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ORIGINAL PAPER

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Reduced renal vascular resistance in response to verapamil during gradated ureter obstruction in pigs

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Abstract Unilateral ureteral obstruction (UUO) is associated with reductions in ipsilateral renal blood flow (RBF) and glomerular filtration rate (GFR) caused by an active preglomerular vasoconstriction, where angiotensin II (ANGII) may be an important mediator. Calcium-channel blockers preferentially dilate preglomerular vessels and abolish the vasoconstrictor actions of ANGII in preglomerular arterioles of the hydronephrotic rat kidney. In this study, we, therefore, examined the effects of the calcium-channel blocker verapamil (3.65 μg/kg per minute i.v.) on RBF, GFR and renal vascular resistance (RVR) in our pig model with UUO, where ultrasonic flow probes are mounted on each renal artery and catheters placed in the abdominal aorta and both renal veins. Verapamil treatment was associated with a 34% reduction in ipsilateral RBF (from 182.6 ± 20.5 ml/min to 120.6 ± 12.2 ml/min, $P < 0.001$), which was similar to the 27% reduction in ipsilateral RBF in controls (from 194.6 ± 13.1 ml/min to 140.6 ± 15.2 ml/min, $P < 0.001$). Ipsilateral GFR was reduced by 70% in the verapamil-treated pigs (from 29.0 ± 2.6 to 8.5 ± 0.9 ml/min, $P < 0.001$) and by 73% in control animals (from 29.2 ± 3.1 to 7.6 ± 2.1 ml/min, $p < 0.001$). However, the increase in RVR was significantly attenuated in the verapamil-treated pigs. Ipsilateral RVR increased by 19% in the verapamil-treated pigs (from 0.585 ± 0.076 to 0.726 ± 0.081 mmHg/min/ml, $P < 0.05$) compared with a 34% increase in control pigs (from 0.560 ± 0.056 to 0.854 ± 0.091 mmHg/min per milliliter, $P < 0.001$), suggesting that an intact calcium-channel may be important for the increase in renal vascular resistance during unilateral ureter obstruction. In conclusion, the present study shows that verapamil is able to modulate the increase in renal vascular resistance in response to increased pelvic pressure.

Keywords Verapamil · Ureteral obstruction · Renal hemodynamics · Angiotensin II · Pigs

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Introduction

Unilateral ureteral obstruction (UUO) is associated with marked changes in renal hemodynamics and renal metabolism of vasoactive hormones [18]. Obstruction ultimately results in a progressive reduction in ipsilateral renal blood flow (RBF) and glomerular filtration rate (GFR) due to an increased renal vascular resistance (RVR). Previously, we demonstrated that gradated UUO in the pig is associated with reductions in RBF and GFR compatible with a preglomerular vasoconstriction [14] and that UUO is associated with an enhanced de novo renal synthesis of ANGII from the ipsilateral kidney [12]. Further, we have shown that the ANGII antagonist losartan is able to reduce ipsilateral vasoconstriction in the obstructed pig kidney [15], suggesting that ANGII plays an important role in the initial renal vasoconstriction in response to obstruction.

Changes in RVR due to ANGII-induced vasoconstriction of preglomerular arterioles is suggested to be mediated in part by a voltage-gated calcium-channel pathway [27]. A rapid Ca^{2+}-influx into smooth muscle cells results in an increase in muscle tone, and the actively maintained large Ca^{2+} gradient between the extracellular and cytosolic environments is essential [6].

Depolarization of the sarcolemma-activating potential-dependent calcium channels allowing Ca^{2+} to enter the cell is one way of increasing delivery of Ca^{2+} to the myoplasm. Receptor occupation may also directly activate Ca^{2+}-influx pathways independently of membrane depolarization [21]. Organic calcium-channel blockers (CCB) primarily interfere with the influx of Ca^{2+} through voltage-gated channels [6], resulting in a reduced inflow of Ca^{2+} into cells [20]. Schnackenberg et al. showed that verapamil alters the preglomerular resistance primarily through blockade of voltage-gated calcium channels [27].

Both systemic and intrarenal arterial infusion of CCB have been shown to increase RBF and GFR, causing an increase in urine output, together with natriuresis [21, 25], and it has been suggested that these changes are mediated through autoregulatory resistance adjustments [24]. Thus, changes in RVR are believed to be localized to the preglomerular sites, predominantly to the preglomerular arteriole [5, 7, 28], and CCB, therefore, seem to be important functional modulators of the preglomerular contractile elements. Moreover, CCB have been shown to preferentially dilate preglomerular vessels in the split hydronephrotic rat kidney [11] and abolish the vasoconstrictor actions of ANGII in the preglomerular arterioles [6, 21]. Recently Kahn et al. demonstrated that verapamil prevented the endothelin-1-dependent renal vasoconstriction during acute UUO in the dog [16], further supporting the view that Ca^{2+}-channels play an important role for regulation of preglomerular vascular resistance in response to UUO.

In most previous studies, the renal functional changes in response to ureteral obstruction have been examined in models with complete ureteral occlusion. Using stepwise increases in pelvic pressure allows us to examine whether changes occur at specific pelvic pressure levels. Thus, the aim of this study was to examine changes in renal hemodynamics and renal handling of solute and water during gradated UUO in a well-characterized pig model, where the expression of the intrarenal renin angiotensin system is increased. Furthermore, we examined whether calcium-channel blockade was able to modulate changes in RBF, GFR and RVR.

Materials and methods

Preparation of animals

Sixteen immature pigs (90 days old) of the Danish Landrace breed (Yorkshire/Lancaster), weighing from 32 to 35 kg, were used. Before the study, the pigs were fed a standard pig diet. From the day before any experimental procedures, the animals had free access to water but were deprived of food. Twenty-four hours prior to any experiments, the pigs were given 300 mg lithium carbonate (Nycomed DAK) orally.

