Kidney International, Vol. 58 (2000), pp. 242–250

Long-term renal effects of unilateral ureteral obstruction and the role of endothelin

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Long-term renal effects of unilateral ureteral obstruction and the role of endothelin.

Background. Angiotensin II (Ang II) and endothelin (ET) are involved in the alteration of renal function in unilateral ureteral obstruction (UUO). The renal response to Ang II following the reversal of a 24-hour UUO and the effect of ET blockade by bosentan during the time of obstruction were investigated.

Methods. Following blockade of the endogenous production of Ang II by captopril, the renal response to Ang II was studied in rats 15 to 18 days after a 24-hour UUO ($N = 10$) or a sham operation ($N = 9$) both with ($N = 10$) and without ($N = 8$) bosentan treatment in the periobstruction period. Similar studies were performed in another group ($N = 9$) two months following the reversal of obstruction.

Results. In the sham-operated group, Ang II reduced renal blood flow (RBF) by 42 ± 9% ($P < 0.01$), glomerular filtration rate (GFR) by 30 ± 8% ($P < 0.01$), urine volume (UV) by 44 ± 9% ($P < 0.001$), and absolute ($U_{Na}V$) and fractional sodium excretion (FE_{Na}) by 52 ± 9% ($P < 0.001$) and 33 ± 9% ($P = 0.054$), respectively. In the previously obstructed kidney, Ang II did not change RBF but increased GFR by 106 ± 40% ($P < 0.01$), UV by 75 ± 21% ($P < 0.001$), $U_{Na}V$ by 190 ± 60% ($P < 0.001$), and FE_{Na} by 40 ± 13% ($P < 0.05$). Bosentan treatment in the obstructed group prevented these Ang II-induced effects and did not have any effect on the sham-operated kidney. Two months following reversal of the obstruction, the response of the kidney was similar to that of the control kidney.

Conclusion. Twenty-four–hour UUO results in a temporary abnormality in the renal response to Ang II, which is due, in part, to the actions of ET at the time of obstruction.

Ureteral obstruction is a relatively common clinical problem in humans that can lead to pain and ultimately to renal damage [1, 2]. The renal damage is due to the interaction of various factors leading to alterations in glomerular and tubular function at the time of obstruction [1–3]. Several animal studies have been performed to investigate the alteration to renal function in the early stages (hours) after ureteral obstruction-[2, 3]; however, few studies have addressed the longer-term effects of reversible ureteral obstruction on renal hemodynamics or tubular function. Nonetheless, Bander et al measured renal function under baseline conditions sequentially for up to 60 days following a reversible 24-hour unilateral ureteral obstruction (UUO) in the rat [4]. In this model, it was shown that renal blood flow (RBF), glomerular filtration rate (GFR), and absolute ($U_{Na}V$) and fractional excretion of sodium (FE_{Na}) return to normal by 14 days after the reversal of the obstruction. However, the sustained low urine osmolality for up to two months following the obstruction reversal was indicative of a persistent functional defect in the distal nephron.

Endothelins (ETs) are a family of three potent vasoconstrictor isopeptides (ET-1, ET-2, and ET-3) [5], for which at least two types of receptors have been identified (ET_A and ET_B) [6]. It has been shown that renal tissue can synthesize ET, and it expresses both ET_A and ET_B receptors [7]. ET plays an important role in several renal pathophysiological conditions [7]. Indeed, complete obstruction of one ureter in three-week-old weanling rats increased ET concentration in renal tissue significantly [8]. Furthermore, prior inhibition of the biological action of ET-1 by a specific antibody significantly attenuated the fall in GFR and effective renal plasma flow (ERPF) in rats after unilateral release of 24-hour bilateral ureteral obstruction [9].

Bosentan, an orally active mixed ET_A/ET_B receptor antagonist, has been shown to have a protective effect in a number of renal pathophysiological conditions, including nephritis, renal tissue loss, and rhabdomyolysis-induced renal failure [10–12]. Therefore, the possibility exists that it may also have a protective role in the long-term renal damage caused by ureteral obstruction.

