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Losartan attenuates renal vasoconstriction in response to acute unilateral ureteral occlusion in pigs

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Abstract Unilateral ureteral obstruction in pigs is associated with an enhanced, de novo generation of angiotensin II from the ipsilateral kidney. In order to further investigate the role of this system during unilateral ureter obstruction, the renal hemodynamic response to the non-peptide angiotensin II antagonist losartan was investigated. Danish land race pigs were operated on under general anesthesia. Catheters were placed in both renal veins by x-ray and ultrasonic flow probes were mounted on the renal arteries. Losartan (2 mg/kg/h) was administered intravenously to an experimental group ($n=9$) continuously over 8 h of unilateral ureteral occlusion. This group was then compared to a matched control group which received only saline ($n=6$). Ipsilateral pelvic pressure, renal blood flow using ultrasound transit time, glomerular filtration rate, mean arterial pressure and heart rate were measured. Renal handling of angiotensin II was examined by determining the renal extraction and secretion rates of immunoreactive angiotensin II. The anticipated reduction in ipsilateral renal blood flow after the onset of obstruction was attenuated in the losartan treated pigs, but the ipsilateral glomerular filtration rate was unaffected as compared with the controls. In the losartan group, the increase in renal vascular resistance was significantly reduced compared with un-treated controls ($141 \pm 25\%$ vs $217 \pm 24\%$, $P<0.05$). Plasma immunoreactive angiotensin II increased significantly from all three sample locations in both groups after the onset of obstruction, being more pronounced in the losartan treated group in which immunoreactive angiotensin II from the ipsilateral renal vein increased from 5.1 ± 0.5 pmol/l to 41.6 ± 19.6 pmol/l, $P=0.027$. In the controls immunoreactive angiotensin II increased from 2.7 ± 0.3 pmol/l to 24.8 ± 10.2 pmol/l. Furthermore, plasma aldosterone was significantly reduced after losartan administration (from 80.4 pmol/l to 36.0 pmol/l, $P=0.005$), indicating effective blockade of the angiotensin II type-1 receptor. The results from the present study suggest that continuous intravenous administration of losartan blocks the angiotensin II receptor mediated effects in the pig. Losartan is able to reduce ipsilateral vasoconstriction in the obstructed kidney during unilateral ureter obstruction supporting the view that angiotensin II is an important mediator of vasoconstriction during unilateral ureter obstruction in the pig model with acute unilateral occlusion of the ureter.

Keywords Losartan · Unilateral ureteral obstruction · Renal hemodynamics · Angiotensin II · Pigs

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Introduction

Characteristically, renal blood flow (RBF) and glomerular filtration rate (GFR) decrease in response to unilateral ureteral obstruction (UUO). Although a variety of factors have been implicated, the pathogenesis of the impaired renal function in urinary tract obstruction is incompletely understood. Kidney function in patients with persisting urinary tract obstruction is characterized by a progressive reduction in renal hemodynamics which is caused by an increased renal vascular resistance (RVR). The mechanism behind this response is believed to be an active preglomerular vasoconstriction [1, 15, 20] mediated by an enhanced renal expression of the renin-angiotensin system (RAS), and the prostaglandin-thromboxane systems. Other vasoactive agents such as endothelin, nitric oxide and platelet activating factor are also thought to be implicated in the renal hemodynamic changes during UUO [18]. In a previous study on pigs, we showed that the renin angiotensin system rapidly

becomes activated in response to obstruction. Unilateral obstruction was associated with an enhanced generation of angiotensin II (ANGII) which could be explained by a de novo production of intrarenal ANGII from the ipsilateral obstructed kidney [10], supporting the view from other studies that the renin-angiotensin system is turned on in response to obstruction [3, 8, 26]. However the functional significance of the renin angiotensin system has been questioned in experiments using both angiotensin II antagonists (saralasin) and angiotensin I converting enzyme inhibitors (ACEI). Prior studies reported that the administration of saralasin [21] or ACEI [30] did not affect the functional response to obstruction, whereas other studies showed that the administration of ACEI reduced the RVR of the obstructed kidney compared to the resistance in the intact kidney [3, 16, 34]. We previously demonstrated that captopril did not significantly change the RVR in the obstructed pig kidney, supporting the view that other vasoactive mediators also participate in the active vasoconstriction of the obstructed kidney [9].

Angiotensin II type 1 receptor (AT1-R) blockers are important tools for examining the direct pathophysiological importance of ANGII and AT1-R seems to be the major site for ANGII action in the renal vessels [28]. Losartan is a non-peptide AT1 antagonist which blocks most of the known physiological effects of ANGII [33], without having the agonistic effects seen using non-peptide antagonists. It does not alter the production of bradykinin and prostaglandins in contrast to angiotensin converting enzyme inhibitors [32]. Losartan may therefore be useful in further delineating the hemodynamic significance of ANGII in kidneys during acute UUO. Indeed, studies in which rats were pretreated with losartan showed that the drug markedly improved post-obstructive renal hemodynamics in response to the release of 24 h of obstruction [25, 26]. Furthermore, UUO was associated with a downregulation of AT1-R mRNA levels [25] suggesting that ANGII plays an important role for the hemodynamic regulation in ureteral obstruction as well as for the adaptational downregulation of AT1-R during conditions with high circulating levels of ANGII [25].

We examined the effects of intravenous losartan administration on the renal hemodynamic changes and on renal secretion of ANGII in response to acute obstruction of the pig kidney, which shows a high resemblance to the human kidney. The main results of our study were that losartan administration attenuates the reduction in ipsilateral RBF after the onset of ureteral occlusion, and reduces the anticipated increase in ipsilateral RVR, supporting the view that ANGII is important in mediating the reduction in the renal function in response to ureteral obstruction of the pig kidney.

