Renal cellular response to ureteral obstruction: role of maturation and angiotensin II

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Chevalier, Robert L., Barbara A. Thornhill, and Jennifer T. Wolstenholme. Renal cellular response to ureteral obstruction: role of maturation and angiotensin II. Am. J. Physiol. 277 (Renal Physiol. 46): F41–F47, 1999.—Renal angiotensin II (ANG II) is increased as a result of unilateral ureteral obstruction (UUO), and angiotensin AT\(_2\) receptors predominate over AT\(_1\) receptors in the early postnatal period. To examine the renal cellular response to 3-day UUO in the neonatal and adult rat, AT\(_1\) and AT\(_2\) receptors were inhibited by losartan and PD-123319, respectively. Additional rats received exogenous ANG II, 0.5 mg·kg\(^{-1}\)·day\(^{-1}\). Renal cellular proliferation and apoptosis were quantitated by proliferating cell nuclear antigen and terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling technique, respectively. In the neonate, UUO reduced proliferation and increased tubular apoptosis. Losartan had no detectable cellular effect, whereas PD-123319 increased cellular proliferation and suppressed apoptosis, and exogenous ANG II stimulated apoptosis. In the adult, UUO increased cellular proliferation as well as apoptosis, whereas losartan, PD-123319, and exogenous ANG II did not alter the cellular response. In conclusion, UUO impairs renal growth in the neonate by reducing proliferation and stimulating apoptosis, at least in part through angiotensin AT\(_2\) receptors. UUO stimulates both renal cellular proliferation and apoptosis in the adult, but these effects are independent of ANG II. We speculate that the unique early responses of the developing kidney to urinary tract obstruction are mediated by a highly activated renin-angiotensin system and preponderance of AT\(_2\) receptors.

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OBSTRUCTIVE NEPHROPATHY is a significant cause of renal insufficiency in neonates and adults. We have shown that unilateral ureteral obstruction (UUO) in the neonatal rat impairs renal growth and development, whereas UUO in the adult induces a marked interstitial infiltrate that is significantly greater than in the neonate (1). In the neonate, chronic UUO impairs cellular proliferation and reduces the renal DNA content, whereas in the adult, UUO stimulates renal cell proliferation and increases renal DNA content (1). Moreover, although UUO induces renal cellular apoptosis in both neonates and adults, the magnitude of apoptosis is significantly greater in the neonate (1). We have demonstrated also that chronic UUO in the neonatal rat markedly stimulates the renin-angiotensin system (RAS), with a persistent increase in renin secretion and intrarenal angiotensin II (ANG II) production (20, 33). The stimulation of renin expression is greater in the neonate than in the adult rat (1).

The AT\(_1\) receptor is known to mediate the vasoconstrictor action of ANG II, as well as many of its growth-promoting actions (30). Expression of this receptor in the kidney increases progressively throughout postnatal life, whereas expression of the AT\(_2\) angiotensin receptor is greatest in the fetal and perinatal period, with a progressive decrease after birth (9, 33). The function of the AT\(_2\) receptor is less well understood than that of the AT\(_1\) receptor, but it appears to mediate the inhibition of cellular proliferation (17, 27) and the stimulation of apoptosis (29, 32). We have shown previously that UUO in the neonatal rat modulates the expression of ANG II receptors: expression of both AT\(_1\) and AT\(_2\) renal receptors is downregulated within the first 24 h of obstruction, whereas AT\(_2\) receptor expression decreases in the first 3–14 days of postnatal life regardless of UUO (33). However, as a result of prolonged UUO, renal AT\(_1\) receptor expression increases progressively, concurrent with an elevation in renal ANG II content (33).

In the present study, we hypothesized that ANG II mediates the early renal cellular response to UUO and that this response is determined by the relative abundance of AT\(_1\) and AT\(_2\) receptors. We further hypothesized that the greater expression of AT\(_2\) receptors in the neonate mediates the enhanced apoptosis and reduced proliferation in the neonatal compared with the adult rat kidney subjected to chronic UUO. The present study was therefore designed to compare the role of ANG II AT\(_1\) and AT\(_2\) receptors in the early renal cellular responses to UUO in neonatal and adult rats. Since the abundance of AT\(_2\) receptors exceeds that of AT\(_1\) receptors in the first several days of life, the studies were performed 3 days following UUO in neonatal rats, then compared with similarly treated adult rats. Immunohistochemistry and morphometry were used to measure glomerular maturation, as well as renal tubular and interstitial cellular proliferation and apoptosis in the obstructed and the intact opposite kidneys. The contribution of AT\(_1\) and AT\(_2\) receptors was examined using selective receptor blockers administered chronically throughout the period of study. To evaluate the renal response to ANG II, exogenous ANG II was infused chronically in additional groups of animals.