Angiotensin II (Ang II) plays an important role in the control of sodium excretion by the kidneys under physiological and pathophysiological conditions [13, 14]. There is evidence to suggest that Ang II synthesis increases during ureteral obstruction [1, 15–17] and that

Key words: bosentan, angiotensin II, captopril, hemodynamics, vasoconstriction.

Received for publication July 13, 1999
and in revised form November 12, 1999
Accepted for publication February 1, 2000

the postobstructed kidney exhibits an abnormal response to Ang II [18, 19]. In the rabbit, Nishikawa, Morrison, and Needleman demonstrated an increase in the Ang II-induced prostaglandin release by the postobstructed kidney [18]. Furthermore, the contractile response of strips of rabbit renal cortex to Ang II is enhanced in the postobstructed kidney [19], indicating an increase in the number and/or affinity of the Ang II receptors under these conditions [20].

The aim of this study was thus to investigate the long-term in vivo renal response to physiological doses of Ang II, which affect both hemodynamic and tubular functions two weeks and two months following reversal of a 24-hour UUO in the rat. Furthermore, the effect of an ET blockade at the time of obstruction on the response to Ang II was investigated.

METHODS

Studies were performed in male Wistar rats weighing 230 to 245 g at the time of ureteral obstruction or sham operation. Animals were supplied by the Department of Laboratory Animals Sciences, University of Otago. Animal procedures were approved by University of Otago Animal Ethics Committee. Rats were kept in a 12-hour light/dark cycle at 20°C and fed a standard rat chow, and the rats had access to water ad libitum.

Ureteral occlusion/sham operation and reversal

The following procedures were carried out under aseptic conditions. Induction of anesthesia was achieved by administering 4% halothane (fluothane, I.C.I.; Zeneca, Macclesfield, UK), nitrous oxide and oxygen at a rate of 2 and 3 L/min, respectively. Surgical anesthesia was maintained by administration of 1.5 to 3.5% fluothane in oxygen at a rate of 1 L/min. The left kidney and ureter were exposed via a flank incision. Using the technique described by Bander et al, the ureter was obstructed by placing a 3 to 4 mm length of bisected PVC tubing (0.58 mm internal diameter) around the left midureter [4]. The ureter was then occluded by constricting the tubing with a 4-0 silk suture. At the end, the wound was closed in layers.

Twenty-four hours later, all animals underwent reversal procedures. Using similar anesthesia as described previously in this article, the left kidney and ureter were approached through the same incision. Using a dissecting microscope, the obstructing tube was identified and removed. Full release of the obstruction was confirmed by observation of a free flow of urine across the site of obstruction. The wound was then closed.

Sham animals underwent identical surgical procedures, but the left ureter was simply manipulated.

Bosentan administration

Bosentan (Ro 47-0203/029; Roche, Zurich, Switzerland), a mixed ET_A/ET_B receptor antagonist, was administered orally (150 mg/kg/day) in the periobstruction or sham procedure periods. Bosentan was given for four days commencing 24 hours prior to obstruction or sham procedures and continued for three days after operation. The drug was mixed with a small quantity of pulverized rat chow (5 g) made into loose pellets by adding water and then drying it under 37°C. The pellets were placed inside the cage each evening, and all other food was removed to ensure that the full dose of bosentan was taken. The following morning, regular rat chow was made available. In this way, the animal would rapidly ingest the bosentan, resulting in a rapid increase in plasma levels. Its long half-life means that the plasma levels remains in the therapeutic range throughout the treatment period [21, 22].