Materials and methods

Preparation of animals

Fifteen immature pigs (90-days-old) of the Danish land race breed (Yorkshire/Lancaster) weighing from 31 to 34 kg were used. Before the study they were fed a standard pig diet which was withheld 12 h before the experimental procedures. Free access to water was allowed until anesthesia. The pigs were allocated to a losartan group ($n=9$) and a control group ($n=6$).

The experiments were carried out with the pigs under general anaesthesia. This was induced by the 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 (4:2). The tidal volume and rate were adjusted according to the analysis of arterial blood samples every hour, keeping the pH between 7.4 and 7.5 (ABL 300, Radiometer, Copenhagen). Anesthesia was maintained by the intravenous administration of midazolam (5 μg/min/kg), ketamine (0.35 mg/min/kg) and pancuronium bromide (Pavulon) (2.5 μg/min/kg) through the central venous catheter in the left jugular vein in isotonic saline, 3 ml/min throughout the experiment.

Through a cut-down 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 into 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, respectively. Finally, another catheter for the measurement of the central venous pressure was placed into 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) and connected to an amplifier and monitor (Medistim CardioMed CM-4008). Both ureters were isolated using a low flank muscle splitting retroperitoneal approach and cannulated with ureteral catheters (Ch 9). The ureters were ligated around the catheters at the ureterotomy. The catheter placed in the left ureter was connected to a pressure transducer (Statham Gould, no. 4523551) and the other catheter was left open for urine flow measurements and urine sampling.

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

Study design

Following surgery, the pigs were allowed an equilibration period of 2 h before occlusion of the left ureter. Urine was sampled from *both* ureters every 30 min during this period. Losartan was given intravenously as a bolus of 2 mg/kg followed by continuous infusion of 2 mg/kg/h 1 h before the occlusion of the left ureter. The *left ureter* was occluded by connecting the catheter to the pressure transducer. The pressure generated in the pelvis by the obstruction of the urinary output was then monitored continuously during the following 8 h. Urine was sampled from the *right ureter* and measured every 30 min throughout the experiment. Blood samples were taken from the aorta and both renal veins every 30 min. Hormone samples were taken at –1.5 h, –0.5 h, 0.5 h, 3 h and 8 h from all three sampling sites. The control group did not receive losartan, but was otherwise exposed to the same treatment as the losartan group.

Measurements

GFR was measured by a continuous infusion clearance technique using ^{51}Cr-EDTA [15]. Subsequent to the operating procedures, the pigs were given 1.1 MBq ^{51}Cr-EDTA (Behring, Marburg, Germany) intravenously as a bolus. This was 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 (Cobra, Packard, USA) to a statistical

accuracy of 1%. The recorded counts were corrected for radioactive decay during counting. GRF 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:

$$EF_R = \left(^{51}CR - EDT_{art} - {}^{51}CR - EDTA_{vein}\right)/{}^{51}Cr - EDTA_{art}$$

which equals filtration fraction (FF) because of the equation:

$$FF = GFR/RPF = EF_R$$

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 aortic blood pressure.

Hormone analysis

ANGII

Blood samples were drawn in glass vials containing EDTA and o-phenanthroline. Immunoreactive ANGII (iANGII) was determined by radioimmunoassay using a modification [24] of the method originally described by Kappelgaard et al. [17]. Radioimmunoassay was performed after plasma extraction by Sep-Pak C_{18} cartridges (Waters Associates, Milford, Mass.). The antibody was obtained from the Department of Clinical Physiology, Glostrup Hospital, Denmark. The minimum detectable level was 2 pmol/l. The coefficients of variation were 12% (interassay) and 8% (intraassay).

Aldosterone

Blood samples were drawn in EDTA-glass vials. Immunoreactive aldosterone was measured by a modification [24] of the method originally described [27]. With a rabbit-anti-aldosterone-antibody (Simoco, Denmark), a radioimmunoassay was performed after the extraction of plasma by Sep-Pak C_{18} cartridges (Waters Associates). The minimum detectable level was 42 pmol/l plasma. The coefficients of variation were 13% (interassay) and 9% (intraassay).

Renal handling of ANGII

Renal extraction ratio (RE) of iANGII was calculated as $RE_{ANG} = (C_A - C_V)/C_A \times 100$ where C_A and C_V are arterial and venous concentrations of ANGII, respectively. Renal secretion rate (RSR) of iANGII was calculated as follows $RSR_{ANG} = (C_V - C_A) \times RPF$ where RPF is renal plasma flow.

Statistical methods

To ascertain whether changes occurred with time and within one kidney, a one way analysis of variance was performed. If there was a significant difference, a Tukey's multiple comparison test was performed to determine which comparisons were significant. A two way analysis of variance was performed to determine any significant differences in the parameters between ipsilateral and contralateral kidneys. A probability of 0.05 or less was considered significant. The data are presented as means ± SEM.

Results

Pelvic pressure increases in response to UUO

After the induction of obstruction, there was an anticipated increase in renal pelvic pressure from 5.8 ± 1.1 mmHg to a maximum of 46.4 ± 3.4 mmHg in the losartan treated pigs and from 5.7 ± 0.6 mmHg to a maximum of 51.2 ± 4.2 mmHg in the controls (Fig. 1). Subsequently, pelvic pressure declined gradually to 30.0 ± 3.0 mmHg at the end of the experiment. The changes in pelvic pressure in the losartan treated pigs did not differ significantly from those in the control group.

