Glomerular and tubular function during AT1 receptor blockade in pigs with neonatal induced partial ureteropelvic obstruction

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Previously, we showed that neonatal induced chronic partial unilateral ureteral obstruction (PUUO) of the multipapillary pig kidney decreased glomerular filtration rate (GFR) of the obstructed kidney. We hypothesized that ANG II and nitric oxide (NO) are important for the changes in renal function and in the present study we examined the effects of chronic AT1 receptor blockade using CV-11974 (0.12 mg/h candesartan from age 23 to 30 days) on kidney function development after PUUO was induced in 2-day-old piglets. Moreover, the effect of superimposed acute NO inhibition using N^G-nitro-L-arginine methyl ester (L-NAME; 15 mg/kg) was examined to identify if this has diagnostic potential. PUUO significantly increased GFR in the non-obstructed contralateral kidney independent of candesartan. In candesartan-treated piglets, the L-NAME-induced GFR reduction seen in normal and nonobstructed kidneys was absent in the partial obstructed kidneys. Urine output and fractional excretion of water were increased from the partial obstructed kidneys. Consistent with this immunohistochemical analyses showed a reduced aquaporin-2 labeling in the collecting duct principal cells. Moreover, renal sodium handling was compromised by PUUO evidenced by an increased fractional excretion of sodium which was enhanced by candesartan treatment. In conclusion, our findings suggest that the counterbalance between AT1 receptor-mediated vasoconstriction and NO-mediated vasodilatation which maintain GFR in normal young porcine kidneys is changed by neonatal induced chronic PUUO. This may have diagnostic potential in children with suspected congenital obstruction. Our results also demonstrate compromised tubular functions in response to chronic PUUO despite preservation of glomerular function.

hydronephrosis; stereology; glomerular filtration rate; aquaporin 2

Prenatal diagnosed unilateral hydronephrosis persisting postnatal may be caused by partial obstruction at the ureteropelvic junction and hydronephrosis is found in ~0.5% of newborns potentially leading to obstructive nephropathy (36). Major challenges exist in the clinical management of the infant with congenital obstructive nephropathy. Renal function of the hydronephrotic kidney is often preserved despite severe dilatation (13). Measuring kidney function may therefore not disclose obstruction-induced impairment at the cellular and molecular levels including the activation of vasoconstrictors and cytokines that has been documented in animal models (6). Thus, for infants with unilateral hydronephrosis, there is a need to predict which patients are candidates for pyeloplasty and to estimate the acceptable duration of observation before proceeding to surgical intervention (13).

In recent studies, we demonstrated that neonatal induced partial unilateral ureteral obstruction (PUUO) in piglets decreases glomerular filtration rate (GFR) and causes a significant reduction in the number of nephrons (11, 12, 14). However, the regulation of these pathophysiological changes has not been addressed in the neonatal pig model. Several studies have demonstrated that ANG II plays important roles for the hemodynamic and cellular changes induced by unilateral ureteral obstruction (2, 5, 6). Moreover, it has been demonstrated that both ANG II and AT1 receptor expression are increased during neonatal ureter obstruction in rats (31, 45). In the developing pig kidney, the AT1 receptor-mediated glomerular vasoconstriction by ANG II, which is an important component of GFR regulation (1), is counteracted by nitric oxide (NO) (39, 40). Thus ANG II is likely to play a significant role in the hemodynamic response of the partially obstructed pig kidney which potentially can be modulated by NO inhibition.

ANG II is also known to play important roles in the renal regulation of sodium and water handling (33). Via the AT1 receptors ANG II mediates sodium reabsorption in the proximal and distal tubules and in the collecting ducts of mature rat kidneys (4, 25, 34). Renal sodium reabsorption is compromised in response to PUUO induced neonatally in rats (37). Thus it is important to examine this in pigs and to elucidate whether it is modulated by AT1 receptor blockade. Renal water reabsorption is tightly regulated by aquaporin water channels, especially via aquaporin-2 (AQP2) located in the collecting duct principal cells (29). AQP2 is predominantly regulated by vasopressin leading to translocation of AQP2 from the intracellular compartment to the apical membrane (17). Recent studies demonstrated reduced abundance of AQP2 which was associated with impaired urinary concentrating ability in rats with neonatally induced PUUO (37, 38). The role of the renin-angiotensin system in the regulation of renal water han-
ANG II AND RENAL FUNCTIONAL CHANGES IN NEONATAL PUUO

In this study, we therefore examined the effects of chronic AT1 receptor blockade on single-kidney GFR and single-kidney tubular sodium and water handling to identify whether this intervention will provide markers of obstruction. PUUO was induced during ongoing nephrogenesis in 2-day-old piglets. After completion of nephrogenesis, which requires an intact ANG II response (19, 20), the AT1 receptor was blocked using candesartan. Hypothesizing that NO-mediated vasodilator counterbalance is more pronounced during obstruction the potential of using NO inhibition for diagnostic purpose in children with suspected unilateral obstruction was subsequently addressed by comparing single-kidney GFR before and after a bolus infusion of Nω-nitro-l-arginine methyl ester (l-NAME).