Experiments were carried out with the pigs under general anesthesia induced by intramuscular administration of ketamine NFN (Ketalar) 10 mg/kg b.w. and midazolam (Dormicum) 3 mg/kg. After orotracheal intubation, the pig was connected to a respirator (Siemens Servo 900 D) and ventilated with a gas mixture of O_2 and N_2O (2:4). Tidal volume and rate were adjusted according to analysis of arterial blood samples every hour, keeping pH between 7.4 and 7.5 (ABL 300, Radiometer, Copenhagen). Anesthesia was maintained by intravenous administration of midazolam (5 μg/min per kilogram), ketamine (0.3 mg/min per kilogram) and pancuronium bromide (Pavulon) (2.5 μg/min per kilogram) through the central venous catheter in the left jugular vein, as well as isotonic saline, 3 ml/min that was administrated throughout the experiment.

Through cut-downs in the femoral groins, the femoral vessels were located. By the use of a modified Seldinger technique and X-ray control, a Teflon-coated catheter was placed in the aorta for arterial blood sampling and monitoring of arterial blood pressure. Subsequently, two catheters were placed with tips in the right and left renal vein. Finally, another catheter for measurement of central venous pressure was placed in the superior caval vein through the right jugular vein. The catheters in the aorta and superior caval vein were connected to pressure transducers (Statham Gould no. 4523551) connected to an amplifier and monitor (Medistim CardioMed CM-4008). The left midureter was isolated using a low flank muscle splitting retroperitoneal approach and cannulated with a ureteral catheter (Ch 9) for urine-flow measurements and urine sampling. The same procedure was used on the right side, but the right ureter was cannulated with a two-channel catheter with the tip placed in the renal pelvis. The ureters were ligated around the catheters at the ureterotomy. One channel of the catheter placed in the right ureter was connected to a pressure transducer (Statham Gould no. 4523551). The other channel was mounted with a three-way stop-cock, which could be switched to an adjustable water column to increase pelvic pressure by elevation of the water column.

Through subcostal flank incisions, the renal arteries were isolated on both sides, and ultrasonic flow probes (Medistim 4 mm), connected to a transit time volume flow meter (Medistim CardioMed CM-4008) for continuous flow reading, were inserted central to any bifurcation. Arterial blood pressure, heart rate, central venous pressure, right pelvic pressure and bilateral renal blood flow were continuously measured. After the study, the pigs were terminated with an overdose of potassium chloride.

Study design

The pigs were allowed a resting period of 90 min following surgery. Systemic administration of verapamil (3.65 μg/kg per minute) was then started as a continuous infusion to the eight pigs in the verapamil-treated group.[1] From the right pelvis, urine was sampled every 30 min during the resting period, and the right pelvic pressure was monitored. Subsequently, gradated obstruction was initiated by connecting the free lumen of the right ureteral catheter to the adjustable water column. Thereafter, the pressure was raised every 30 min in steps of 10 mmHg. The pressure generated in the right ureter by the urine flow was monitored simultaneously in the same period. From the left ureter, urine was sampled and measured every 30 min throughout the experiment. Blood samples were taken from the aorta and both renal veins every 30 min.

Calculations

GFR was measured by a continuous infusion clearance technique using ^{51}Cr-EDTA. Subsequent to operating procedures, the pigs were given 1.1 MBq ^{51}Cr-EDTA (Behring, Marburg, Germany) as

[1]Prior to the experiments included in this study, dose-response studies were performed using three selected doses of verapamil. The effects on arterial blood pressure and renal hemodynamics of 2.45 μg/kg per minute, 3.65 μg/kg per minute and 4.90 μg/kg per minute verapamil were examined. Administration of 2.45 μg/kg per minute did not change arterial blood pressure or renal hemodynamics. Administration of 3.65 μg/kg per minute resulted in a slight increase in renal blood flow, without changes in arterial blood pressure. Administration of 4.90 μg/kg per minute verapamil markedly decreased arterial blood pressure.

a bolus, followed by a continuous infusion (1.13 MBq/h) of ^{51}Cr-EDTA during the remainder of the experiment. Plasma samples from the aorta and renal veins were counted in a gamma counter (Packard Cobra, GMI Inc., Clearwater, Minn., USA) to a statistical accuracy of 1%. The recorded counts were corrected for radioactive decay during counting. Glomerular filtration rate was calculated as:

$$GFR = RPF \times EF_R$$

where RPF is the renal plasma flow, and EF_R is the renal extraction fraction of ^{51}Cr-EDTA. RPF was calculated from the equation:

$$RPF = RBF \times (1 - Hct)$$

where RBF is renal blood flow measured by transit time ultrasound and Hct is the hematocrit; EF_R was calculated by the following equation:

$$EF_R = \frac{\left(^{51}CrEDTA_{art} - {}^{51}CrEDTA_{vein}\right)}{^{51}CrEDTA_{art}}$$

which is equivalent to filtration fraction (FF):

$$EF_R = GFR/RPF = FF$$

FF is equivalent to the renal extraction of EDTA, knowing that EDTA is filtered freely and neither excreted, absorbed nor retained [9].

With the assumption that blood pressure in the renal vein was zero, renal vascular resistance (RVR) was calculated from the following equation:

$$RVR = MAP/RBF$$

where MAP is mean arterial blood pressure.

Plasma concentration of ANG II

Blood samples were drawn in glass vials containing EDTA and o-phenanthroline. Plasma ANG II was determined by a slight modification of the method described by Kappelgaard et al. [17]. Radioimmunoassay was performed after plasma extraction by means of Sep-Pak cartridges using methanol-water. Separation was performed on Sephadex G-25 fine micro columns. Recovery was $80 \pm 3\%$. The coefficients of variations were 12% (interassay) and 8% (intra-assay) (in 15 double determinations). The lowest detectable level was 2.0 pM.

Renal secretion of ANG II

Renal secretion rate of ANG II was calculated as follows:

$$RSR_{ANG} = (C_V - C_A) \times RPF$$

where C_A and C_V are arterial and venous concentrations of ANG II respectively, and RPF is the renal plasma flow.

Lithium clearance

With the assumption that the lithium ion is absorbed solely in the proximal tubules in approximately the same proportions as sodium and water, the lithium clearance (C_{Li}) calculated as the ratio between the urinary excretion rate of lithium and the interpolated mean plasma concentration is thought to give an estimate of the delivery of fluid from the proximal tubules into the thin segment of Henle [29]. Using GFR, C_{Li}, renal plasma clearance of sodium (C_{Na}), urine flow rate (U_V), and plasma concentration of sodium (P_{Na}), we performed the following calculations to obtain estimates of segmental renal handling of sodium and water.

