhibition of ureteral contractile activity normally mediated by nitric oxide produced in the urothelium (20). Although urine flow could not be measured in this study, increased peristalsis in the iNOS−/− mice may reduce hydronephrosis in the partially obstructed kidney by enhancing drainage of pelvic urine, thereby allowing normal renal papillary development.

Normal maturation of ureteral peristalsis is necessary for renal papillary development, a process dependent on an intact renin-angiotensin system. Because ureteral peristaltic activity
reduces the hydrostatic gradient between renal pelvis and distal ureter, the renal papilla becomes atrophic in animals lacking a functional angiotensin AT1 receptor (22). Expression of renal pelvic angiotensin and its receptor are normally upregulated in mice at birth, and angiotensin stimulates the production of α-SMA by cultured wild-type ureteral cells (23). Conversely, angiotensin AT1 receptor inhibition in rats with partial UUO decreases ureteral peristaltic frequency and worsens hydronephrosis (11). In contrast to the salutary effects of loss of iNOS in partial UUO in the present study, renal parenchyma was reduced in iNOS/−/− mice with complete UUO (Fig. 1, A and B), consistent with a study in adult iNOS/−/− mice (14). An analogous finding was reported in mice lacking a functional AT1 receptor: compared with wild-type animals, complete UUO in mutants was no more severe (22). In mice lacking functional calcineurin in the developing urinary tract, ureteral peristalsis is impaired, and animals develop bilateral uretero-pelvic junction obstruction and renal failure (4). Thus it appears that any process interfering with the normal development of ureteral peristalsis can aggravate the progression of renal injury due to congenital partial UUO.

In the present study, renal apoptosis was not affected by lack of iNOS following 7 days of partial UUO, but tubular and interstitial apoptosis were both markedly increased following complete UUO in iNOS/−/− compared with wild-type mice. Renal apoptosis is also increased following complete UUO in adult iNOS/−/− mice, and apoptosis stimulated by in vitro stretching of renal tubular cells (mimicking obstructive tubular dilatation) is reduced by increasing nitric oxide and augmented by nitric oxide inhibition (21). This indicates that nitric oxide can act as a survival factor for tubular cells severely injured by UUO. Increased apoptosis in iNOS/−/− mice with complete UUO may contribute to the reduced parenchymal thickness of the obstructed kidney at 7 days. Inhibition of NOS in rats subjected to renal ablation reduces tubular VEGF and accelerates progression of renal disease (16). Reduction of immunoreactive VEGF following partial UUO in iNOS/−/− mice in the present study is consistent with a salutary effect of nitric oxide on the renal parenchymal response to UUO. However, the response of endogenous VEGF by the obstructed kidney is complex (1), and exogenous VEGF may selectively inhibit interstitial apoptosis in the partially obstructed neonatal rat kidney (2).

Compared with 7 days of obstruction, following 21 days of partial UUO tubular apoptosis had declined, and interstitial apoptosis had increased slightly. Following 21 days of complete UUO, both tubular and interstitial apoptosis increased markedly (Table 5). This is a reflection of the ongoing severe...
### Table 5. Fractional distribution (%) of renal immunostaining, partial UUO, 21 days of age

<table>
<thead>
<tr>
<th>Group</th>
<th>Tubular Apoptosis, count/10 fields</th>
<th>Interstitial Apoptosis, count/10 fields</th>
<th>Renin, % positive glomeruli</th>
<th>VEGF</th>
<th>FSP-1</th>
<th>α-SMA</th>
<th>F4/80</th>
<th>Collagen</th>
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<td></td>
<td>UOO CL</td>
<td>UOO CL</td>
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</tr>
<tr>
<td>WT</td>
<td>3.2±1.1</td>
<td>1.8±0.4</td>
<td>10.8±2.2</td>
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<td>iNOS−/−</td>
<td>5.8±2.4</td>
<td>2.2±0.5</td>
<td>8.2±1.9</td>
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<td>28.5±1.7</td>
<td>333.3±3.1</td>
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</table>

**Effect of iNOS genotype:** NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS

**Effect of UUO severity:** § NS § NS § NS § NS § NS § NS § NS § NS

**Effect of iNOS genotype-UUO severity:** NS NS § NS § NS § NS § NS § NS § NS § NS

*Abbreviations and symbols are defined as in Table 2.*

### Table 6. Fractional distribution (%) of renal immunostaining, complete UUO, 21 days of age

<table>
<thead>
<tr>
<th>Group</th>
<th>Tubular Apoptosis, count/10 fields</th>
<th>Interstitial Apoptosis, count/10 fields</th>
<th>Renin, % positive glomeruli</th>
<th>VEGF</th>
<th>FSP-1</th>
<th>α-SMA</th>
<th>F4/80</th>
<th>Collagen</th>
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<tr>
<td>WT</td>
<td>50.0±6.6</td>
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<td>334.3±3.2</td>
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<td>0.6±0.1</td>
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</table>

**Effect of iNOS genotype:** NS NS NS NS NS NS NS NS NS NS NS NS NS NS NS

**Effect of UUO severity:** § NS § NS § NS § NS § NS § NS § NS § NS § NS

**Effect of iNOS genotype-UUO severity:** NS NS § NS § NS § NS § NS § NS § NS § NS § NS

*Abbreviations and symbols are defined as in Table 2.*
injury resulting from complete compared with partial UUO. By 21 days, there is no longer an effect of iNOS genotype on apoptosis, which is likely due to a progressive downregulation of endogenous renal NOS activity as a result of UUO. Following complete UUO in the adult mouse, renal iNOS production increases by 7 days, then decreases by 14 days (21). This may be related to the finding that the inner medullary collecting duct is responsible for most iNOS production in the kidney (29): this segment undergoes the most rapid dilatation and apoptosis following 7 days UUO, with a return to low levels of apoptosis by 21 days (3, 26).