MATERIALS AND METHODS

Experimental design and pharmacological intervention. The experiments were performed on 2-day-old female Danish Landrace pigs weighing 1.8 ± 0.4 kg who were randomized to four experimental groups of which siblings were equally distributed. At age 23 days a 2-ml osmotic mini-pump (ALZET Osmotic Pump, DURECT, Cupertino, CA) was implanted subcutaneously under light ketamine sedation and subcutaneous lidocaine anesthesia in the groin of each piglet releasing candesartan (kindly provided by Astra-Zeneca Sweden) dissolved in physiological saline (groups sham+ can and PUUO+c) or saline only (groups sham and PUUO). All animals were transported and housed under the same conditions. The study complied with the Danish regulations for the care and use of experimental animals and was approved by the Danish Animal Experiments Inspectorate.

Experimental groups. The Sham group involved (n = 12) sham operation of the left ureter at age 2 days. The Sham+c group involved (n = 10) sham operation of the left ureter at age 2 days and candesartan treatment at a dose of 0.12 mg/h from day 23 to 30. The PUUO group involved (n = 11) partial obstruction of the left ureter at age 2 days. The PUUO+c group involved (n = 12) partial obstruction of the left ureter at age 2 days and candesartan treatment at a dose of 0.12 mg/h from day 25 to 30.

During investigations at age 30 days, 15 mg/kg l-NAME (Sigma) dissolved in physiological saline was given as an intravenous bolus. Ambulatory blood pressure measurements (Veterinary Blood Pressure Monitor, CAS Medical Systems) were performed once a day, three times before (from age 15 to 22 days), and three times during treatment with candesartan or saline (from age 23 to 30 days) and mean values were calculated.

Induction of PUUO at 2 days of age. The operative procedure has previously been described in detail (11, 44). In brief, under isoflurane anesthesia a 3- to 3.5-cm flank incision was made on the left side using sterile technique. The muscle layers were divided and the peritoneum was gently pressed medially. The left ureter was identified at the lower renal pole, and ~3 cm were carefully mobilized, preserving the periureteral adventitial tissue. A 2- to 2.5-cm longitudinal slit was made in the adjacent psoas muscle. The piglet was then randomized to one of the four experimental groups. The obstruction was created using the method of Ulm and Miller (44) embedding the mobilized ureter in the muscle slit and closing the muscle with three 5–0 nylon sutures. In the sham-operated groups, the ureter was exposed but not obstructed.

Examinations at 30 days of age. The pigs were sedated with an intramuscular injection of 5 mg/kg ketamine and 0.5 mg/kg midazolam. An additional intravenous injection of 5 mg/kg ketamine was given and orotracheal intubation was established (tube size 3.5 to 4). The pigs remained anesthetized with an intravenous infusion of 0.3 mg·kg⁻¹·min⁻¹ ketamine, 5 µg·kg⁻¹·min⁻¹ midazolam, and 2.5 µg·kg⁻¹·min⁻¹ pancuronium and were ventilated with a gas mixture of O₂ and N₂O (1:2), tidal volume 1.8, 26 breaths per minute adjusted according to pH. A standardized hydration regimen with intravenous isotonic saline 12 ml·kg⁻¹·h⁻¹ was continued throughout the experiment.

Through bilateral flank incisions, the ureters were located retroperitoneal and cannulated with ureteral catheters (Ch 6) for urine collection including sampling for urine culture. Through a neck incision, the right carotid artery and jugular vein were isolated and catheters were inserted by use of a Seldinger technique; the venous catheter was used for infusion of ⁵¹Cr-Ethylendiaminetetraacetic acid (⁵¹Cr-EDTA) and l-NAME, the arterial catheter was used for continuous blood pressure monitoring using a pressure transducer (Cardio Med) and for blood sampling.