METHODS

Experimental protocol. Experiments were performed using 82 neonatal and 95 adult Sprague-Dawley rats. Animals were anesthetized with halothane and oxygen and subjected to UUO as described previously (7). Briefly, the left ureter was...
Sprague-Dawley rats
Neonates: UUO within first 48 h of birth
Adults: UUO in males

Saline
Losartan [AT1 inhibitor]
PD123319 [AT2 inhibitor]
Losartan [AT1 inhibitor]
Saline
Angiotensin II (0.5 mg/kg/d)
PD123319

Study 72 h later

Fig. 1. Scheme showing experimental design. Six groups of animals were studied for both neonatal and adult rats. Left: animals received either saline (control group), losartan (AT1 receptor inhibitor, 40 mg·kg⁻¹·day⁻¹), or PD-123319 (AT2 receptor inhibitor, 10 mg·kg⁻¹·day⁻¹). Right: three additional groups of animals received a continuous infusion of ANG II (0.5 mg·kg⁻¹·day⁻¹). UUO, unilateral ureteral obstruction.

Exposed through an abdominal incision, ligated, and the incision was closed. Neonatal animals were returned to their mothers, whereas adult animals were returned to their cages following recovery.

As shown in Fig. 1, animals in each age range were divided into six groups and treated with either saline vehicle injection, losartan, PD-123319, ANG II, ANG II plus losartan, or ANG II plus PD-123319. Losartan (gift of Merck, Rahway, NJ), a selective AT1 receptor blocker, was administered at 40 mg·kg⁻¹·day⁻¹ via daily subcutaneous injections. This dose of losartan was chosen because it effectively blocks the vasoconstrictor response to ANG II, and no toxic effects were observed in rats receiving 45 mg·kg⁻¹·day⁻¹ over 1–3 mo (31). PD-123319 (gift of Parke-Davis, Ann Arbor, MI), a selective AT2 receptor inhibitor, was administered from timed-released pellets (Innovative Research of America, Sarasota, FL) placed intraperitoneally at the time of ureteral obstruction. Use of pellets, rather than osmotic minipumps, was necessary in neonatal rats due to the small size of the animals. The pellets have been shown to release compounds reliably in a number of studies (8, 18, 26), and were designed necessary in neonatal rats due to the small size of the animals. The pellets have been shown to release compounds reliably in a number of studies (8, 18, 26), and were designed necessary in neonatal rats due to the small size of the animals. These were injected daily with normal saline vehicle, whereas animals not receiving PD-123319 or ANG II were given placebo timed-release pellets such that every animal had the same number of injections and pellets.

In adult rats, losartan, PD-123319, and ANG II were administered in the same doses as in the neonatal animals (factored for body weight). Losartan was administered through daily subcutaneous injections, whereas PD-123319 and ANG II were administered using osmotic minipumps (models 1003D and 2001; Alzet Pharmaceuticals, Palo Alto, CA). Control animals were injected daily with saline vehicle and received osmotic minipumps containing bovine serum albumin (vehicle). The distribution of rats in experimental groups is shown in Fig. 1.

Seventy-two hours after UUO, rats were killed by lethal dose of intraperitoneal pentobarbital sodium. Both kidneys were removed, decapsulated, weighed, and fixed in 10% buffered Formalin following dehydration through graded alcohols and xylene, then embedded in paraffin for sectioning at 4 µm.

Glomerular maturation. Late glomerular maturation was scored in 10 nonoverlapping fields spanning the renal cortex of midcoronal sections stained using the periodic acid-Schiff technique. Glomerular maturation and calculation of the weighted glomerular maturation index were determined as published previously (19).