Losartan attenuates the reduction in ipsilateral RBF in response to UUO

In the obstructed kidneys, RBF was significantly reduced during 8 h of obstruction in both losartan treated and control pigs, but renal blood flow reduction differed significantly between the losartan group and the control group ($P < 0.001$) (Fig. 2A). The relative RBF was consistently higher in the losartan group throughout the experiment. After the onset of obstruction, the reduction rate in RBF was more dramatic during the first 60–90 min in the untreated pigs compared with losartan treated pigs, suggesting an attenuation in the RVR in response to losartan. The baseline ipsilateral value of RBF in the losartan treated pigs was 203 ± 17 ml/min decreasing to 105 ± 19 ml/min after 8 h of obstruction. In the control group the initial ipsilateral flow was 225 ± 17 ml/min, decreasing to 109 ± 11 ml/min after 8 h of obstruction. In both groups there was a significant interaction between RBF and time ($P < 0.001$).

Contralateral RBF is unchanged in response to UUO

In the losartan treated pigs the baseline RBF of the contralateral non-obstructed kidneys was 176 ± 14 ml/

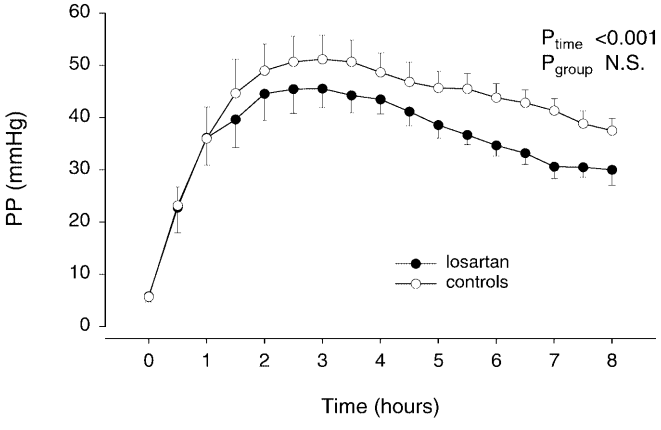

Fig. 1. Pelvic pressure is rapidly increased in response to UUO in both groups. Pelvic pressure increase rate and maximum pelvic pressure did not differ significantly between the two groups. Values are mean ± SEM

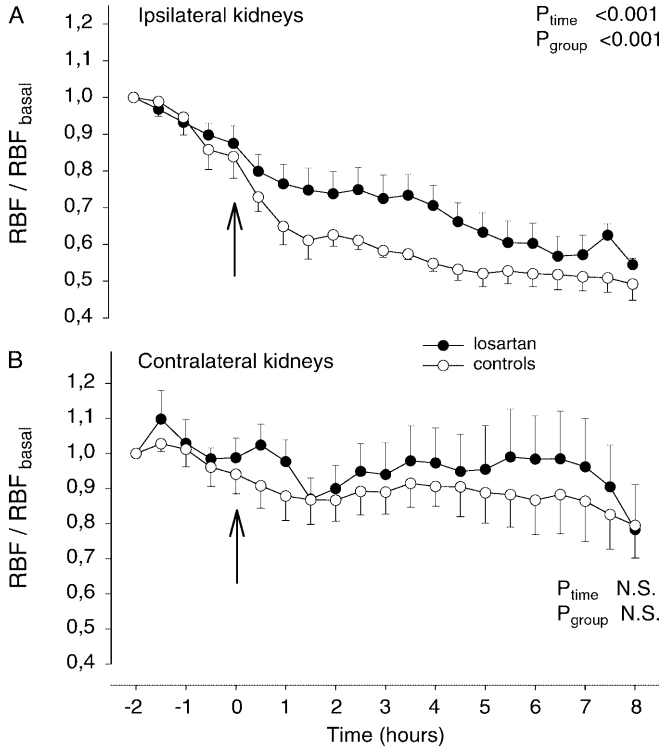

Fig. 2A, B. Renal blood flow (RBF) changes before and during the 8 h of UUO. **A** In the ipsilateral kidney RBF is reduced in both groups after onset of UUO. In the losartan treated pigs the reduction is attenuated compared with the reduction in the more rapid reduction in control pigs. **B** In contralateral, kidneys RBF did not differ between the two groups. ↑ indicates the onset of UUO. Values are mean ± SEM

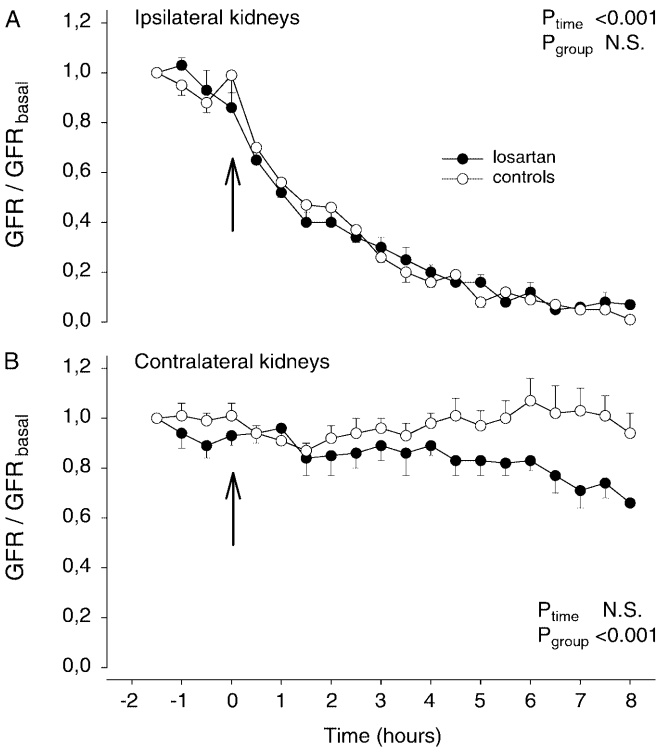

Fig. 3. Glomerular filtration rate (*GFR*) is rapidly reduced in the ipsilateral kidneys (**A**) and there was a difference between the two groups. **B** In the contralateral kidneys GFR remained unchanged in the non-treated pigs. Compared with this GFR was significantly reduced in the losartan treated pigs. ↑ indicates the onset of UUO. Values are mean ± SEM