Renal function measurements. The day before clearance measurements 75 mg lithium carbonate (Nycemed DAK) was given orally to obtain measurable plasma lithium concentrations without affecting kidney function. Single-kidney GFR was measured by a continuous infusion clearance technique using ⁵¹Cr-EDTA (priming injection 5 MBq followed by a sustained infusion 8 MBq/h). After a 60-min equilibration period, urine was collected from each kidney separately and arterial blood samples were taken at 30-min intervals. Mean arterial pressure and heart rate were measured continuously. Blood samples were centrifuged and 200-µl plasma samples were counted together with plasma blanks and 200-µl urine samples in a well crystal scintillation detector (Cobra, Packard) to a statistical accuracy of 1% and corrected for background activity and radioactive decay during counting. Plasma and urine concentrations of lithium were measured by atomic absorption (Perkin-Elmer atomic absorption spectrophotometer 290B). Urine and plasma sodium concentrations were measured in a continuous flow system (ABL 300 Radiometer). The urine output volumes were measured by weighing the samples. At the end of experiments, the kidneys were antegrade perfusion fixed via the abdominal aorta using 4% phosphate-buffered formalin. Kidneys were removed and the animals were euthanized under anesthesia using potassium-chloride.

Immunohistochemistry. After removal, the kidneys were weighed, cut in 4-mm-thick horizontal slices, and stored in 4% formalin. The slices were numbered sequentially, dehydrated, and paraffin-embedded. Random slices from the middle third of the kidneys were chosen and cut in 2.5-µm-thick sections using a calibrated microtome (Microm HM 355, Walldorf, Germany). Sections were single labeled with AQP2 antibody (7661ap) diluted 1:3,000 in PBS with 0.1% BSA and 0.3% Triton X-100, incubated with horseradish peroxidase-conjugated second antibody (P448, goat anti-rabbit immunoglobulin, DAKO), and visualized with 0.05% 3,3'-diaminobenzidine tetrachloride (Kem-en Tek, Copenhagen, Denmark) for identification of the primary antibody. Using a Leica DMRE light microscope, the sections were examined in a blinded fashion describing the labeling intensity of the collecting duct principal cells as homogenous or heterogeneous and the presence of AQP2 labeling in the apical plasma membrane.

To examine the density of principal cells in the collecting ducts, double labeling with AQP2 and [H]ATPase antibodies was performed as follows. Sections were incubated with rat polyclonal AQP2 antibodies raised to chicken (cc256ap kindly provided by Dr. M. Knepper, LKEM, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD) diluted 1:10,000 on the first day.
of the procedure. After overnight incubation, this was followed by a second incubation with peroxidase secondary antibodies (Abcam ab 6877–1) and visualized with 3,3-diaminobenzidine (DAB; Kem-En-Tec). After blockade of remaining peroxidase, the sections were incubated overnight with the primary rabbit [H+]ATPase antibodies raised polyclonal to the H7659 15 COOH-terminal peptide in rabbit 1:100 dilution followed by a second incubation with goat-anti-rabbit P448 (DAKO, Glostrup, Denmark) and visualized by SK-4700 (Vector Laboratories). Sections from partial obstructed and sham-operated kidneys were labeled at the same time with the same solutions to allow for comparison.

Quantification of principal cells. To ensure that principal cells that expressed even very low levels of AQP2 antibody were detected, sections were double labeled with antibody against AQP2 and [H+]ATPase (intercalated cells in the collecting ducts) as described above and nuclear staining was omitted. Cell counting was performed using a microscope (Olympus BX-50) equipped with a motorized object stage enabling movement from one field of view to another in the x to y direction in a systematic uniformly random fashion (step length 1,800 μm). Images of the sections were viewed on a computer screen using a ×40 objective lens and a 3 CCD color video camera. Stereological software (CAST, Visiopharm, Hillerød, Denmark) was used to superimpose two point grids with 4 and 256 test points. The cortex and medulla were separated using the arcuate arteries and the presence of glomeruli and proximal tubules in the cortex. The antibody against [H+]ATPase also stains the brush border and subvillar invaginations of proximal tubules (22) which were not included in the counting. The volume fraction of AQP2 or [H+]ATPase-positive cells in the cortex and medulla, respectively, was calculated as

\[
V(V_{\text{structure}}) = \frac{\sum P_{\text{structure}}}{\sum P_{\text{total}}}
\]

where \(V(V_{\text{structure}})\) is the volume fraction of the structure (e.g., AQP2-positive cells) in the reference space (e.g., cortex), \(P_{\text{structure}}\) is the number of points falling on the component, and \(P_{\text{total}}\) the number of points falling on the reference space (including the component). The whole kidney volume densities were converted to absolute quantities by multiplying the total kidney volume estimated by the Cavalieri principle of a quarter of the kidney sampled using systematically random sampling (32). The sections were corrected for paraffin-induced shrinkage. Small kidney samples were weighed and the weight was converted to volume by multiplying with 1.04 cm³/g (volume before). The volume of kidney samples after paraffin embedding (volume after) was estimated by point counting and the principle of Cavalieri (32). Tissue deformation was equal to 1 – (volume after/volume before) which was accounted for.

For all tissue preparation and stereological quantification, the observer was naive to the origin of the histological sections.