Surgical procedure in the terminal experiment

Rats were fasted overnight but had water ad libitum. Each animal was anesthetized with pentobarbitone sodium (60 to 70 mg/kg, intraperitoneally; Nembutal, Virbac, New Zealand), and the trachea was cannulated. Following cannulation of a femoral vein with polyethylene tubing (PE-50), anesthesia was maintained by a continuous infusion of pentobarbitone sodium (12.5 mg/kg/hour), and a sustaining infusion of 0.9% saline was established at a rate of 50 μL/min using an infusion pump (IITC Life Science, Woodland Hills, CA, USA). A femoral artery was cannulated, and the tip of the cannula was positioned just below the level of the renal artery. The cannula was connected to a pressure transducer (Statham P23AC, Hato Rey, Puerto Rico). The blood pressure signal was amplified using a MacLab bridge Amp (ADInstruments, Castle Hill, Australia), digitized using a MacLab/4 and version 3.5.4 software (ADInstruments) and displayed on a Macintosh LCIII computer. The measured pressure was used as an estimate of the mean renal arterial pressure (RAP). The arterial cannula was also used to obtain blood samples throughout the procedure as required. The left kidney was exposed through a flank incision, and its upper ureter was cannulated with polyethylene tubing (PE-10) for the collection of urine in preweighed microcapped tubes. The urine volume (UV) was determined gravimetrically. The renal nerves were visualized and sectioned to eliminate the effect of renal nerves on kidney function. The renal artery and vein were completely stripped of connective tissue and painted with absolute alcohol to achieve complete denervation [23, 24]. The right ureter was cannulated to provide a continuous drainage of urine, thus preventing reno-renal responses caused by increased right ureteral pressure. A length of thread was looped around the aorta above the level of renal arteries and attached to a screw device. This adjustable constrictor was used to control the RAP during Ang II infusion. The thread on the screw device had a fine pitch that

provided a precise control of blood pressure such that the mean pressure over the period of Ang II infusion was ±2 mm Hg from the desired pressure, that is, the pressure during captopril infusion only.

On completion of the surgery, the sustaining infusion was replaced by one containing inulin (1.5% weight/volume) and para-aminohippuric acid (PAH; 0.2% weight/volume). A priming dose of 2 mL of the same solution was infused over two minutes. Animals were allowed two hours to equilibrate before being subjected to the experimental protocol.

Experimental protocol and assays

The experimental protocol consisted of seven 15-minute clearance periods. Following two baseline clearance periods, captopril (900 μg/kg/hour; Sigma, St. Louis, MO, USA), an angiotensin I-converting enzyme (ACE) inhibitor, was added to the infusion to block the production of endogenous Ang II. Captopril infusion was continued thereafter until the end of the protocol. Thirty minutes were allowed for the captopril action to take effect. The effectiveness of captopril was determined by infusing 200 ng of angiotensin I (Sigma) dissolved in 0.2 mL of normal saline before and 30 minutes after the start of the captopril infusion. If the pressor response of angiotensin I was reduced to less than 10% of the response prior to captopril, blockade of Ang II production was considered to be effective [25].

Following two clearance periods in the presence of captopril, Ang II (acetate salt, 25 ng/kg/min; Sigma) was introduced to the infusion. Fifteen minutes were allowed to servo-control the RAP at a pre-angiotensin level and for the passage of the preformed urine. Once stabilized, a further 15-minute urine collection was performed. The infusion of Ang II was then terminated, and two recovery collections were made. Finally, the animals were euthanized with an overdose of barbiturate and the kidneys weighed.

Arterial blood samples (0.4 mL) taken at the beginning and end of the basal, captopril, Ang II and recovery periods were immediately centrifuged. Plasma samples (125 μL) were frozen to be assayed later. The plasma was replaced by an equal volume of saline, and the erythrocytes were resuspended by gentle vortexing and were returned to the animal. The hematocrit was determined.

Urine and plasma samples were assayed for sodium using a flame photometer (SEAC, Florence, Italy). Inulin and PAH content were determined using modified techniques described by Bojesen [26] and Bratton and Marshall [27], respectively. GFR was estimated from the clearances of inulin. RBF was calculated using the formula [RBF = ERPF/(1 − hematocrit)], where the clearance of PAH was used as an estimate to ERPF. All renal data were corrected for kidney weight.