Determination of cellular proliferation. Proliferating nuclei were identified in sections by proliferating cell nuclear antigen (PCNA, 1:400; Vector Laboratories, Burlingame, CA) using the method described previously (2). Sections were counterstained in Gill's hematoxylin, dehydrated through graded alcohols and xylene, and mounted. Nuclei appeared as dark brown staining and were present in tubules and interstitium (Fig. 2A).

Determination of apoptosis. Apoptosis was quantitated using the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) technique (Apoptag; Oncor, Gaithersburg, MD) as described previously (2). Slides were counterstained in Gill's hematoxylin, dehydrated through graded alcohol and xylene, and mounted. In addition to brown staining, apoptotic nuclei were identified by condensed nuclear material and vacuolization of the cytoplasm (Fig. 2B).

Both tubular and interstitial proliferating and apoptotic cells were quantitated by counting the number of positively stained nuclei in 10 nonoverlapping fields viewed at ×450 magnification. Care was taken to distribute the fields across cortex and medulla of both poles and the center of each kidney. There was no detectable variation in the density of

Fig. 2. Representative sections of neonatal rat kidney 3 days following ipsilateral UUO. A: proliferating cells are identified by positive nuclear staining for proliferating cell nuclear antigen (PCNA). These are dark-staining nuclei adjacent to asterisks in dilated tubules. B: apoptotic cells are identified by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling technique (TUNEL), which stains fragmented nuclear DNA adjacent to asterisks in dilated tubules. Note the condensed nuclear material characteristic of apoptotic cells.
Table 1. Body weight at time of study

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Losartan</th>
<th>PD-123319</th>
<th>Angiotensin</th>
<th>Angiotensin + Losartan</th>
<th>Angiotensin + PD-123319</th>
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<tbody>
<tr>
<td>Neonates</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Body wt, g</td>
<td>7.89 ± 0.47</td>
<td>8.38 ± 0.34</td>
<td>6.83 ± 0.36</td>
<td>7.99 ± 0.32</td>
<td>7.31 ± 0.42</td>
<td>7.41 ± 0.40</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
<td>12</td>
<td>12</td>
<td>17</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body wt, g</td>
<td>215.48 ± 6.28</td>
<td>213.24 ± 4.98</td>
<td>196.92 ± 4.63</td>
<td>202.44 ± 5.44</td>
<td>213.35 ± 5.05</td>
<td>190.52 ± 2.76</td>
</tr>
<tr>
<td>n</td>
<td>23</td>
<td>20</td>
<td>6</td>
<td>22</td>
<td>18</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals.

PCNA or TUNEL-positive cells between fields in an individual kidney.

Statistical analysis. The effects of AT1 and AT2 receptor inhibitor and of exogenous ANG II were analyzed using two-way ANOVA with Tukey pairwise multiple comparison. Differences between obstructed and intact opposite kidneys were determined using Student’s t-test for paired data. Statistical significance was defined as being less than 0.05. Data are expressed as means ± SE.

RESULTS

As shown in Table 1, there was no significant effect of angiotensin receptor antagonists or ANG II infusion on body weight in neonatal or adult rats. As shown in Table 2, UUO resulted in an increase in kidney weight-to-body weight ratio in both neonatal and adult groups. Weight of the obstructed and intact opposite kidneys in neonates was reduced by losartan (P < 0.01 by 2-way ANOVA). Administration of ANG II increased the weight of the obstructed kidney (P < 0.02 by 2-way ANOVA) but not of the intact opposite kidney. There was no effect of ANG II or of angiotensin receptor inhibition on kidney weight in adult rats. As shown in Table 3, there was a reduction in the glomerular maturation index in the cortex of the obstructed compared with the intact opposite kidney (P < 0.001 by 2-way ANOVA). There was no effect of angiotensin receptor inhibition or of ANG II infusion on glomerular maturation of the left or right kidney of any of the groups.

As shown in Fig. 3A, compared with the intact opposite kidney, UUO reduced renal tubular cell proliferation in the neonate. There was a tendency for losartan to reduce further tubular cell proliferation, but this was not significant. However, infusion of PD-123319 doubled renal tubular cell proliferation in both obstructed and intact opposite kidneys (P < 0.05). Administration of exogenous ANG II had no significant additional effects on tubular cell proliferation.