Calculations and statistics. Clearance values (C\(_X\)) were calculated as the product of urinary solute concentration (U\(_X\)) and urine output (UO) divided by the plasma concentration (P\(_X\)) over time as milliliters per minute per kilogram of body weight. The fractional excretions (FE\(_X\)) were calculated as (C\(_X\)/GFR)*100. The tubular handling of sodium and water was investigated using the lithium clearance technique (42). The fractional excretion of lithium represents the fractional delivery of sodium and water at the end of the proximal tubules. Using the renal clearances of \(51\)Cr-EDTA, sodium, and lithium, the following parameters were calculated

\[
\text{GFR: } \text{GFR}_{\text{Cr-EDTA}} = \frac{U_{\text{Cr-EDTA}}}{P_{\text{Cr-EDTA}}} \times \text{UO}
\]

\[
\text{FE of sodium: } \text{FE}_{\text{Na}} = \frac{(C_{\text{Na}})}{(C_{\text{GFR-EDTA}})} \times 100
\]

\[
\text{FE of water: } \text{FE}_{\text{H}} = \frac{(U_{\text{O}})}{(C_{\text{GFR-EDTA}})} \times 100
\]

\[
\text{PFE of water and sodium from the proximal tubules: } \text{PFE}_{\text{H,Na}} = \frac{(C_l)}{(C_{\text{GFR-EDTA}})} \times 100
\]

\[
\text{FE of water from the distal tubules: } \text{DE}_{\text{H}} = \frac{(U_{\text{O}}/C_{\text{l}})}{100}
\]

\[
\text{FE of sodium from the distal tubules: } \text{DE}_{\text{Na}} = \frac{(C_{\text{Na}}/C_{\text{l}})}{100}
\]

Values are presented as mean and coefficient of variation (CV; SD/mean) and compared using ANOVA and post hoc Student’s t-test (unpaired for comparison between groups, paired for comparison within individual pigs). In addition, comparison was done after log transforming the data. Volume fractions and absolute AQP2 values are compared using unpaired Student’s t-test. Values were considered statistically significant at \(P < 0.05\).

RESULTS

All pigs had macroscopically normal appearing left kidneys at study entry and right kidneys at study end.\(^1\) Urine culture at 30 days did not demonstrate urinary tract infection. Body weight did not differ between groups (NS) and all partial obstructed kidneys were found to have significant hydronephrosis (grade three to four) at age 30 days. Mean arterial pressure was measured ambulatory once a day three times before (from age 15 to 22 days) and three times during candesartan or saline treatment (from age 23 to 30 days) and did not differ between groups (data not shown).

PUUO increased GFR in nonobstructed contralateral kidneys independent of AT1 receptor blockade. In the PUUO groups, single-kidney GFR was reduced in the partial obstructed kidney compared with the contralateral nonobstructed kidney independent of candesartan treatment (see Table 2). Moreover, in the PUUO pigs single-kidney GFR increased significantly in the nonobstructed contralateral kidneys compared with sham-operated controls which was also independent of candesartan treatment (see Table 2).

AT1 receptor blockade uncovered the single-kidney GFR response to l-NAME after PUUO. To identify whether NO inhibition may have a diagnostic potential in children with congenital hydronephrosis, single-kidney GFR was measured before and after a bolus infusion of l-NAME in the four experimental groups (see Table 2). As a clinical useful approach, l-NAME was given intravenously, and this significantly increased mean arterial pressure in all groups (Table 1). Importantly, the increase was of identical magnitude in all four groups. l-NAME did not change single-kidney GFR in the kidneys of saline-treated, sham-operated and PUUO pigs compared with the value before l-NAME was given. However, in pigs pretreated with candesartan, l-NAME decreased single-kidney GFR in the nonobstructed kidneys of the PUUO group and in the kidneys of the sham-operated pigs. In contrast, l-NAME did not change single-kidney GFR in the partially obstructed kidneys of the candesartan-treated pigs (Fig. 1 and Table 2).