Clearance of sodium and lithium were calculated as:

$$C_{Na/Li} = U_V \times U_{Na/Li}/P_{Na/Li}$$

where $U_{Na/Li}$ is the concentration of sodium or lithium in urine and $P_{Na/Li}$ the interpolated mean concentration of sodium or lithium in plasma.

Filtered load of sodium (FL_{Na}), proximal absolute reabsorption of sodium (PAR_{Na}), proximal absolute reabsorption of water (PAR_{H2O}), proximal fractional reabsorption of sodium and water ($PFR_{Na/H2O}$), proximal output of sodium (PO_{Na}), distal absolute reabsorption of sodium (DAR_{Na}), distal absolute reabsorption of water (DAR_{H2O}), distal fractional reabsorption of sodium (DFR_{Na}), distal fractional reabsorption of water (DFR_{H2O}) was calculated as previously described [14].

Sodium urine and plasma samples were measured in a continuous flow system (ABL 300 Radiometer, Copenhagen). Plasma and urinary concentrations of lithium were measured by atomic absorption (Perkin Elmer atomic absorption spectrophotometer 290B) [1].

Statistics

Statistical analyses were performed on the hemodynamic and renal function data. To ascertain whether any changes that occurred with time and within one kidney were significant, a One-Way Analysis of Variance (ANOVA) was performed. If there was a significant difference, multiple comparisons vs. a control group (Tukey's or Bonferroni's method) were performed to see at what time points the changes were significant.

A two-way analysis of variance (ANOVA) was performed to test a difference between two series or groups of observations. A *t*-test was performed to determine whether mean values at selected time points differed between two groups. $P < 0.05$ was considered significant. The data are presented as means $\pm$ SEM.

Results

Renal blood flow

Figure 1 depicts changes in RBF. Verapamil treatment was associated with a marked RBF decrease in both the ipsilateral and the contralateral kidney. After onset of obstruction, ipsilaterally, RBF decreased an average of 34% (from 182.6 ± 20.5 ml/min to 120.6 ± 12.2 ml/min, $P < 0.001$). In the contralateral kidney, RBF decreased by 29% (from 187.8 ± 15.4 ml/min to 133.1 ± 14.5 ml/min, $P < 0.001$). In the control group, ipsilateral RBF decreased consistently by 27% (from 194.6 ± 13.1 ml/min to 140.6 ± 15.2 ml/min, $P < 0.001$), whereas contralateral RBF did not show any changes. Changes were gradual and could not be ascribed to a specific size of the pelvic pressure.

Comparison of RBF changes between verapamil- and non-treated pigs showed that verapamil treatment was associated with a more pronounced RBF reduction, which differed significantly from controls in both the ipsilateral (end point values: 120.6 ± 12.2 and 140.6 ± 15.2 ml/min, $P < 0.05$) and in the contralateral non-obstructed kidney (end point values: 133.1 ± 14.5 and 202.1 ± 17.2 ml/min, $P < 0.05$).

Glomerular filtration rate

In the verapamil-treated pigs ipsilateral GFR decreased an average of 70% (from 29.0 ± 2.6 to 8.5 ± 0.9 ml/min, $P < 0.001$) after onset of obstruction (Fig. 2a). Reductions in GFR were not apparent until ureteral pressure

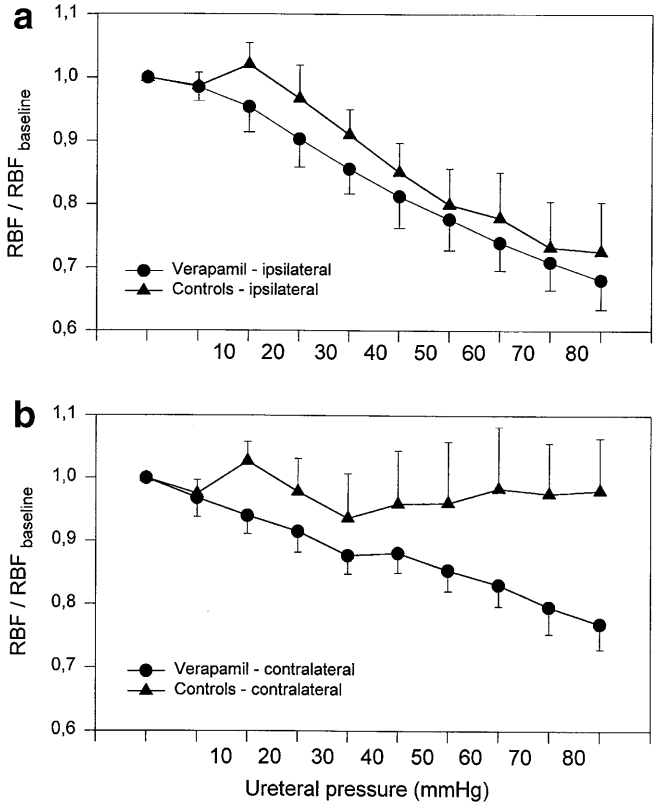

Fig. 1a, b Changes in renal blood flow (RBF) are shown as changes in fraction of baseline ($RBF/RBF_{baseline}$) in ipsilateral obstructed kidneys (**a**) and contralateral non-obstructed kidneys (**b**). In the ipsilateral kidneys, RBF declined consistently in both the verapamil-treated ($P < 0.001$) and control pigs ($P < 0.001$) in response to gradated pressure increase in the ureter. In the contralateral kidney RBF declined significantly in response to verapamil treatment ($P < 0.001$). In the controls, contralateral RBF did not change. Values are mean ± SEM