As shown in Fig. 3B, tubular cell apoptosis was markedly increased in the obstructed compared with the intact opposite kidney in all groups of neonatal rats. Administration of losartan tended to decrease tubular apoptosis, but this was not statistically significant. However, administration of PD-123319 significantly decreased apoptosis in the obstructed kidney by ~50%, whereas infusion of exogenous ANG II significantly enhanced apoptosis in the obstructed kidney.

As shown in Fig. 3C, interstitial cell proliferation was unaffected by UUO in neonatal rats, but increased more than twofold in the intact kidney as a result of inhibition of AT2 receptors. There was no significant effect of exogenous ANG II on interstitial cell proliferation.

As shown in Fig. 3D, renal interstitial cell apoptosis was undetectable in the intact kidney but increased in the obstructed kidney of neonatal rats. There was a further increase in interstitial cell apoptosis in the obstructed kidney as a result of ANG II infusion, but no significant effect of inhibition of either AT1 or AT2 receptors.

As shown in Fig. 4A, UUO had the opposite effect on renal tubular cell proliferation in the adult compared with the neonatal rat: there was a consistent increase in tubular cell proliferation in the obstructed kidney, although the overall prevalence of tubular cell proliferation was less than in the neonate (P < 0.05). There was no effect of AT1 or AT2 inhibition or exogenous ANG II infusion on renal tubular cell proliferation in either the obstructed or the intact opposite kidneys.

As shown in Fig. 4B, there was a significant increase in renal tubular cell apoptosis as a result of ureteral obstruction, although the magnitude of apoptosis was lower in the adult than the neonate (P < 0.05). As with tubular cell proliferation, there was no modulation of
this effect as the result of either AT1 or AT2 receptor inhibition or of ANG II infusion.

As shown in Fig. 4C, compared with the intact opposite kidney, UUO resulted in a consistent increase in renal interstitial cell proliferation, a response not observed in the neonate. There was no additional effect of AT1 or AT2 inhibition or of ANG II infusion on renal interstitial cell proliferation in adult rats. As shown in Fig. 4D, UUO induced a significant increase in renal interstitial cell apoptosis compared with the intact opposite kidney. Unlike the neonate, in which apoptosis was greater in tubules than intersti-
tium, in the adult, the prevalence of apoptotic interstitial nuclei was similar to that of tubular nuclei. There was no effect of AT1 or AT2 receptor inhibition or of ANG II infusion on renal interstitial cell apoptosis in either kidney.

**DISCUSSION**

The major findings in this study highlight the importance of the RAS in modulating the early renal cellular response to chronic urinary tract obstruction in the neonate, but not the adult. Acting via AT2 receptors, ANG II significantly inhibited cellular proliferation and stimulated apoptosis in renal tubules of the obstructed neonatal rat kidney: exogenous ANG II further aggravated tubular apoptosis. Although we demonstrated a transient reduction in renal AT1 and AT2 receptor binding 24 h following ipsilateral UUO in the neonatal rat, receptor binding was not different from the intact opposite kidney after 3-day obstruction (33). The relative abundance of renal mRNA for AT2 receptor is 10-fold greater than that of AT1 receptor at 1 day of age, approximately equal at 7 days of age, and over 30-fold less than AT1 at 14 days (16). Since we have shown that renal AT1 and AT2 receptor binding parallels steady-state mRNA content (33), it is likely that the dramatic effects of AT2 receptor inhibition on renal cell proliferation and apoptosis in the neonate are due to a preponderance of this class of receptors coupled with the marked activation of the RAS in the perinatal period (10). Renal renin mRNA is significantly greater...
in the obstructed kidney than in the intact opposite or sham-operated kidney 3, 7, 14, and 28 days following UUO in the neonatal rat (6, 33). Moreover, compared with its normal juxtaplomerular localization, immunoreactive renin extends along the length of the afferent arteriole following 5 days of UUO in the neonatal rat (3). These findings indicate an early and persistent activation of the RAS following neonatal UUO. Increased generation of endogenous ANG II would therefore likely contribute to the renal tubular cellular changes of the present study.