\(^1\)The number of animals included varied between the renal function and immunohistochemical analyses and the exact number of animals included for each analysis is given in the tables and legends. Seven animals were lost due to anesthesia-related death (n = 3) or failure to thrive (n = 4). Four animals were excluded from the renal function studies because of loss of osmotic pumps immediately before investigation (n = 2), unstable urine production (n = 1), and urinary catheter occlusion (n = 1). A further four animals had renal function measured by a different procedure and are therefore excluded from the function studies. In four animals, the plasma lithium concentrations were inadequate for valid lithium clearance calculation. In four animals the perfusion fixation failed.
PUUO increased urine output and fractional water excretion which is associated with reduced AQP2 labeling and preserved number of principal cells. The measurements of tubular function were done before the piglets were given l-NAME. Partial obstruction significantly increased urinary output (UO) and fractional water excretion (FEw) compared with sham-operated kidneys (Table 3). The increase was partly due to an increase in DFEw, although this did not reach statistical significance. The trend toward an increased DFEw raises the possibility that AQP2 expression may be downregulated and plays a role in the PUUO-induced increase in diuresis. We therefore analyzed AQP2 abundance by semiquantitative scoring of AQP2 staining intensity by systematic analysis of a cross section containing both cortex and medulla from the middle part of each left kidney. As UO and FEw did not differ between candesartan- and saline-treated partial obstructed kidneys (Table 3), only the saline-treated groups were studied. In the partial obstructed kidneys, there was a consistent heterogeneous and reduced AQP2 distribution in the collecting duct principal cells (Fig. 2A), whereas the distribution of AQP2 in the sham-operated kidneys was clearly more homogenous (Fig. 2B). To study whether the compromised urinary concentrating ability in the partial obstructed kidneys could be explained by tubular cell destruction and thereby a reduced number of principal cells, we quantitated the volume of AQP2-positive cells by use of stereology. To ensure that even principal cells with very low AQP2 staining were included, [H]ATPase staining for intercalated cells were added and nuclear staining was omitted (Fig. 3). The volume density of AQP2-positive cells in the cortex and in the medulla did not differ between partial obstructed or sham-operated kidneys (Table 4). Furthermore, the absolute volumes of principal cells in the whole kidney did not differ between the two groups (Table 4).

PUUO increased sodium clearance and fractional sodium excretion. PUUO significantly increased CNa and FENa compared with sham-operated kidneys (Table 3). Candesartan did not change CNa and FENa in the partial obstructed kidneys (Table 3) but significantly increased DFEw compared with controls suggesting an enhanced AT1 receptor involvement in distal sodium reabsorption during neonatal PUUO.

**DISCUSSION**

The main results of the present study, using a well-established neonatal pig model with high analogy to the human kidney, demonstrated that chronic PUUO led to 1) a GFR increase in the nonobstructed contralateral kidney independent of AT1 receptor blockade, 2) a change in the GFR response to intravenous NO inhibition during AT1 receptor blockade, 3) increased water excretion associated with reduced AQP2 abundance without a reduction in the volume of principal cells, and 4) increased tubular sodium excretion. AT1 receptor blockade increased the fractional excretion of sodium from the distal tubules in the partial obstructed kidneys demonstrating that ANG II plays a role in distal sodium reabsorption during neonatal PUUO.

**GFR increase in nonobstructed kidneys is not AT1 receptor mediated.** In PUUO pigs, GFR increased in the nonobstructed contralateral kidney. Previously, we demonstrated in this pig model with PUUO that contralateral nonobstructed kidney GFR does not increase uniformly despite a significant GFR reduction of the partial obstructed kidney (14). Thus, consistent with observations in neonatal rat models, changes in contralateral nonobstructed GFR are not an unambiguous finding (23, 24), suggesting that an increase in contralateral kidney GFR is not only caused by stimuli present when there is significant decrease in GFR of the partial obstructed kidney.

AT1 receptor blockade did not prevent the GFR increase in the contralateral kidney in PUUO pigs, suggesting that counterregulatory vasoactive mediators are activated. This is consistent with findings in the unilateral obstructed neonatal guinea pig where contralateral kidney vascular resistance was unaffected by ANG I-converting enzyme inhibition (7). Alternatively, sympathetic nerve activity may play a role in the contralateral kidney adaptive vasoactive response as indicated in the neonatal rat model of unilateral occlusion (9).

**AT1 receptor blockade uncovered the single-kidney GFR response to l-NAME after PUUO.** The balance between ANG II-mediated vasoconstriction and NO-mediated vasodilatation normally influences GFR. We studied the GFR response to systemic NO inhibition during neonatal PUUO where the ANG

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**Table 1. Body weight, MAP, and plasma sodium in the four experimental groups**

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 9)</th>
<th>Sham + Can (n = 7)</th>
<th>PUUO (n = 7)</th>
<th>PUUO + Can (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>4.86 (0.21)</td>
<td>4.84 (0.14)</td>
<td>4.86 (0.35)</td>
<td>4.66 (0.27)</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>72 (0.13)</td>
<td>60 (0.13)*</td>
<td>69 (0.11)</td>
<td>63 (0.11)</td>
</tr>
<tr>
<td>MAP after l-NAME</td>
<td>102 (0.09)</td>
<td>83 (0.13)*</td>
<td>102 (0.12)†</td>
<td>89 (0.15)†</td>
</tr>
<tr>
<td>PNa, mmol/l</td>
<td>135 (0.02)</td>
<td>134 (0.03)</td>
<td>134 (0.02)</td>
<td>135 (0.02)</td>
</tr>
</tbody>
</table>