min and remained unchanged until the end of experiments when RBF was 142 ± 3 ml/min ($P > 0.05$) (Fig. 2B). In the untreated pigs RBF was initially 186 ± 10 ml/min and at the end of the study it was 143 ± 11 ml/min ($P > 0.05$).

Glomerular filtration rate decreases in response to obstruction

After the onset of obstruction, GFR decreased consistently during the course of the study (Fig. 3A). In both losartan and untreated pigs, GFR decreased slightly during the first 2 h of the experiment before the onset of obstruction. GFR of the obstructed kidneys did not differ significantly between the pigs treated with losartan and the untreated controls (Fig. 3A). In the losartan treated pigs, GFR of the obstructed kidneys was initially 33.1 ± 4.6 ml/min, decreasing significantly to 2.3 ± 0.6 ml/min after 8 h of obstruction ($P < 0.001$). Almost identical GFR changes were seen in the obstructed kidneys in the control group (35.1 ± 2.6 ml/min initially and 1.7 ± 0.2 ml/min after 8 h; $P < 0.001$). The renal extraction ratio of ^{51}Cr-EDTA did not differ between the two groups (Table 1).

In the losartan treated pigs, GFR of the contralateral non-obstructed kidneys was 34.9 ± 3.8 ml/min under baseline conditions and 22.6 ± 4.5 ml/min after 8 h of obstruction ($P < 0.05$) (Fig. 3B). In the control group, contralateral GFR was 31.2 ± 1.6 ml/min under baseline conditions and 28.6 ± 2.3 ml/min after 8 h of obstruction ($P = 0.887$). GFR of the contralateral non-obstructed kidneys differed significantly between the two groups by the end of the experiment ($P < 0.05$). Likewise the renal extraction ratio of ^{51}Cr-EDTA differed significantly between the two groups (Table 2) equivalent to the difference in the FF of the non-obstructed kidney.

Table 1. Renal extraction of EDTA in both losartan treated and untreated control pigs

Hours	Obstructed kidney		Non-obstructed kidney	
	Losartan	Controls	Losartan	Controls
–2	0.233 ± 0.019	0.234 ± 0.010	0.235 ± 0.016	0.240 ± 0.010
–1	0.235 ± 0.021	0.235 ± 0.014	0.246 ± 0.013	0.251 ± 0.017
0	0.223 ± 0.021	0.257 ± 0.014	0.243 ± 0.018	0.263 ± 0.011
1	0.156 ± 0.016	0.210 ± 0.017	0.248 ± 0.018	0.256 ± 0.010
2	0.112 ± 0.017	0.164 ± 0.019	0.252 ± 0.019	0.254 ± 0.011
3	0.082 ± 0.014	0.119 ± 0.015	0.249 ± 0.016	0.261 ± 0.015
4	0.065 ± 0.009	0.069 ± 0.015	0.235 ± 0.016	0.255 ± 0.014
5	0.045 ± 0.009	0.059 ± 0.007	0.226 ± 0.015	0.270 ± 0.013
6	0.026 ± 0.009	0.052 ± 0.007	0.223 ± 0.022	0.290 ± 0.015
7	0.019 ± 0.001	0.032 ± 0.007	0.215 ± 0.046	0.288 ± 0.013
8	0.025 ± 0.006	0.023 ± 0.002	0.223 ± 0.035	0.285 ± 0.017

Losartan reduces renal vascular resistance in the ipsilateral kidney

Changes in mean arterial blood pressure, heart rate and urine volume from the contralateral kidney are shown in Table 2. RVB of the obstructed kidneys increased significantly in both groups throughout the experiment (Fig. 4A). RVR differed significantly between the losartan treated and the control group ($P < 0.001$). Baseline RVR of the obstructed kidney was 0.62 ± 0.07 mmHg×min/ml in the losartan group and 1.02 ± 0.26 mmHg×min/ml after 8 h of obstruction ($P < 0.05$). In the control group, the baseline RVR was 0.47 ± 0.05 mmHg×min/ml at the start of the experiment and 0.98 ± 0.09 mmHg×min/ml after 8 h of obstruction ($P < 0.001$).

The RVR of the contralateral non-obstructed kidneys in the losartan treated pigs decreased from 0.64 ± 0.06 mmHg×min/ml to 0.50 ± 0.06 mmHg×min/ml (NS) (Fig. 4B). In the nontreated controls contralateral RVR increased slightly from 0.53 ± 0.05 mmHg×min/ml to 0.74 ± 0.02 mmHg×min/ml (NS).