Experimental groups

Animals were divided into five groups.

(1) Sham group (N = 9). These rats underwent manipulation of the left ureter only and acted as a control group.

(2) UUO group (N = 10). These rats underwent left ureteral obstruction.

(3) B/sham group (N = 8). These rats underwent manipulation of the left ureter and received bosentan treatment.

(4) B/UUO group (N = 10). These rats underwent left ureteral obstruction and received bosentan treatment.

These four groups had their renal function measured 15 to 18 days following reversal of the obstruction.

(5) UUO/LT group (N = 9). These rats had ureteral obstruction, and the renal function was measured 59 to 63 days following reversal of the obstruction. Renal function of the postobstructed left kidney was compared with the right kidney.

Statistical analysis

Statistical analysis was performed with the StatView IV system (Abacus Concepts, Inc., Berkeley, CA, USA) designed for the Macintosh computer. All data are expressed as mean ± SEM. Analysis of variance (ANOVA) with repeated measures was used to identify changes in each variable as a consequence of treatment with captopril and Ang II. Where appropriate, intragroup comparison between basal and captopril treatment and between captopril and Ang II were carried out using a Student's paired *t*-test. A comparison between groups was achieved using one-way factorial ANOVA. A value of $P < 0.05$ was considered significant.

RESULTS

The basal blood pressure and renal function variables in the sham, UUO, B/sham, and B/UUO groups were similar and are shown in Table 1.

Response of the sham group

Captopril administration resulted in a 7 ± 1% ($P < 0.01$) decrease in RAP. RBF and GFR increased by 34 ± 12% and 34 ± 9%, respectively ($P < 0.05$ in both cases). Similarly, urine flow rate (UV), $U_{Na}V$, and FE_{Na} excretion increased by 60 ± 20% ($P < 0.001$), 236 ± 86% ($P < 0.001$), and 169 ± 79% ($P < 0.01$), respectively (Table 1).

During the infusion of Ang II, RAP was successfully maintained at a preinfusion level by adjusting the aortic constrictor. Ang II reduced RBF and GFR by 42 ± 9% and 30 ± 8%, respectively ($P < 0.01$ in both cases), and reduced UV and $U_{Na}V$ by 44 ± 9% ($P < 0.001$) and 52 ± 9% ($P < 0.001$), respectively. FE_{Na} decreased by 33 ± 9%, but this reduction just failed to reach significance ($P = 0.054$; Figs. 1 and 2).

Table 1. Blood pressure and renal hemodynamic and tubular data of the left kidney from the sham, unilateral ureteral obstruction (UUO), B/Sham and bosentan (B)/UUO groups during the basal conditions and with captopril infusion

	Sham ($N = 9$)		UUO ($N = 10$)		B/Sham ($N = 8$)		B/UUO ($N = 10$)	
	Basal	Captopril	Basal	Captopril	Basal	Captopril	Basal	Captopril
BP *mm Hg*	99 ± 3	92 ± 2[b]	102 ± 1	93 ± 2[c]	103 ± 3	94 ± 4[b]	103 ± 4	96 ± 4[a]
RBF *mL/min/g*	4.40 ± 0.45	6.14 ± 0.87[a]	4.63 ± 0.62	4.99 ± 0.48	4.83 ± 0.66	7.34 ± 1.05[b]	4.99 ± 0.59	6.70 ± 0.71[b]
GFR *mL/min/g*	0.79 ± 0.09	1.01 ± 0.10[a]	0.84 ± 0.06	0.71 ± 0.07	0.73 ± 0.14	1.09 ± 0.14[b]	0.73 ± 0.09	1.11 ± 0.11[c]
UV *μL/min/g*	12.53 ± 1.87	17.72 ± 2.20[c]	13.52 ± 1.60	9.94 ± 1.66[c]	11.78 ± 1.93	16.34 ± 2.56[a]	9.70 ± 1.84	14.40 ± 2.49[a]
$U_{Na}V$ *μmol/min/g*	1.37 ± 0.34	2.86 ± 0.50[c]	1.32 ± 0.27	1.21 ± 0.33	1.47 ± 0.34	3.14 ± 0.45[b]	1.06 ± 0.23	2.46 ± 0.49[b]
FE_{Na} %	1.66 ± 0.57	2.19 ± 0.48[b]	1.19 ± 0.26	1.14 ± 0.30	1.66 ± 0.40	2.33 ± 0.50[a]	1.23 ± 0.33	1.80 ± 0.46[a]