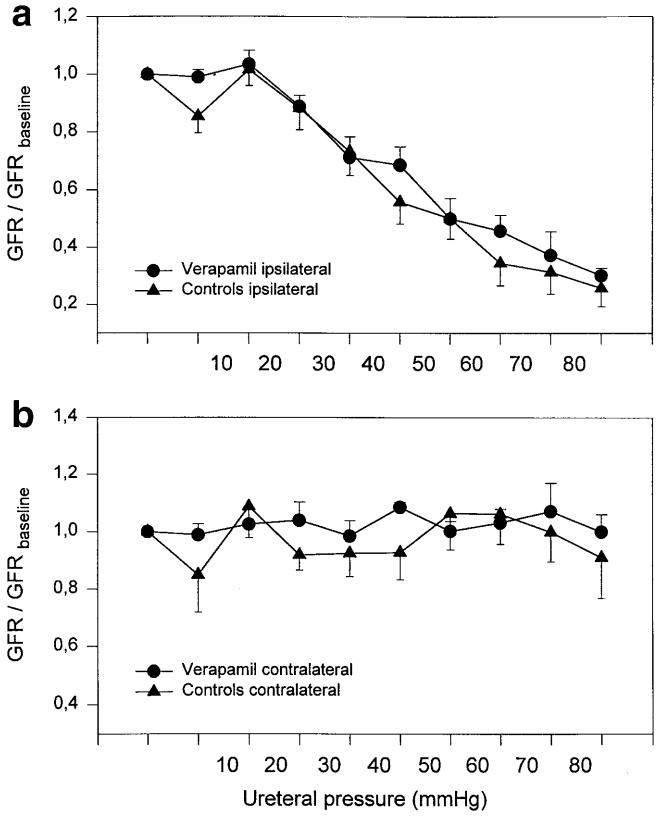

Fig. 2a, b Glomerular filtration rate (GFR) changes are shown as changes in fraction of baseline ($GFR/GFR_{baseline}$) in ipsilateral obstructed kidneys (**a**) and contralateral non-obstructed kidneys in response to increasing ureteral pressure (**b**). Ipsilateral GFR declined consistently in both the verapamil-treated ($P < 0.001$) and control pigs ($P < 0.001$). In the contralateral kidneys, there were no changes in either group. Values are mean ± SEM

(UP) exceeded 20 mmHg corresponding to 60 min after onset of pelvic-pressure increase. Thus, reductions in GFR could not be ascribed to a specific threshold level of pelvic pressure, as there were large variations in the results between individual animals. In the contralateral kidney, GFR did not change during experiments. In the control group, GFR changes paralleled those observed in the verapamil group: In the non-treated ipsilateral kidney, GFR decreased by 73% (from 29.2 ± 3.1 to 7.6 ± 2.1 ml/min, $P < 0.001$), and in the contralateral kidney, there were no changes in GFR. There was no significant difference between the ipsilateral decrease in the verapamil group and the control group.

Renal vascular resistance

In the verapamil-treated pigs, ipsilateral RVR increased by 19% from 0.585 ± 0.076 mmHg/min per milliliter at baseline to 0.726 ± 0.081 mmHg/min per milliliter ($P < 0.05$) (Fig. 3). In the contralateral kidney, RVR did not show any changes. In the control group, ipsilateral RVR increased by 34% (from 0.560 ± 0.056 to 0.854 ± 0.091 mmHg/min per milliliter, $P < 0.001$), whereas RVR did not change in the contralateral kidney (from 0.559 ± 0.068 to 0.576 ± 0.048 mmHg/min per milliliter at maximum UP. The increase in ipsilateral RVR was significantly reduced in the verapamil-treated pigs (0.726 ± 0.081 and 0.854 ± 0.091 mmHg/min per milliliter, $P < 0.05$).

Mean arterial pressure and heart rate

Mean arterial pressure (MAP) was constant during the baseline period in both groups (Fig. 4). After verapamil administration, MAP decreased markedly immediately from 105.1 ± 3.9 to 96.9 ± 2.0. Subsequently, MAP decreased steadily to 87.8 ± 4.1 mmHg at end of experiments ($P < 0.05$). In the control pigs, MAP was unchanged from 106.4 ± 3.9 at the start to 109.6 ± 3.3 mmHg at the end of the experiment (NS).

Heart rate (HR) changed non-significantly from 80.8 ± 4.3 at baseline to 90.0 ± 4.7 at UP of 20 mmHg and to 91.6 ± 8.4 beats/min at end of the experiments in the verapamil group. In the control group, HR increased non-significantly from 77.9 ± 6.9 to 88.5 ± 5.4 beats/min.

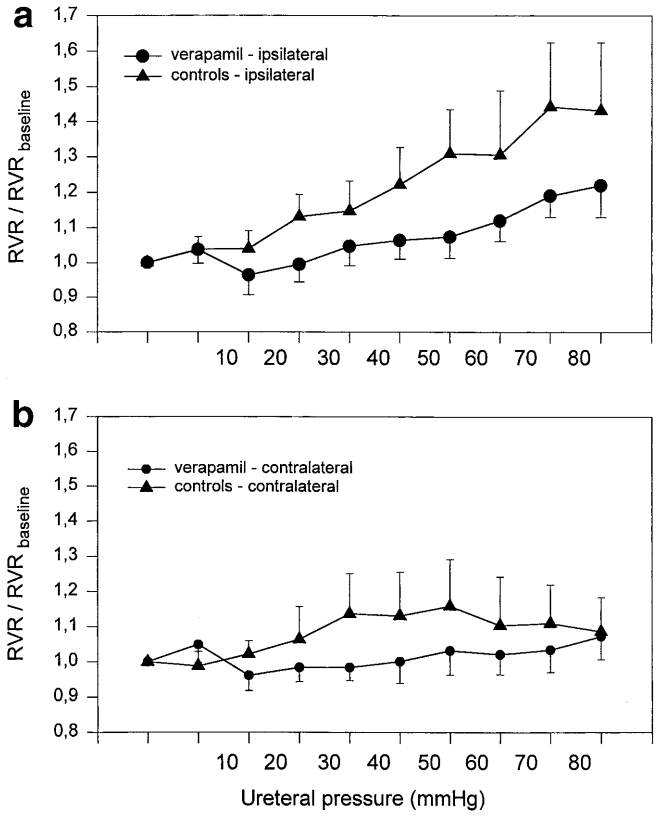

Fig. 3a, b Changes in renal vascular resistance (RVR) are shown as changes in fraction of baseline ($RVR/RVR_{baseline}$) in ipsilateral obstructed kidneys (**a**) and contralateral non-obstructed kidneys (**b**). In the ipsilateral kidney, RVR increased consistently in the verapamil-treated pigs ($P<0.05$) but was significantly ($P<0.05$) attenuated compared to the increase in the control pigs ($P<0.001$) in response to gradated pressure increase in the ureter. In the contralateral kidneys, there were no changes in either group. Values are mean ± SEM

