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

Received: 4 September 2000 / Accepted: 11 July 2001 / Published online: 22 September 2001
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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 catheres 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

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 graded 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 Ca²⁺-influx into smooth muscle cells results in an increase in muscle tone, and the actively maintained large Ca²⁺ 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 pregglomerular 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 graded 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$_2$O (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 administered 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. From the right pelvis, urine was sampled every 30 min during the resting period, and the right pelvic pressure was monitored. Subsequently, graded 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

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1Prior 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.