Values are mean (CV). *P < 0.05 candesartan (can) vs. saline. †P < 0.01 before l-NAME vs. after l-NAME. MAP, mean arterial pressure; PNa, plasma sodium; PUUO, partial unilateral ureteral obstruction.
II system is enhanced, to identify whether this approach may be clinically useful. Thereby the potential of using the vasoactive counterbalance between ANG II and NO for diagnostic purpose in suspected neonatal obstruction could be explored. Because of the intravenous administration route, l-NAME increased systemic blood pressure. This may explain the unchanged GFR levels in control pigs as opposed to the findings by Solhaug and colleagues (40) where intrarenal L-NAME administered directly via the renal artery reduced GFR by 30% in the normal piglet. However, this approach is not clinically feasible. The absence of GFR changes despite systemic blood pressure increase in the present study may be due to activation of renal autoregulation modulating afferent arteriolar tone by a combination of tubuloglomerular feedback and myogenic effects (27). The complex autoregulatory mechanisms may to some extent be influenced by ANG II and NO as suggested in the studies from the normal adult rat kidney (18). Importantly, the piglets in the present study were studied under strictly standardized conditions and the l-NAME-induced moderate blood pressure increase did not differ between experimental groups.

During AT1 receptor blockade, NO inhibition decreased single-kidney GFR in control kidneys. Likewise, NO inhibition decreased single-kidney GFR in the nonobstructed kidney but not in the partially obstructed kidney of the candesartan-treated PUUO groups. GFR regulation is complex and involves interaction between a variety of hormone systems and neural factors. This interaction is modulated by the ureter obstruction making interpretation of GFR changes induced by pharmacological manipulation during obstruction even more complex. In addition to ANG II, previous studies in adult obstructive rats models have shown increased expression of the vasoconstrictor endothelin (21). Furthermore, ANG II synthesis inhibition by enalapril demonstrated an increased expression of the vasoconstrictor endothelin receptors in adult mice with ureter obstruction (28). Vasoconstriction maintained by endothelin even in the presence of ANG II blockade is compatible with the observations in the present study suggesting that GFR is regulated by other mediators during partial ureter obstruction. It may be speculated that the vasoconstrictor thromboxane A2 (TxA2) or the vasodilator prostaglandins also are involved in GFR regulation in response to PUUO. However, previous studies of chronic neonatal PUUO in guinea pigs (8) and of chronic UUO in pigs (16) did not demonstrate changes in renal function after TxA2 blockade, whereas TxA2 blockade improved the postobstructive function in rats (35), suggesting both species- and design-dependent differences in the response to TxA2 blockade.

The present observation that administration of l-NAME-induced changes in the single-kidney GFR response during AT1 receptor blockade in piglets with PUUO indicates that simultaneous blockade of ANG II and NO may have a potential to identify infants who are candidates for pyeloplasty among infants with unilateral hydronephrosis. Furthermore, our data may suggest that the individual GFR changes after NO inhibition could be related to the level of functional deterioration in the obstructed kidneys. This aspect needs to be further addressed in animal models together with the challenge to estimate the acceptable duration of observation before proceeding to surgical intervention. A characteristic of the multipapillary kidney model is the variation in the GFR response to a standard obstruction procedure. This is in agreement with the variation in renal function observed in children with hydronephrosis (13), suggesting that the pig model is valuable when results are compared with the clinical situation. However, this variation may also make statistical comparisons difficult in clinical studies.

PUUO is associated with reduced AQP2 abundance and impaired tubular water reabsorption. In the present study, we demonstrated that water excretion was significantly increased.

### Table 2. GFR before and after l-NAME in the four experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham (n = 9)</th>
<th>Sham + Can (n = 7)</th>
<th>PUUO (n = 7)</th>
<th>PUUO + Can (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left kidney GFR pre ml·min⁻¹·kg body wt⁻¹</td>
<td>1.53 (0.16)</td>
<td>1.49 (0.26)</td>
<td>1.71 (0.16)</td>
<td>1.74 (0.15)</td>
</tr>
<tr>
<td>Right kidney GFR post ml·min⁻¹·kg body wt⁻¹</td>
<td>1.47 (0.23)</td>
<td>1.45 (0.27)</td>
<td>1.50 (0.23)</td>
<td>1.45 (0.21)</td>
</tr>
</tbody>
</table>

Values are mean (CV). *P < 0.05 PUUO vs. sham. †P < 0.05 obstructed vs. nonobstructed (nonobs.). +P < 0.05 PUUO vs. sham. ‡P < 0.05 Can vs. saline.