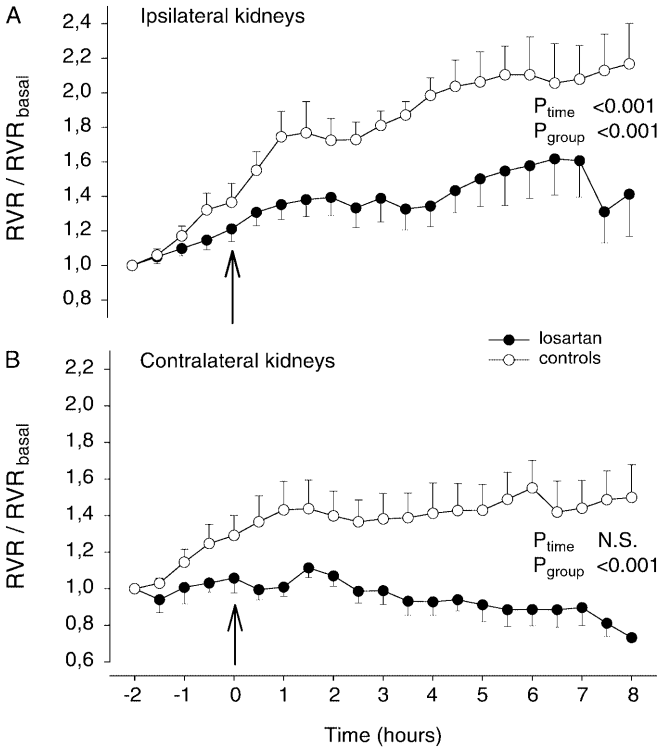

Fig. 4A, B. Renal vascular resistance (*RVR*) is shown as a ratio with the baseline RVR. **A** In the ipsilateral kidney losartan administration markedly prevented the increase in RVR compared with untreated control pigs, suggesting that ANGII may be important for the increase in RVR in response to UUO. **B** In the contralateral kidneys RVR/RVR_{basal} increased slightly in the untreated pigs whereas losartan administration was associated with a modest reduction during the course of the experiment. ↑ indicates the onset of UUO. Values are mean ± SEM

Ipsilateral renal secretion rate of iANGII increases in response to obstruction

Renal handling of angiotensin II was examined in detail by measuring the plasma levels of iANGII from all three sample locations simultaneously. From these data the renal extraction and the renal secretion rates of iANGII were calculated.

iANGII

The variation in plasma iANGII in the losartan treated and control pigs is shown in Fig. 5. There was a pronounced increase in plasma iANGII levels from all three sample locations in both the losartan group and the control group (Fig. 5A, B). But in the losartan group the net increase was markedly higher, confirming the blockade of the AT1 receptor (see below). In the losartan treated pigs, the increase was almost identical in the samples from the artery (5.1 ± 0.5–41.6 ± 19.6 pmol/l) and the ipsilateral renal vein (3.1 ± 0.4–39.4 ± 18.1 pmol/l), whereas the increase in iANGII levels was slightly lower in the contralateral renal vein (3.3 ± 0.7–31.6 ± 15.3 pmol/l) (Fig. 5A). The increase was significant from all sample sites ($P < 0.05$). Similarly, in the control group iANGII increased significantly in the

Table 2. Values for mean arterial pressure (MAP), heart rate (HR) and urine volume (U_{vol}) from the contralateral kidney are given for both losartan treated and untreated control pigs, for every hour throughout the study. There were no significant differences in the parameters between the two groups

Hours	MAP (mmHg)		HR (beats/min)		U_{vol} (ml/min)	
	Losartan	Controls	Losartan	Controls	Losartan	Controls
–2	112.0 ± 4.2	101.3 ± 5.3	77.6 ± 4.8	95.3 ± 4.8	–	–
–1	114.3 ± 5.7	110.3 ± 5.0	71.9 ± 3.4	80.7 ± 3.4	1.1 ± 0.3	0.8 ± 0.2
0	115.8 ± 5.7	111.7 ± 3.7	69.1 ± 3.4	80.3 ± 5.7	1.0 ± 0.2	0.8 ± 0.2
1	111.6 ± 4.9	109.5 ± 2.9	76.7 ± 3.5	82.5 ± 5.7	1.3 ± 0.2	1.0 ± 0.3
2	109.0 ± 4.6	106.5 ± 3.3	79.0 ± 4.7	83.3 ± 5.4	1.2 ± 0.2	1.3 ± 0.4
3	104.1 ± 4.3	105.8 ± 4.9	79.0 ± 3.7	84.3 ± 5.6	1.6 ± 0.3	1.4 ± 0.4
4	100.0 ± 4.1	109.2 ± 6.3	80.0 ± 5.7	83.3 ± 5.1	1.5 ± 0.3	1.3 ± 0.3
5	100.6 ± 5.5	104.8 ± 4.1	80.0 ± 4.9	84.2 ± 7.5	1.3 ± 0.3	1.6 ± 0.6
6	99.3 ± 6.3	103.0 ± 4.5	77.5 ± 4.9	86.0 ± 11.9	1.3 ± 0.4	1.7 ± 0.3
7	94.8 ± 7.4	102.7 ± 3.9	73.4 ± 3.0	90.0 ± 10.5	0.9 ± 0.2	2.2 ± 0.5
8	93.0 ± 14.0	101.7 ± 5.2	77.7 ± 5.1	83.2 ± 6.5	1.0 ± 0.4	1.5 ± 0.2