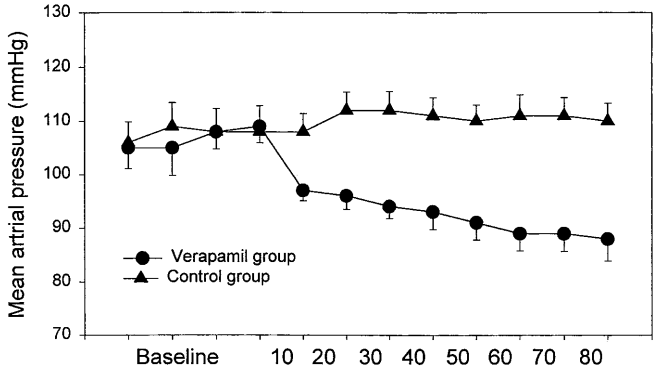

Fig. 4 Mean arterial pressure (MAP) was constant during the baseline period in both groups. After verapamil administration, MAP decreased markedly ($P<0.05$). In the control pigs, MAP was unchanged (NS)

Angiotensin II

Plasma ANGII levels increased significantly from all three sample sites in the verapamil group (Fig. 5) The most dramatic increase was from the ipsilateral renal

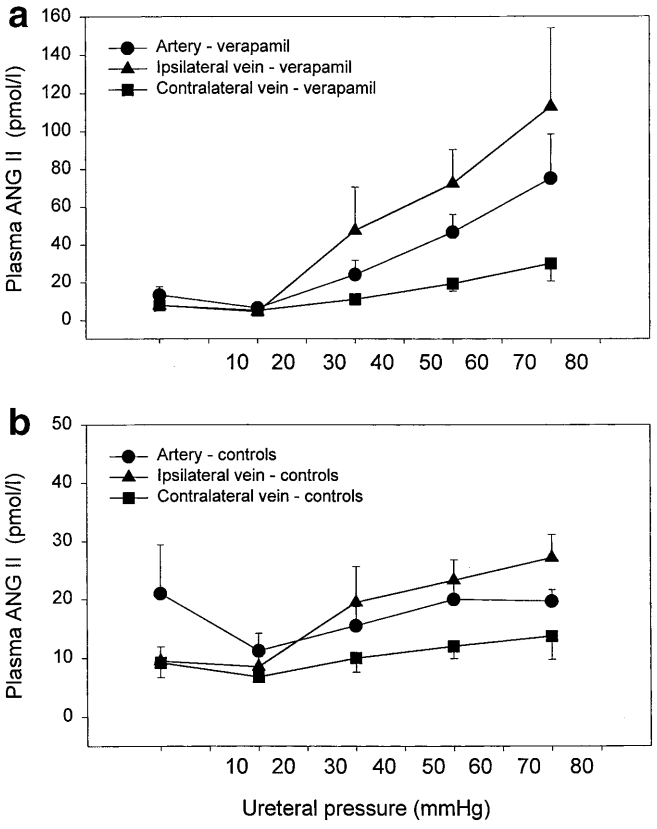

Fig. 5a, b Plasma immunoreactive angiotensin II (ANG II) levels were measured in arterial blood and both renal veins at baseline and at increased ureteral pressure levels of 20, 40, 60 and 80 mmHg. **a** Plasma ANG II levels increased persistently in the verapamil-treated pigs from all three sampling sites ($P<0.05$). **b** In control pigs, plasma ANG II levels increased moderately in the ipsilateral vein ($P<0.05$) whereas in the artery and contralateral vein, levels did not increase. Values are in mean ± SEM

vein (from 7.8 ± 2.3 to 112.9 ± 41.0 pmol/l). In the renal artery, plasma-ANGII increased from13.4 ± 4.3 to 75 ± 23.4 pmol/l, $P<0.01$ and in the contralateral renal vein from 7.8 ± 2.3 to 29.9 ± 9.5 pmol/l, $P<0.01$. In the control group, samples from the ipsilateral vein increased significantly (from 9.5 ± 2.4 to 27.2 ± 1.9, $P<0.05$), whereas both arterial and venous plasma-ANGII levels in the contralateral kidney did not change significantly.

Renal handling of ANGII

The renal secretion rate of ANGII (RSR_{ANG}) increased significantly from the ipsilateral kidney in the verapamil group (from –0.6 ± 0.2 to 3.2 ± 1.5 pmol/min, $P<0.05$) (Fig. 6). The RSR_{ANG} in the ipsilateral kidney in the controls did not change significantly during UUO (–1.3 ± 0.7 pmol/min at onset of obstruction, and 0.6 ± 0.4 pmol/min at the end of the experiment). In the contralateral kidney in the verapamil-treated pigs, RSR_{ANG} decreased persistently (from –0.6 ± 0.3 to –3.3 ± 1.1 pmol/min, $P<0.05$), whereas in the contra-

lateral kidneys of the controls, there was no change in RSR_{ANG}.

Tubular sodium handling

FL_{Na}, PO_{Na}, PAR_{Na}, DAR_{Na}, and DFR_{Na}, were estimated (Table 1). Tubular sodium handling in the contralateral kidney did not change significantly with time either before or after increasing renal pelvic pressure. PAR_{Na} tended to decrease in the verapamil group and DFR_{Na} tended to decrease in the controls, but they did not reach statistical significance.

Tubular water handling

The relative changes in GFR (Fig. 2) were estimated as well as $PFR_{Na/H2O}$, PAR_{H2O}, DAR_{H2O}, DFR_{H2O} and urine volume were estimated (Table 2). No significant changes were detected in tubular water and sodium handling either in verapamil-treated pigs or in the controls. PAR_{H2O} in the verapamil group was slightly reduced but did not reach statistical significance ($P = 0.07$ by ANOVA).