### Table 3. UO and renal clearance data in the four experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham (n = 9)</th>
<th>Sham + Can (n = 7)</th>
<th>PUUO (n = 7)</th>
<th>PUUO + Can (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UO, μl·min⁻¹·kg body wt⁻¹</td>
<td>14.3 (0.55)</td>
<td>13.6 (0.47)</td>
<td>24.5 (0.52)</td>
<td>23.4 (0.40)</td>
</tr>
<tr>
<td>FEw, %</td>
<td>0.95 (0.50)</td>
<td>0.97 (0.45)</td>
<td>1.47 (0.58)</td>
<td>1.43 (0.54)</td>
</tr>
<tr>
<td>CNa, μl·min⁻¹·kg body wt⁻¹</td>
<td>5.3 (1.05)</td>
<td>5.4 (0.89)</td>
<td>10.1 (0.78)</td>
<td>9.5 (0.79)</td>
</tr>
<tr>
<td>FENa, %</td>
<td>0.33 (0.93)</td>
<td>0.35 (0.76)</td>
<td>0.62 (0.87)</td>
<td>0.57 (0.82)</td>
</tr>
<tr>
<td>PFENa, %</td>
<td>26 (0.56)</td>
<td>25 (0.42)</td>
<td>35 (0.54)</td>
<td>34 (0.52)</td>
</tr>
<tr>
<td>DFEw, %</td>
<td>6.0 (0.89)</td>
<td>4.9 (0.69)</td>
<td>4.7 (0.38)</td>
<td>4.8 (0.45)</td>
</tr>
<tr>
<td>DFESn, %</td>
<td>1.7 (0.72)</td>
<td>1.5 (0.70)</td>
<td>1.7 (0.63)</td>
<td>1.7 (0.61)</td>
</tr>
</tbody>
</table>

Values are measured before the administration of l-NAME and given as mean (CV). *P < 0.05 PUUO vs. sham. †P < 0.05 Can vs. saline. ‡P < 0.05 obstructed vs. nonobstructed. UO, urine output; FEw, fractional excretion of water; CNa, sodium clearance; FENa, fractional excretion of sodium; PFEw and PFESn, proximal fractional excretion of water and sodium, respectively; DFEw and DFESn, distal fractional excretion of water and sodium, respectively. §N = 7, n = 5, and n = 6 for PFEw and PFESn in sham, PUUO, and PUUO+can groups, respectively; n = 7 for CNa in sham group.
from the PUUO kidneys. The water excretion from the proximal and the distal tubules, respectively, was quantitated using the lithium clearance technique. This technique has been widely used and is generally accepted as being the best indirect method available for estimating the delivery of sodium and water from the proximal tubules to the loop of Henle (42).

Although a great number of experimental studies have tested the lithium clearance method against micropuncture measurements (42), this is not possible in the pig model. Our results indicate that distal tubule water excretion was increased, although this did not reach statistical significance. To further address the mechanisms involved in the compro-

Fig. 2. Immunohistochemical staining with primary antibodies that recognize AQP2 (brown) in collecting duct cells. Heterogeneous AQP2 labeling intensity (arrows) in partial obstructed (A) and homogeneous AQP2 labeling intensity (arrows) in sham-operated kidneys (B).

Fig. 3. Double labeling of sections from saline-treated piglets. Labeling was performed with primary antibodies that recognize AQP2 (brown) and [H\(^+\)]ATPase (blue). The AQP2 staining intensity was reduced in the medullary [A and C (high magnification of inset in A)] and cortical (E) collecting ducts of partial obstructed kidneys compared with the staining intensity of medullary (B and D) and cortical (F) collecting ducts in sham-operated kidneys.
nised tubular water reabsorption, AQP2 abundance was systematically examined using specific immunostaining techniques. Analyses of the kidney sections were done semiquantitatively by viewing the entire section in a blinded fashion. This demonstrated that AQP2 expression was reduced in the partial obstructed kidneys. Recent studies in the neonatal obstructed rat model documented reduced AQP2 expression which was associated with decreased solute-free water reabsorption demonstrating a relationship between molecular and functional changes in the obstructed kidney (37, 43). Our study is the first to show a similar functional association between the AQP2 expression and the ability to reabsorb water in the multipapillary kidney.

In the partial obstructed kidneys, there were heterogeneous AQP2 labeling and distribution as opposed to the homogenous distribution found in the control kidneys. Our findings are consistent with the first original observation demonstrating that urinary tract obstruction is associated with downregulation of AQP2, which is more severe in some parts of the collecting ducts than others (15). It could therefore be speculated that local ANG II generation in the obstructed kidney, which has been shown to interact with AQP2 regulation in rat kidneys subjected to neonatal PUUO (43), is heterogeneously affected by the obstruction.