Data are means ± SEM. Abreviations are: BP, blood pressure; GFR, glomerular filtration rate; RBF, renal blood flow; UV, urine flow rate; $U_{Na}V$, absolute sodium excretion; FE_{Na}, fractional excretion of sodium.
[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$, captopril vs. basal in each group

Response of the unilateral ureteral obstruction group

Captopril infusion reduced RAP by 9 ± 2% ($P < 0.001$) but did not change RBF or GFR (Table 1). UV decreased by 28 ± 6% ($P < 0.001$), but $U_{Na}V$ and FE_{Na} were unchanged during captopril infusion. Compared with the sham group, the changes in RAP and RBF were similar in the two groups. However, changes in GFR were different (−6 ± 14% vs. 34 ± 9%, $P < 0.05$). UV, $U_{Na}V$, and FE_{Na} responses to captopril were also different when compared with the sham group (−28 ± 6% vs. 60 ± 20%, $P < 0.001$; −18 ± 10% vs. 236 ± 86%, $P < 0.01$; and −1 ± 15% vs. 169 ± 79%, $P < 0.05$), respectively.

During Ang II infusion, RAP was maintained at preinfusion level by adjusting the aortic constrictor. Ang II infusion did not change RBF; however, GFR, UV, $U_{Na}V$, and FE_{Na} increased by 106 ± 40% ($P < 0.01$), 75 ± 21% ($P < 0.001$), 190 ± 60% ($P < 0.001$), and 40 ± 13% ($P < 0.05$), respectively. These renal function responses to Ang II in the UUO group were different from those obtained in the sham group: RBF −6 ± 12% vs. −42 ± 9% ($P < 0.05$); GFR 106 ± 40% vs. −30 ± 8% ($P < 0.01$); UV 75 ± 21% vs. −44 ± 9% ($P < 0.001$); $U_{Na}V$ 190 ± 60% vs. −52 ± 9% ($P < 0.01$) and FE_{Na} 40 ± 13% vs. −33 ± 9% ($P < 0.01$; Figs. 1 and 2).

Response of the sham group treated with bosentan

Captopril infusion reduced RAP by 9 ± 3% ($P < 0.01$), whereas RBF and GFR were increased by 48 ± 11% ($P < 0.01$) and 77 ± 36% ($P < 0.01$), respectively (Table 1). Captopril also increased UV, $U_{Na}V$, and FE_{Na} by 58 ± 40% ($P < 0.05$), 203 ± 77% ($P < 0.01$) and 76 ± 45% ($P < 0.05$), respectively. All of these responses were similar to those observed in the sham group.

During Ang II infusion, RAP was successfully maintained at pre-Ang II level. Ang II infusion decreased RBF by 45 ± 8% ($P < 0.01$) and GFR by 28 ± 8% ($P < 0.05$; Fig. 1). Ang II reduced UV, $U_{Na}V$, and FE_{Na} by 43 ± 4% ($P < 0.001$), 53 ± 4% ($P < 0.001$), and 31 ± 7% ($P < 0.05$), respectively (Fig. 2). These responses were similar to those observed in the sham group.