Filtration fraction

During the baseline period, FF did not change. Following onset of obstruction ipsilateral, FF increased slightly from 0.222 ± 0.014 to 0.234 ± 0.014 at 20 mmHg in the verapamil group. Then FF decreased by 61.9% to 0.089 ± 0.008, $P < 0.001$ (Table 3). The contralateral FF in the verapamil-treated pigs increased significantly by 20% from 0.195 ± 0.011 to 0.237 ± 0.016, $P < 0.05$. In the control group, the ipsilateral FF decreased significantly by 62.3% (from 0.218 ± 0.017 to 0.082 ± 0.021, $P < 0.001$). Contralateral FF in the control group did not change during the experiment. FF in the ipsilateral kidney did not differ between verapamil-treated pigs and control.

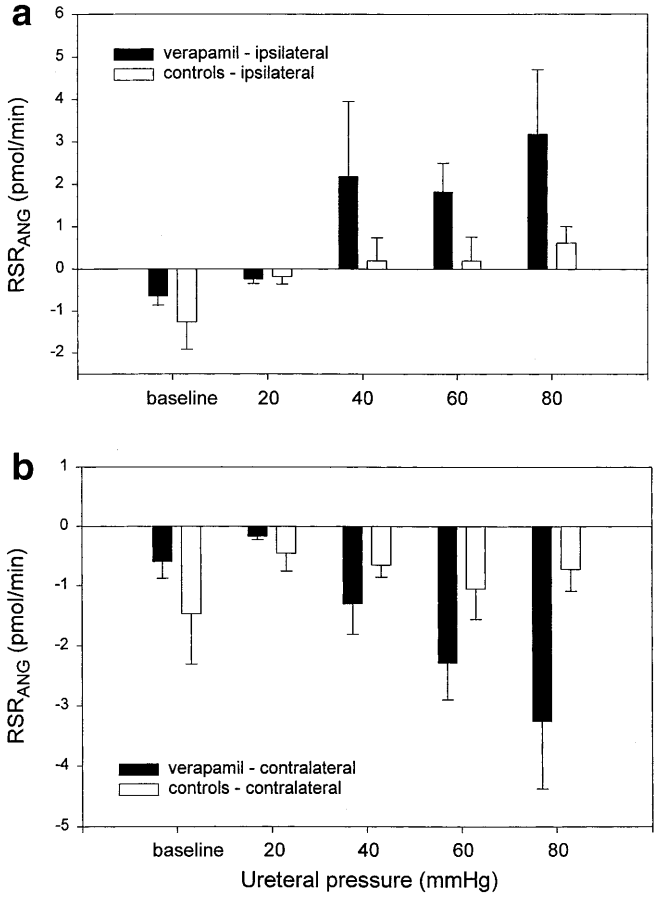

Fig. 6a, b The figure shows the renal secretion rate of angiotensin II (RSR_{ANG}) in absolute numbers in response to increasing ureteral pressure in ipsilateral kidneys from verapamil-treated and control pigs (**a**) and contralateral non-obstructed kidney (**b**). In the verapamil-treated pigs, RSR_{ANG} increased ($P < 0.05$) and contralateral RSR_{ANG} decreased ($P < 0.05$). In the control pigs, no changes were observed. Values are mean ± SEM

Discussion

The main finding of this study was that verapamil treatment in pigs with gradated UUO partially prevented the anticipated RVR increase in the obstructed kidney. Contralateral RBF decreased in the verapamil-treated pigs compared with the control group in which contralateral RBF was stable throughout the experiment. Ipsilateral GFR decreased significantly during obstruction, but GFR did not differ between the two groups. There was a significant decrease in MAP in the verapamil group. These results were associated with increased levels of plasma ANG II as well as an increased secretion rate of ANG II from the ipsilateral kidney in the verapamil-treated pigs. Thus, the present results suggest that an intact calcium channel may be important for the increase in RVR during UUO.

Verapamil reduces renal vascular resistance

Calcium-channel blockers (CCB) are important systemic and renal vasodilators, and it has been shown that CCB maintain or increase RBF [6, 8, 21]. This seems to be a unique effect of these agents, even in conditions where blood pressure is reduced [11, 26]. Furthermore, CCB have been shown specifically to dilate the afferent vessels in the kidney [11] and abolish the vasoconstriction induced by ANGII or endothelin at this site [6, 21, 25]. A recent study by Kahn et al. supports the view that verapamil antagonizes the renal vasoconstrictor effects of ANGII (and possibly also of endothelin) on both the preglomerular and postglomerular arterioles but with a more pronounced effect on the preglomerular vessels in response to acute UUO of the dog kidney [16].

Table 1 Tubular handling of sodium during unilateral ureteral obstruction in the contralateral kidney based on urine sampling in the verapamil-treated pigs and in controls. Data are means ± SEM. *UP* ureteral pressure, FL_{Na} filtered load of sodium, PAR_{Na} proximal absolute reabsorption of sodium, PO_{Na} proximal output of sodium, DAR_{Na} distal absolute reabsorption of sodium, DFR_{Na} distal fractional reabsorption of sodium

