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

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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 AT₁ 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 AT₂ angiotensin receptor is greatest in the fetal and perinatal period, with a progressive decrease after birth (9, 33). The function of the AT₂ receptor is less well understood than that of the AT₁ 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 AT₁ and AT₂ renal receptors is downregulated within the first 24 h of obstruction, whereas AT₂ receptor expression decreases in the first 3–14 days of postnatal life regardless of UUO (33). However, as a result of prolonged UUO, renal AT₁ 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 AT₁ and AT₂ receptors. We further hypothesized that the greater expression of AT₂ 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 AT₁ and AT₂ receptors in the early renal cellular responses to UUO in neonatal and adult rats. Since the abundance of AT₂ receptors exceeds that of AT₁ 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 AT₁ and AT₂ 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...
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 ANG II AT1 receptor blocker, was administered at 40 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. ANG II was administered also via timed-release pellets, releasing 0.5 mg·kg⁻¹·day⁻¹, a nonhypertensive dose (34). Control animals 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.

Determination of apoptosis. Apoptotic nuclei were identified by positive nuclear staining, using the method described previously (2). Slides were counterstained in Gill’s hematoxylin, dehydrated through graded alcohols and xylene, and mounted. Nuclei identified by TUNEL were identified in sections by proliferating cell nuclear antigen (PCNA, 1:400; Vector Laboratories, Burlingame, CA) as described previously (2). Sections were counterstained in Gill’s hematoxylin, dehydrated through graded alcohols and xylene, and mounted. In addition to brown staining and were present in tubules and interstitium (Fig. 2A).

Determination of cellular proliferation. Proliferating nuclei were identified in sections by proliferating cell nuclear antigen (PCNA, 1:400; Vector Laboratories, Burlingame, CA) as described previously (2). Sections were counterstained in Gill’s hematoxylin, dehydrated through graded alcohols and xylene, and mounted. Nuclei identified by TUNEL were identified in sections by proliferating cell nuclear antigen (PCNA, 1:400; Vector Laboratories, Burlingame, CA) as described previously (2). Sections were counterstained in Gill’s hematoxylin, dehydrated through graded alcohols and xylene, and mounted. In addition to brown staining, apoptotic nuclei were identified by condensed nuclear material and vacuolization of the cytoplasm (Fig. 2B).
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>
</tr>
</thead>
<tbody>
<tr>
<td>Neonates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>23</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 inhibitors and of exogenous ANG II were analyzed using two-way ANOVA with Tukey pairwise multiple comparison. Differences between obstructed and intact opposite kidneys in neonates was reduced by losartan (P < 0.05 vs. contralateral kidney).

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 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 in obstructed kidneys as a result of ureteral obstruction, although the magnitude of apoptosis was lower in the adult than the neonate (P < 0.05). 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-

Table 3. Neonatal rat glomerular maturation index

<table>
<thead>
<tr>
<th>Kidney</th>
<th>Saline</th>
<th>Losartan</th>
<th>PD-123319</th>
<th>Angiotensin</th>
<th>Angiotensin + Losartan</th>
<th>Angiotensin + PD-123319</th>
</tr>
</thead>
<tbody>
<tr>
<td>UUO</td>
<td>1.51±0.07</td>
<td>1.47±0.04</td>
<td>1.36±0.08</td>
<td>1.40±0.05</td>
<td>1.42±0.03</td>
<td>1.41±0.05</td>
</tr>
<tr>
<td>Intact opposite*</td>
<td>1.59±0.05</td>
<td>1.60±0.07</td>
<td>1.51±0.06</td>
<td>1.52±0.07</td>
<td>1.56±0.04</td>
<td>1.57±0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.001 vs. UUO by two-way ANOVA.
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

Fig. 4. Relative number of renal tubular (A and B) and interstitial (C and D) proliferating (A and C) and apoptotic (B and D) cells in the obstructed and intact opposite kidneys of adult rats. Legends and symbols are the same as Fig. 3.
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 juxtaglomerular localization, immuno-reactive 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 AT2 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 AT2 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 AT1 receptors under the stimulus of p53 (13, 23). The renal distribution of AT1 receptors is similar to that of AT2 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 AT1 and AT2 receptors on cellular proliferation and apoptosis in the adult rats of the present study likely reflects the overwhelming preponderance of AT1 over AT2 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 AT1-mediated antiproliferative effect of endogenous ANG II in the neonate, compared with a predominantly proliferative response to AT1 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 AT1-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 angiotensinogen gene, although the effect was not found to be statistically significant until 1 wk of age (19). It is likely that the preponderance of AT2 receptors prior to 1 wk of age serves to limit the proliferative action of ANG II, whereas the rapid dominance of the AT1 receptors after that time accounts for the trophic effect of ANG II on later glomerular matura-

In a recent report, adult mice with a null mutation of the AT2 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 AT1 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 AT2 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 AT2 over AT1 receptors in the neonatal kidney contribute to the greater renal damage in the developing kidney consequent to chronic urinary tract obstruction.

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