Response of the unilateral ureteral obstruction group treated with bosentan

Captopril infusion reduced RAP by 6 ± 2% ($P < 0.05$). This response was similar to that recorded in the UUO group. Captopril elevated RBF by 35 ± 10% ($P < 0.01$) and GFR by 57 ± 10% ($P < 0.001$). The responses of the RBF and GFR were similar to those observed in the B/sham group. Comparing the B/UUO with the UUO group, the change in RBF was similar in the two groups. However, the change in GFR was different (57 ± 10% vs. −6 ± 14%, $P < 0.01$). Captopril also increased UV, $U_{Na}V$, and FE_{Na} by 64 ± 32% ($P < 0.05$), 185 ± 62% ($P < 0.01$), and 67 ± 27% ($P < 0.05$). These changes were similar to those observed in the B/sham group but different from those in the UUO group: UV 64 ± 32% vs. −28 ± 6% ($P < 0.05$); $U_{Na}V$ 185 ± 62% vs. −18 ± 10% ($P < 0.01$); and FE_{Na} 67 ± 27% vs. −1 ± 15% ($P < 0.05$).

During Ang II infusion, RAP was successfully maintained at a preinfusion level. Ang II reduced RBF and GFR by 46 ± 9% ($P < 0.01$) and 36 ± 8% ($P < 0.001$), respectively (Fig. 1). The percentages of changes were not different from those observed in the B/sham group but were different from the UUO group (−46 ± 9% vs. −6 ± 12% ($P < 0.05$) and −36 ± 8% vs. 106 ± 40% ($P < 0.01$), respectively. Ang II also reduced UV, $U_{Na}V$, and FE_{Na} by 46 ± 6% ($P < 0.001$), 51 ± 6% ($P < 0.001$), and 21 ± 6% ($P < 0.05$), respectively (Fig. 2). These responses were similar to the B/sham group but were different from the UUO group ($P < 0.001$ in all cases): −46 ± 6% vs. 75 ± 21%, −51 ± 6% vs. 190 ± 60%, and −21 ± 6% vs. 40 ± 13%, respectively.

In all four groups, upon discontinuation of Ang II, all variables returned to values comparable to those found during captopril infusion alone.

Response of the unilateral ureteral obstruction/ long-term group

The blood pressure and renal function data from the UUO/LT group throughout the experimental protocol are given in Table 2. In this group, the baseline RAP

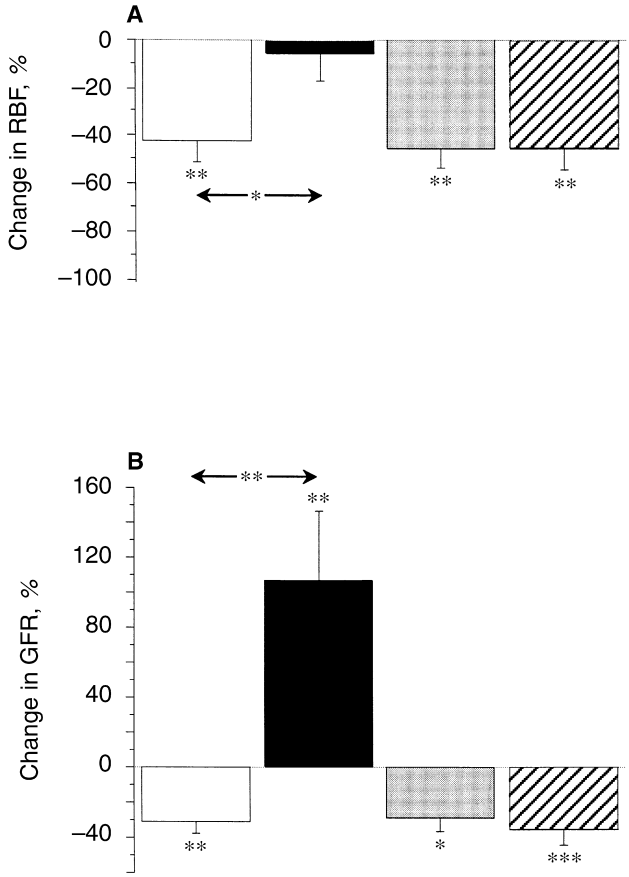

Fig. 1. The percentage change of renal blood flow (RBF) (*A*) and glomerular filtration rate (*B*) to Ang II infusion. Values represent mean ± SEM. Symbols are: (□) Sham group; (■) UUO group; (▩) B/sham group; (▨) B/UUO group; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