UP (mmHg)	FL_{Na}		PO_{Na}		PAR_{Na}		DAR_{Na}		DFR_{Na}	
	Verapamil	Controls	Verapamil	Controls	Verapamil	Controls	Verapamil	Controls	Verapamil	Controls
Baseline	3.79 ± 0.40	3.59 ± 0.35	1.46 ± 0.16	1.45 ± 0.15	2.68 ± 0.30	2.33 ± 0.31	1.35 ± 0.16	1.33 ± 0.13	91.79 ± 1.71	92.82 ± 1.33
Baseline	3.33 ± 0.32	3.85 ± 0.29	1.58 ± 0.14	1.41 ± 0.24	2.02 ± 0.24	2.43 ± 0.42	1.44 ± 0.12	1.27 ± 0.22	91.68 ± 1.50	88.50 ± 3.54
10	3.31 ± 0.35	3.43 ± 0.28	1.45 ± 0.12	1.44 ± 0.35	1.86 ± 0.33	1.86 ± 0.56	1.34 ± 0.10	1.28 ± 0.33	92.76 ± 1.18	82.25 ± 9.25
20	3.41 ± 0.35	3.99 ± 0.29	1.84 ± 0.28	1.45 ± 0.23	1.57 ± 0.30	2.54 ± 0.35	1.68 ± 0.28	1.30 ± 0.25	90.26 ± 2.26	83.31 ± 6.98
30	3.34 ± 0.21	3.48 ± 0.23	1.87 ± 0.32	1.52 ± 0.26	1.48 ± 0.23	1.96 ± 0.32	1.66 ± 0.31	1.33 ± 0.25	88.95 ± 1.93	82.12 ± 6.38
40	3.19 ± 0.23	3.51 ± 0.33	1.58 ± 0.18	1.78 ± 0.28	1.61 ± 0.18	1.73 ± 0.35	1.39 ± 0.17	1.57 ± 0.26	87.45 ± 2.65	81.43 ± 8.82
50	3.62 ± 0.35	3.52 ± 0.38	1.69 ± 0.21	1.70 ± 0.29	1.93 ± 0.28	1.82 ± 0.37	1.51 ± 0.18	1.48 ± 0.27	89.68 ± 1.66	77.90 ± 9.85
60	3.30 ± 0.29	4.05 ± 0.52	1.81 ± 0.21	1.83 ± 0.28	1.49 ± 0.17	2.22 ± 0.53	1.61 ± 0.18	1.59 ± 0.26	89.73 ± 1.62	79.25 ± 9.05
70	3.37 ± 0.27	4.01 ± 0.39	1.74 ± 0.23	1.87 ± 0.27	1.63 ± 0.15	2.14 ± 0.32	1.54 ± 0.19	1.62 ± 0.26	89.55 ± 1.58	79.73 ± 8.14
80	3.39 ± 0.28	3.79 ± 0.42	1.66 ± 0.18	1.75 ± 0.22	1.73 ± 0.14	2.35 ± 0.38	1.45 ± 0.15	1.46 ± 0.21	82.62 ± 1.69	78.37 ± 7.41

Table 2 Urine volume (V_{URINE}) and tubular handling of water in the contralateral kidney during unilateral ureteral obstruction (UUO) in the verapamil-treated pigs and in controls. Data are means ± SEM. *UP* ureteral pressure, PAR_{H20} proximal absolute reabsorption of water, $PFR_{H2O/Na}$ proximal fractional reabsorption of water and water, DAR_{H20} distal absolute reabsorption of water, DFR_{H20} distal fractional reabsorption of water

UP (mmHg)	V_{URINE}		PAR_{H2O}		$PFR_{H2O/Na}$		DAR_{H2O}		DFR_{H2O}	
	Verapamil	Controls	Verapamil	Controls	Verapamil	Controls	Verapamil	Controls	Verapamil	Controls
Baseline	3.79 ± 0.40	0.82 ± 0.18	19.15 ± 2.14	16.49 ± 2.18	64.22 ± 4.36	60.44 ± 6.02	9.32 ± 0.99	9.36 ± 0.96	89.41 ± 2.01	91.37 ± 1.30
Baseline	3.33 ± 0.32	1.23 ± 0.32	14.39 ± 1.70	17.24 ± 3.00	56.13 ± 3.74	60.10 ± 8.71	9.99 ± 0.86	8.76 ± 1.53	89.39 ± 2.25	86.30 ± 3.12
10	3.31 ± 0.35	1.55 ± 0.43	13.20 ± 2.32	13.17 ± 4.05	52.09 ± 6.23	51.64 ± 12.19	9.38 ± 0.70	8.59 ± 2.19	91.26 ± 1.71	79.39 ± 7.52
20	3.41 ± 0.35	1.22 ± 0.16	11.11 ± 2.12	17.96 ± 2.47	44.98 ± 5.93	62.25 ± 7.71	11.88 ± 2.00	9.01 ± 1.72	89.91 ± 2.29	82.26 ± 5.80
30	3.34 ± 0.21	1.62 ± 0.43	10.48 ± 1.61	13.85 ± 2.24	45.89 ± 6.94	55.92 ± 7.23	11.91 ± 2.22	9.11 ± 1.63	89.44 ± 1.68	80.47 ± 5.78
40	3.19 ± 0.23	1.88 ± 0.57	11.39 ± 1.24	12.24 ± 2.49	50.37 ± 4.24	47.63 ± 7.47	9.99 ± 1.26	10.72 ± 1.69	88.05 ± 2.47	80.50 ± 7.17
50	3.62 ± 0.35	1.85 ± 0.54	13.68 ± 2.00	12.84 ± 2.65	52.53 ± 4.20	50.27 ± 8.40	10.79 ± 1.32	10.18 ± 1.85	90.08 ± 1.41	76.90 ± 8.60
60	3.30 ± 0.29	2.02 ± 0.53	10.60 ± 1.20	15.69 ± 3.75	45.66 ± 3.35	50.83 ± 8.34	11.56 ± 1.32	10.88 ± 1.71	90.45 ± 1.22	78.22 ± 7.68
70	3.37 ± 0.27	2.13 ± 0.53	11.54 ± 1.08	15.11 ± 2.26	49.34 ± 3.86	53.41 ± 6.14	11.08 ± 1.42	11.04 ± 1.71	90.13 ± 1.19	78.32 ± 7.15
80	3.39 ± 0.28	2.41 ± 0.63	12.30 ± 1.00	16.61 ± 2.66	51.58 ± 2.51	56.60 ± 6.97	10.45 ± 1.10	9.93 ± 1.44	89.30 ± 1.25	76.33 ± 6.91

Table 3 The table shows values for filtration fraction calculated as the renal extractions of ^{51}Cr-EDTA, for pigs treated with verapamil ($n=8$) and the control group ($n=8$) during baseline and gradated increase in ureteral pressure. Values are means ± SEM