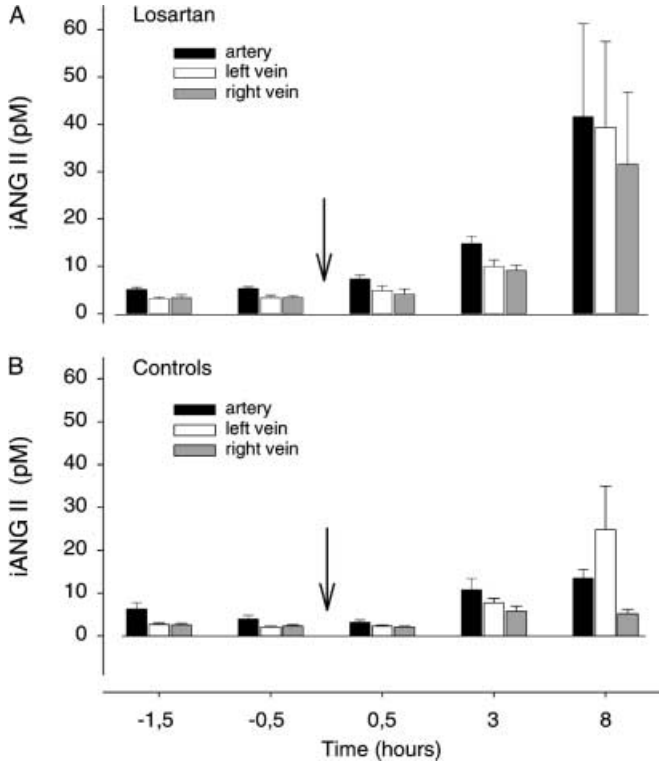

Fig. 5. Immunoreactive angiotensin II (iANGII) levels from the three sample locations are shown for losartan treated pigs (**A**) and untreated controls (**B**). In losartan treated pigs, iANGII levels were constant before the onset of obstruction. After the induction of UUO, there was a rapid increase in iANGII levels for all three sampling sites which was maximal in the final sample (**A**). In the control pigs iANGII levels were also constant before obstruction, and after the onset of UUO there was a less marked increase in iANGII compared with the losartan treated pigs. ↑ indicates the onset of UUO. Values are mean ± SEM

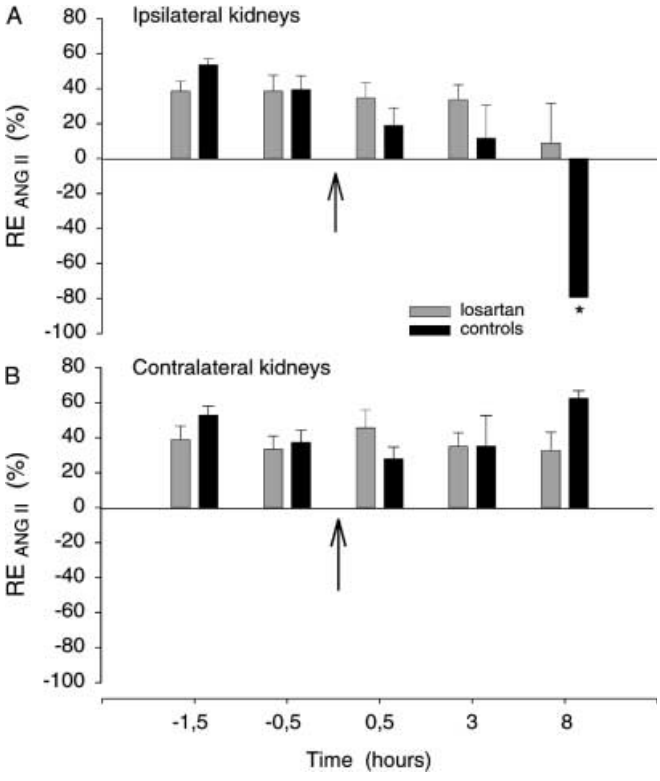

Fig. 6A, B. Renal extraction (*RE*) of angiotensin II (*ANGII*) show an almost identical pattern for both losartan treated pigs and control pigs. In both groups RE_{ANGII} from the ipsilateral kidney (**A**) decreased slightly after the onset of UUO (an *asterisk* for the last solid bar shows the SEM as ± 79%). In the contralateral kidney (**B**), RE_{ANGII} was almost constant during the course of the experiment. ↑ indicates the onset of UUO. Values are mean ± SEM

ipsilateral renal vein (2.7 ± 0.3–24.8 ± 10.2 pmol/l) ($P < 0.05$), whereas iANGII levels both in the aorta and contralateral renal vein did not increase significantly (Fig. 5B).

RE_{ANG}

In the losartan treated pigs, the renal extraction ratio of ANGII (RE_{ANG}) was positive in both ipsilateral and contralateral kidneys during the entire experiment (Fig. 6A). In the control animals, RE_{ANG} from the ipsilateral kidney was initially 54 ± 3% but slowly became negative with a final extraction of –79 ± 79% (not significant compared with losartan treated pigs). In the contralateral control kidney RE_{ANG} was positive throughout the experiment (Fig. 6B).

RSRang

In the losartan treated animals, the renal secretion ratio of ANGII (RSR_{ANG}) from the ipsilateral kidney remained negative during the entire experiment (Fig. 7). In contrast, RSR_{ANG} from the ipsilateral kidney in the control group showed a net secretion in iANGII 8 h after the onset of obstruction (10.8 vs -3.2 pmol/l/min in losartan treated ipsilateral kidney; $P < 0.05$). From the contralateral kidneys, RSR_{ANG} remained negative in both groups during the entire experiment, and changes were insignificant.