Immunohistochemical studies have shown that AT<sub>2</sub> receptors are present in vessels, glomeruli, and tubules of the 19-day fetal and neonatal rat kidney (21). Others have demonstrated that activation of the AT<sub>2</sub> receptor leads to dephosphorylation of Bcl-2, an oncoprotein that inhibits apoptosis (11). This is consistent with our observation that chronic UUO reduces Bcl-2 expression in dilated apoptotic tubules of the obstructed kidney (4). An alternate angiotensin-dependent stimulus for apoptosis through reduction in Bcl-2 may be triggered by renal tubular stretch and mediated by the AT<sub>1</sub> receptors under the stimulus of p53 (13, 23). The renal distribution of AT<sub>1</sub> receptors is similar to that of AT<sub>2</sub> receptors, with preponderance in the microvasculature and proximal tubules (22).

Although all components of the renal RAS are activated immediately following UUO in the adult (24, 25), the lack of effects of inhibition of AT<sub>1</sub> and AT<sub>2</sub> receptors on cellular proliferation and apoptosis in the adult rats of the present study likely reflects the overwhelming preponderance of AT<sub>1</sub> over AT<sub>2</sub> receptors, as well as the attenuated cellular dynamics of the mature kidney compared with the developing kidney. Other factors are therefore responsible for the stimulation of tubular proliferation and apoptosis by UUO in the adult kidney. Although we found a consistent increase in weight of the obstructed compared with the intact contralateral kidney in both neonates and adults, this is presumably due to accumulation of edema in the early phase of obstruction. This is consistent with our previous report showing an increase in the weight of the obstructed neonatal rat kidney 1 and 3 days following UUO (6). Although changes in DNA content of the obstructed kidney are not detectable within the first week of obstruction (6), we have also shown previously that with prolonged UUO (14 days), the DNA content of the obstructed kidney is reduced in the neonate but augmented in the adult (1). This is consistent with a predominant AT<sub>1</sub>-mediated antiproliferative effect of endogenous ANG II in the neonate, compared with a predominantly proliferative response to AT<sub>1</sub> stimulation in the adult. Our finding that losartan reduced wet kidney weight while ANG II increased kidney weight in the neonate is likely due to suppression and stimulation of AT<sub>1</sub>-mediated sodium retention demonstrated previously in the neonatal rat (5).

Whereas 3-day UUO delayed maturation of the neonatal kidney, administration of ANG II did not alter maturation. Others have shown that glomerular maturation is delayed in mice homozygous for a null mutation in the angiotensinen gene, although the effect was not found to be statistically significant until 1 wk of age (19). It is likely that the preponderance of AT<sub>2</sub> receptors prior to 1 wk of age serves to limit the proliferative action of ANG II, whereas the rapid dominance of the AT<sub>1</sub> receptors after that time accounts for the trophic effect of ANG II on later glomerular maturation.

In a recent report, adult mice with a null mutation of the AT<sub>3</sub> receptor gene were subjected to chronic UUO (15). Although cellular proliferation was not different in the mutant mouse obstructed kidneys, there were more interstitial fibroblasts as well as greater interstitial collagen deposition in the mutants (15). This may be explained at least in part by increased intrarenal angiotensin-converting enzyme activity in the mutant hydronephrotic kidneys (28), which would enhance stimulation of fibrogenic cytokines through activation of AT<sub>1</sub> receptors, as well as deplete antiproliferative factors such as bradykinin or nitric oxide. Thus, whereas loss of tubular cells contributes to tubular atrophy, depletion of interstitial cells may be beneficial with respect to progression of interstitial fibrosis.

In summary, UUO in the neonatal rat suppresses cellular proliferation and induces apoptosis in tubules of the ipsilateral kidney. These effects are modulated by ANG II primarily through stimulation of the AT<sub>2</sub> receptor. In the adult, UUO stimulates both proliferation and apoptosis in the obstructed kidney, events which are mediated by factors other than ANG II. It is likely that the increased activity of the RAS and preponderance of AT<sub>2</sub> over AT<sub>1</sub> receptors in the neonatal kidney contribute to the greater renal damage in the developing kidney consequent to chronic urinary tract obstruction.

This research was supported in part by National Institutes of Health (NIH) Research Center of Excellence in Pediatric Nephrology and Urology Grants DK-44756 and DK-52612, NIH O’Brien Center of Excellence in Nephrology and Urology Grant DK-45179, and NIH Child Health Research Center Grant HD-28810.

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Received 10 December 1998; accepted in final form 18 March 1999.

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ANGIOTENSIN IN NEONATAL URETERAL OBSTRUCTION