The reduction of intrarenal renin following 7 days of partial UUO in both kidneys of iNOS −/− compared with wild-type mice indicates that endogenous iNOS initially contributes to upregulation of intrarenal renin. The microvascular expression of renin is increased in fetal renal development and normally disappears, but it persists with neonatal UUO (9, 19). By 21 days of obstruction, there is no longer an effect of iNOS on renin distribution, which is similarly upregulated with both partial and complete UUO. This suggests that factors other than iNOS mediate long-term upregulation of renin in the obstructed kidney. However, by 21 days of partial UUO, contralateral renal renin expression is not different from that of the obstructed kidney, whereas the kidney contralateral to complete UUO is markedly suppressed. This is similar to the effect of complete UUO in the neonatal rat, in which contralateral renal renin remains suppressed (9). These differences in the response of both obstructed and contralateral kidneys to partial vs. complete UUO underscore the significant differences in renal cellular response to the severity of obstruction.

There are considerable data showing that activation of fibroblasts (expressing FSP-1), myofibroblasts (expressing α-SMA), and macrophages plays a role in the progression of renal obstructive injury following UUO (5). These responses therefore appear to be maladaptive in the obstructed kidney, with greater increments in these parameters following complete than partial UUO. The utility of FSP-1 staining has been challenged on the basis of its identity with S100A4, an intermediate filament component (18). Since intermediate filaments are found in a wide variety of cells, intense staining can be seen in such clearly nonfibroblastic entities as arterial endothelium (30). The reduction in α-SMA in the partially obstructed kidney of iNOS −/− mice after 21 days of partial obstruction suggests that endogenous iNOS contributes to the continued expression of this contractile protein by maturing interstitial cells (9). The role of α-SMA-expressing interstitial cells in the obstructed kidney is not entirely clear, however, as mice lacking functional α-SMA develop more severe obstructive renal lesions than wild-type animals (25).

Chronic UUO in the neonatal mouse leads to progressive renal interstitial infiltration with macrophages, which promote tubular cell apoptosis and interstitial fibrosis (17). In the present study, renal interstitial infiltration by macrophages in the obstructed kidney was decreased following 21 days of partial UUO in iNOS −/− compared with wild-type mice but was increased with complete UUO. Others have reported increased numbers of macrophages in the kidneys of adult iNOS −/− mice subjected to complete UUO, leading to the conclusion that endogenous nitric oxide serves a salutary role in obstructive nephropathy (13). There is a dissociation of macrophage- and myofibroblast-specific staining in serial sections of the obstructed kidney (6), suggesting that the renal inflammatory response to UUO is not directly linked to activation of fibroblasts.

There was no net impact of iNOS deletion on renal interstitial fibrosis resulting from partial or complete UUO at either 7 or 21 days in the neonatal mouse. This contrasts with the response of the adult mouse kidney subjected to 14 days of complete UUO, in which renal collagen content was 40% greater in iNOS −/− than wild-type obstructed kidneys (13). In this regard, compared with its progression in older children and adults with urinary tract obstruction, fibrosis is far less common in neonates and infants with obstructive nephropathy (15). The dissociation of collagen deposition from iNOS-dependent changes in apoptosis, renin distribution, and macrophage infiltration likely reflects this maturational effect. In the present study, collagen accumulation in the obstructed kidney following complete UUO significantly exceeded that resulting from partial UUO and parallels increases in renal FSP-1, α-SMA, and F4/80. The lack of an effect of iNOS genotype on other parameters measured in neonatal kidneys subjected to 7 days of complete UUO is likely attributable to the overwhelming injurious stimulus of complete obstruction, with activation of alternate pathways masking iNOS effects. This finding underscores the utility of the model of partial UUO, which parallels clinical congenital obstructive nephropathy.

In conclusion, this study shows that endogenous iNOS slows ureteral peristalsis, reduces renal parenchymal thickness, initially increases renal renin and fibroblast infiltration, and later increases macrophages in the partially obstructed neonatal kidney. Suppression of peristaltic activity by iNOS in the obstructed ureter is the converse of stimulation of ureteral peristalsis by angiotensin, a paradigm that is consistent for a number of contrasting actions of these compounds: nitric oxide is a vasodilator and inhibits proliferation, while angiotensin is a vasoconstrictor and stimulates proliferation. Endogenous iNOS in the neonatal obstructed kidney also inhibits tubular apoptosis, presumably a salutary response. Contrasting renal cellular effects have been described for a number of modulators of obstructive nephropathy: osteopontin reduces apoptosis, but activates myofibroblasts, while death-associated protein kinase stimulates apoptosis and inhibits interstitial fibrosis in the obstructed kidney (30–32). An improved understanding of the physiological and cellular effects of compounds regulating the response to ureteral obstruction may lead to new targeted therapeutic approaches to slowing or preventing progressive renal injury.

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DISCLOSURES

No conflicts of interest are declared by the authors.
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