Losartan reduces aldosterone levels

Immunoreactive aldosterone levels were 77.6–80.4 pmol/l in the first sample 1.5 h before the onset of obstruction (–1.5 h). Following the administration of losartan, aldosterone levels were gradually reduced during the remainder of the experiment. In the arterial samples, aldosterone levels declined almost immediately and were 47.1 ± 5.5 pmol/l at the 0.5 h sample (0.5 h after the onset of obstruction), 41.5 ± 8.3 pmol/l at the 3 h sample and 36.0 ± 5.5 pmol/l at the 8 h sample at the end of obstruction ($P < 0.05$). In contrast, aldosterone levels in the renal veins were reduced more slowly, being only 72.0 ± 8.3 pmol/l in the right renal vein and 66.4 ± 11.1 pmol/l in the left renal vein at the 0.5 h

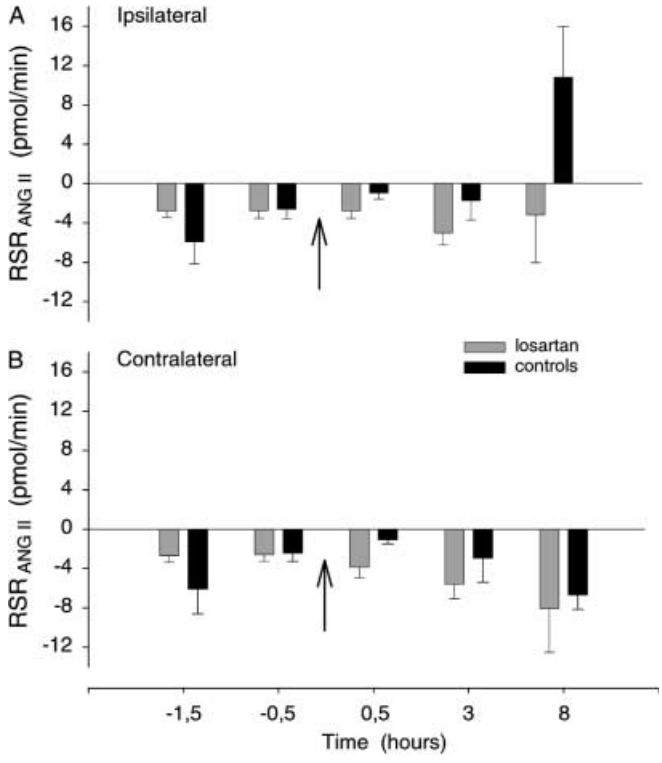

Fig. 7. Renal secretion rate (*RSR*) of angiotensin II (*ANGII*) shows only net secretion in the ipsilateral control kidneys ($P < 0.05$). There are no changes in losartan treated pigs (**A**). In the contralateral kidneys (**B**), values remained negative indicating no net secretion. ↑ indicates the onset of UUO. Values are mean ± SEM

sample. At the final sample, 8 h after the onset of obstruction, renal vein levels were reduced similarly to those found in the aorta (both $P < 0.05$).

Discussion

The main results of the present study, in which pigs with UUO were treated with losartan intravenously and compared to pigs without treatment, were: (1) A reduction in ipsilateral RBF which was less severe in the losartan treated pigs compared with the reduction in RBF observed in untreated control pigs, (2) a dramatic reduction in ipsilateral GFR which did not differ significantly from the controls, (3) an attenuation of ipsilateral RVR in the losartan treated pigs compared with the control pigs, (4) a significant increase in iANGII levels in the aorta and in both renal veins in the losartan treated animals, and (5) a significant reduction in aldosterone levels in the losartan treated pigs after the administration of losartan, indicating the effective blockade of the ANGII type-1 receptor (AT1-R). These results suggest that ANGII is an important vasoconstrictor during UUO in this pig model, and that the vasoconstriction can, in part, be prevented by the administration of losartan.

Losartan attenuates the ipsilateral reduction in RBF, and increase in RVR in response to UUO

UUO for 8 h was associated with a marked reduction in ipsilateral RBF and GFR and a corresponding increase in ipsilateral RVR. This is consistent with previous reports using rats and dogs which have shown a severe reduction in ipsilateral RBF in response to obstruction [18, 20, 22]. The mechanisms behind this reduction are still incompletely understood. A previous study, using a similar pig model in which changes in RBF and GFR were measured, showed that the hemodynamic changes could be explained primarily by a vasoconstriction of the preglomerular arteriole [15]. The contribution of ANGII as a mediator of this vasoconstriction has previously been examined, both by blocking the angiotensin II receptor mediated effects [3, 5, 20] and by the systemic inhibition of the angiotensin I converting enzyme (ACE) [3, 34]. In a previous study using pigs, we showed that 15 h of UUO was associated with an enhanced secretion of iANGII suggesting the de novo intrarenal generation of ANGII from the ipsilateral obstructed kidney [10]. However, the administration of the ACE inhibitor captopril failed to increase ipsilateral RBF in response to obstruction when compared to control animals [9], indicating that other renal vasoconstrictors play a pronounced role in the increased RVR in response to ureteral obstruction. Therefore, the blockade of the ANGII mediated effects at the receptor level may more directly rule out the hemodynamic importance of ANGII in the obstructed kidney. Pimentel and coworkers found that 24 h UUO induces the genes encoding for parts of RAS in the kidney [25]. The molecular changes were associated with marked reductions in RBF and GFR in the postobstructed kidney, similar to those observed during obstruction in the present study. The reduction in ipsilateral RBF and the increase in ipsilateral RVR were partially prevented in pigs treated with the AT1 receptor antagonist losartan in the present study, supporting the view that ANGII is a mediator of major importance for the increased vasoconstriction seen in response to UUO in pigs.