UP	Verapamil-treated group		Control group	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral
Baseline 1	0.196 ± 0.031	0.210 ± 0.011	0.211 ± 0.028	0.198 ± 0.023
Baseline 2	0.221 ± 0.011	0.216 ± 0.015	0.218 ± 0.017	0.190 ± 0.021
Baseline 3	0.222 ± 0.014	0.195 ± 0.011	0.219 ± 0.015	0.204 ± 0.016
10	0.223 ± 0.015	0.200 ± 0.014	0.192 ± 0.020	0.195 ± 0.022
20	0.234 ± 0.014	0.213 ± 0.016	0.213 ± 0.018	0.205 ± 0.014
30	0.211 ± 0.013	0.222 ± 0.015	0.202 ± 0.018	0.183 ± 0.014
40	0.180 ± 0.017	0.213 ± 0.012	0.175 ± 0.021	0.190 ± 0.012
50	0.178 ± 0.011	0.242 ± 0.014	0.147 ± 0.023	0.187 ± 0.013
60	0.137 ± 0.021	0.234 ± 0.015	0.142 ± 0.023	0.216 ± 0.020
70	0.130 ± 0.014	0.243 ± 0.015	0.091 ± 0.025	0.207 ± 0.009
80	0.089 ± 0.008	0.237 ± 0.016	0.082 ± 0.021	0.179 ± 0.024

Similar to previous UUO studies [14, 21, 23], the present study showed that RVR increased in the ipsilateral obstructed kidney compared with the contralateral side. Verapamil treatment significantly attenuated the increase in ipsilateral RVR. Thus, the anticipated increase in RVR in response to UUO was modulated by verapamil. UUO is associated with preglomerular vasoconstriction resulting in increased RVR, which is partially ANGII-dependent [14]. Verapamil is thought to modulate the ANGII mediated vasoconstriction by blocking the voltage-gated calcium channels, which in turn may result in vasodilation [6, 27]. The reduction in MAP, as we observed in this study during verapamil treatment, may be explained by a general vasodilation with a concomitant increase in plasma ANGII levels. Despite the increased ANG II levels in the renal venous effluent or plasma and the increased renal secretion rate of ANG II, verapamil treatment partially antagonized the increase in ipsilateral RVR consistent with previous findings [6]. Furthermore, the results support the view that the vasodilation in response to CCB treatment is more pronounced in conditions associated with ANG II-mediated vasoconstriction [6, 4, 13, 22]. Verapamil treatment was not associated with significant changes in RVR in the contralateral kidney.

Verapamil reduces renal blood flow in non-obstructed kidneys

In pigs treated with verapamil, ipsilateral and contralateral RBF decreased persistently as ureteral pressure increased. In control animals, ipsilateral RBF decreased as anticipated during UUO, whereas contralateral RBF was unchanged [14]. Thus, the reduction in RBF could not be determined by a certain increase in pelvic pressure. The decrease in contralateral RBF during verapamil treatment is most likely caused by a general reduction in vascular resistance, which was associated with a reduction in systemic MAP consistent with results from previous studies [8, 10]. It is unlikely that the reduction in MAP accounts for the reductions in RBF in the verapamil group, since MAP was not reduced below the level for renal blood flow autoregulation [2, 3].

GFR is reduced in response to obstruction

In both the verapamil-treated group and the control group, ipsilateral GFR was progressively reduced in response to gradated obstruction of the ureter, and the reduction did not differ between the two groups. As discussed above, this finding may indicate that verapamil administration is associated with a reduction of primarily the preglomerular arterioles in the obstructed kidney. The observed reduction in contralateral RBF in response to verapamil could be associated with a concomitant GFR reduction. However, due to the increased FF in the contralateral non-obstructed kidney GFR was maintained. This finding may be explained by a relative increase in postglomerular resistance due to an increased local generation of ANGII. Consistent with previous observations, our study suggests that verapamil abolishes the preglomerular vasoconstrictor effects of ANGII, whereas the postglomerular response to ANGII is relatively unaffected [6, 16].

Renal ANGII secretion is increased from the obstructed kidney

Net ANGII secretion, as well as total plasma-ANGII levels from the ipsilateral kidney, increased more pronouncedly in the verapamil-treated pigs compared with controls. The explanation for this is most likely related to a compensatory ANGII increase. In a previous study from our group, it was shown that complete ureteral obstruction was associated with a net secretion of ANGII from the obstructed kidney [12]. In the present study with gradated obstruction, renal secretion rate of ANGII did not increase significantly in controls, but there was a significant increase in plasma ANG II from the ipsilateral renal vein indicating elevation of ANGII levels. Thus, there is a marked difference in the renal handling of ANGII in the presented model with gradated UUO compared with complete UUO.

This study does not allow specific documentation on the ANGII effects within the kidney vasculature during verapamil treatment. Reduced MAP may lead to

increased levels of ANGII in the ipsilateral kidneys. However, the changes in MAP may also influence the level of other vasoactive substances, such as endothelin, nitric oxide and prostacyclin. At least in part, ANGII-mediated effects seem to be blocked by verapamil at the site of preglomerular arterioles, whereas postglomerular vasoconstriction is maintained by increasing FF in the face of a reduced MAP. This suggests that verapamil reduces preglomerular vasoconstriction and RVR in the obstructed kidney.

Renal salt and water handling

Using the lithium clearance technique, renal proximal and distal tubular salt and water handling was estimated. Verapamil did not significantly alter the reabsorption or excretion of salt or water in the contralateral kidney, which may have been anticipated. Kopp et al. showed in a rat study that acute UUO increased contralateral urine output and urinary sodium excretion [19]. This was explained by activation of renal pelvic mechanoreceptors with the net result being an inhibitory renorenal reflex response. We were not able to reproduce this response in our pig model [14].

Conclusion

The present study shows that verapamil administration to pigs with increased ureteral pressure is associated with a reduction in the increase of renal vascular resistance of the obstructed kidney. The changes are most likely explained by a calcium channel blocker-mediated reduction in the ANGII-dependent increase in preglomerular vasoconstriction in response to ureteral obstruction. Our model does not allow us to conclude that changes in renal functions are directly dependent on specific pelvic pressure levels.

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