Candesartan treatment did not change the water excretion parameters compared with saline in the partial obstructed kidneys. Therefore, the AQP2 analyses were restricted to the latter. However, in a recent study in rats candesartan treatment prevented the obstruction-induced reduction in solute-free water reabsorption after 10 wk of severe partial obstruction (43). Consistent with the improved water reabsorption capacity, candesartan treatment was also associated with prevention of PUUO-induced dysregulation of AQP2 suggesting that candesartan prevented the obstruction-induced effects localized to the distal tubules. The results from the present study demonstrate that the fractional proximal water excretion tended to increase after candesartan treatment in the partial obstructed kidneys. In the present study, pigs were examined after 30 days of mild obstruction, whereas the effects of AT1 receptor blockade were studied after 10 wk of severe obstruction in the rat model. Thus it is possible that the response to AT1 receptor blockade in the obstructed pig kidney may change with time to a response more similar to the rat study.

To address whether the compromised ability to reabsorb water was due to tubular destruction and thereby a decrease in the volume of principal cells, we quantitated these by use of stereological technique. The results demonstrated that the volume of principal cells was not reduced indicating that the compromised water reabsorption was not due to a decreased number of principal cells but rather to a reduction in AQP2 protein abundance. In theory, this could indicate reversibility of the compromised water reabsorption supporting the view that normalization of AQP2 levels takes place several weeks after release of bilateral occlusion in rats (26).

AT1 receptor blockade increased distal fractional sodium excretion from the partial obstructed kidney. PUUO significantly increased sodium excretion consistent with observations in obstructed rat kidneys (37). Although AT1 receptor blockade did not change overall sodium excretion, it increased distal tubule fractional sodium excretion, indicating that ANG II may play a role in distal tubule sodium reabsorption during neonatal PUUO in this model. This finding is in contrast to the decreased sodium excretion observed from obstructed weaning rat kidneys in response to enalapril treatment (3). Moreover, the suggested potential renoprotective effect of ANG II inhibition in the weanling rat may not be present in the neonatal obstructed multipapillary pig kidney. This would limit the therapeutic potential of blocking the renin-angiotensin system postnatally to prevent renal tubular damage in children.

The increased distal tubule sodium output from partial obstructed kidneys during AT1 receptor blockade demonstrates an upregulation of ANG II-mediated sodium reabsorption in this segment during neonatal PUUO. In vitro studies have shown that renal epithelial sodium channels, mediating sodium reabsorption in collecting duct cells, are activated by AT1 receptors (34). It may therefore be speculated that PUUO-induced chronic maturation led to an upregulation of distal tubule epithelial sodium channel subunits to compensate for the increased sodium load presented from the proximal tubules. This aspect may be important in predicting tubular damage during congenital hydronephrosis and needs to be addressed in more detail in a future study.

In conclusion, our findings in this study using a multipapillary kidney model with high analogy to human congenital urinary tract obstruction demonstrate that the changes in kidney function are not solely ANG II dependent and suggest that the counterbalance between AT1 receptor-mediated vasoconstriction and NO-mediated vasodilatation which maintain GFR in normal young porcine kidneys is changed by neonatal induced chronic PUUO, possibly by the activation of other vasoactive mediators. The impact of ANG II and the NO system on single-kidney GFR may offer possibilities to detect significant obstruction in children with urinary tract dilatation and suspected obstruction. Moreover, the study demonstrates that tubular functions are likely to be compromised despite preservation of GFR during neonatal induced PUUO. The decreased AQP2 abundance associated with increased water excretion in response to partial ureter obstruction indicates that downregulation of AQP2 protein abundance may play a role in renal function in neonatal PUUO in the multipapillary kidney. As opposed to the intact neonatal kidney, ANG II mediates distal tubule sodium reabsorption during chronic PUUO.

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Table 4. Volume fractions (medulla and cortex) and whole kidney absolute volumes of AQP2-positive cells in partial obstructed and sham-operated kidneys from saline-treated pigs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (n = 8)</th>
<th>PUUO (n = 7)</th>
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<tbody>
<tr>
<td>$V_v(AQP2,\text{cortex})$</td>
<td>12.6 $\times$ 10^{-3} (0.32)</td>
<td>13.8 $\times$ 10^{-3} (0.68)</td>
</tr>
<tr>
<td>$V_v(AQP2,\text{medulla})$</td>
<td>33.0 $\times$ 10^{-3} (0.59)</td>
<td>30.3 $\times$ 10^{-3} (0.50)</td>
</tr>
<tr>
<td>AQP2-positive cell volume, mm³</td>
<td>507 $\times$ 10^{-3} (0.60)</td>
<td>631 $\times$ 10^{-3} (0.50)</td>
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</table>

Values are mean (CV). $V_v(AQP2,\text{cortex})$, volume fraction of AQP2-positive cells in the cortex; $V_v(AQP2,\text{medulla})$, volume fraction of AQP2-positive cells in the medulla.
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