Renal blood flow is unchanged in the contralateral non-obstructed kidney

RBF to the contralateral kidney was maintained after the onset of obstruction at levels which did not differ significantly from RBF levels prior to obstruction. This suggests that functional adaption is delayed in response to UUO in the pig. In the losartan treated pigs, RBF to the contralateral kidney did not differ significantly from untreated controls. Previously, we found that captopril treatment of pigs with UUO increased RBF to the contralateral kidney [9] suggesting that a compensatory increase in RBF may be caused by an ANGII mediated renal vasoconstriction of the contralateral kidney since ACEI treatment reduced RVR [2, 7, 9]. In the present study, the AT1 receptor blockade did not change RBF

to the contralateral kidney, supporting the view that ANGII is not directly involved in the contralateral hemodynamic response to UUO.

Glomerular filtration rate is reduced in response to ureteral obstruction

In the ipsilateral obstructed kidney, GFR and FF were rapidly reduced after the onset of obstruction. The administration of losartan did not significantly prevent this reduction, but in the losartan treated pigs RE_{EDTA} (i.e. FF) is more rapidly reduced compared with the controls. This may be explained by the isolated attenuated reduction in ipsilateral RBF in the losartan treated pigs after the onset of obstruction. These alterations in renal hemodynamics may suggest that losartan causes parallel reductions in both pre- and postglomerular vascular resistance in the obstructed pig kidney. ANGII is able to contract both pre- and postglomerular vessels [19], in spite of the fact that its preferential site of action is thought to be at the postglomerular site [13]. Previous studies have shown that losartan has a beneficial effect on GFR changes by maintaining or increasing GFR both in the normal kidney and in the postobstructed kidney [25, 26]. In the present study we were not able to show similar changes in GFR during obstruction. Interestingly, using losartan and a non-selective AT-receptor antagonist it was recently demonstrated that both these antagonists prevented the increase in RVR in response to ANGII in the rat kidney, whereas only the non-selective antagonist prevented the increase in GFR [14]. Thus, the angiotensin receptor mediating the increase in GFR may be dissociated from that mediating the increase in RVR, providing functional evidence of angiotensin receptor subtypes, at least in the rat kidney.

Surprisingly, GFR in the contralateral non-obstructed kidney decreased significantly in the losartan treated pigs compared with the controls, whereas FF was unchanged in response to losartan and slightly increased in the untreated control pigs during the course of the study. The reason for these changes is unclear. Contralateral RBF was almost unchanged, suggesting limited, direct effects of the RBF on GFR. In a recent study using a slightly different model, Pimentel and colleagues found that losartan did not alter GFR or FF in the contralateral kidney after the release of acute UUO [26]. The importance of a reno-renal reflex mechanism during conditions with increased ureteral pressure is well recognized [6], and in neonatal rats denervation of the obstructed kidney was associated with an attenuated vasodilatation in the contralateral kidney during UUO, suggesting that the sympathetic nervous system plays an important role in the regulation of the renal hemodynamics in the contralateral intact kidney [4]. The importance of the relationship between the sympathetic nervous system and the renal renin angiotensin system, with and without blockade, needs further attention.

Ureteral obstruction increases renal secretion of ANGII

Immunoreactive ANGII levels increased in both losartan-treated and un-treated control pigs throughout the study, supporting the view that ureteral obstruction is associated with increased levels of p-ANGII [10, 11]. However, ANGII levels increased markedly more in response to losartan administration. The reason for this could be explained by an effective AT1-R blockade evidenced as a pronounced reduction in aldosterone levels after losartan administration leading to higher levels of circulating ANGII [29]. In the losartan treated pigs, iANG levels were highest in the arterial blood samples, whereas in the control animals iANGII levels were highest from the ipsilateral renal vein [10, 11].

There is now substantial evidence that much of the intrarenal ANGII is formed locally [23]. Several studies have provided evidence that the intrarenal tissue levels of ANGII expressed per gram kidney wet weight are much higher than the plasma ANGII concentrations expressed per milliliter of plasma [12, 31], suggesting that the intrarenal ANGII levels are not only due to the amount of ANGII delivered to the kidney but are also a consequence of the amount synthesized in the renal tissue. In support of this view, we recently demonstrated that UUO in the pig was associated with negative extractions and a net renal secretion of ANGII in response to 15 h of obstruction, suggesting an enhanced de novo synthesis of ANGII from the obstructed kidney [10]. In order to indirectly examine whether losartan administration to pigs with an 8 h UUO was associated with changes in the intrarenal metabolism of ANGII we calculated the renal extraction ratio and the renal secretion rate of ANGII. In the control pigs there was a net secretion of ANGII from the ipsilateral kidney 8 h after the onset of UUO. This may be explained by an enhanced de novo synthesis of intrarenally formed ANGII from the obstructed kidney. In the losartan treated pigs the secretion rate was negative during the entire experiment. The intrarenal formation and metabolism of ANGII is complex and a number of pathways are involved in this process [23]. From the indirect methods used in this study, it is not possible to examine the contribution of each of these pathways, but it is likely that losartan administration may influence the intrarenal metabolism of ANGII. Finally, a negative secretion of ANGII from the obstructed kidney does not necessarily reflect the lack of intrarenal ANGII production; rather this may be a consequence of a higher ANGII metabolism/consumption than de novo formation in these kidneys.

In conclusion, this study showed that losartan was able to reduce ipsilateral RVR in losartan treated pigs. We found a significant increase in iANGII levels and a significant reduction in aldosterone levels after the administration of losartan, indicating the effective blockade of the ANGII type-1 receptor. Our study supports the view that ANGII is an important vasoconstrictor

during UUO in the pig model and that renal vasoconstriction, in part, can be reduced by administering losartan.

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