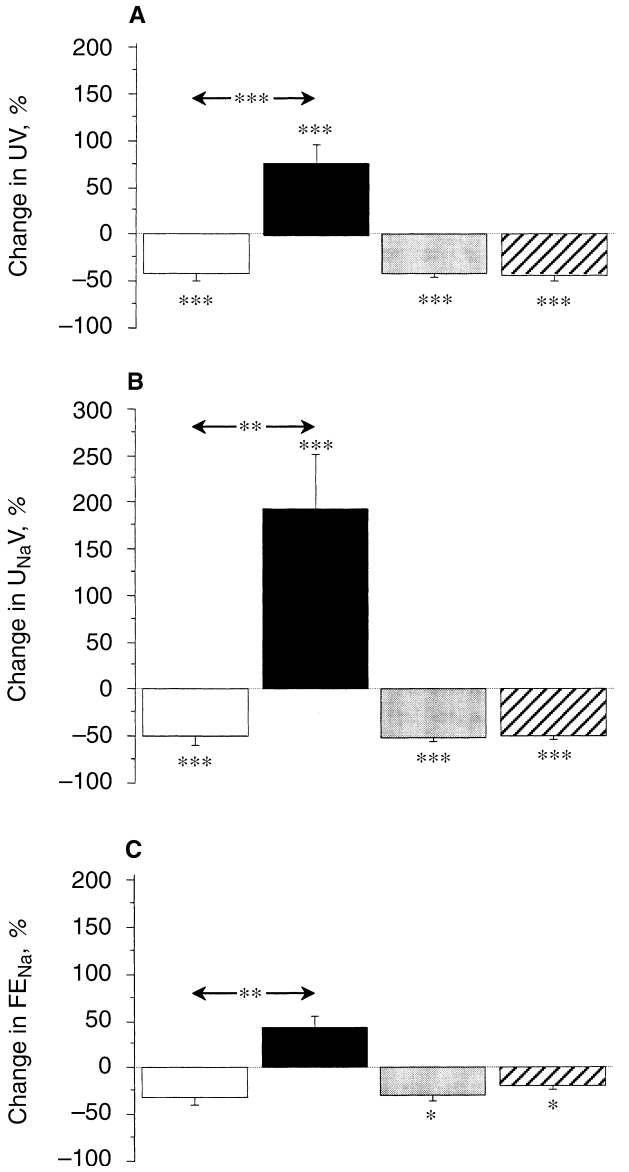

Fig. 2. The percentage of (*A*) urine flow rate (UV), (*B*) absolute sodium excretion ($U_{Na}V$), and (*C*) fractional excretion of sodium (FE_{Na}) to Ang II infusion. Values represent mean ± SEM. Symbols are: (□) sham group; (■) UUO group; (▨) B/sham group; (▨) B/UUO group; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

was higher than the other groups. Comparing it with the UUO group, for example, the blood pressure was 6% higher ($P < 0.05$).

Under baseline conditions, RBF, GFR, UV, $U_{Na}V$, and FE_{Na} in the left, previously obstructed kidney were similar to the right control kidney.

Captopril decreased RAP by 7 ± 1% ($P < 0.01$). In the right control kidney, captopril increased RBF and GFR by 43 ± 12% ($P < 0.05$) and 33 ± 7% ($P < 0.01$), respectively. Captopril also increased UV, $U_{Na}V$, and FE_{Na} by 30 ± 4%, 150 ± 32%, and 82 ± 21% ($P < 0.001$ in all the cases), respectively. In the left, previously obstructed, kidney, RBF and GFR rose during captopril infusion by 45 ± 12% and 32 ± 6%, respectively ($P < 0.01$ in both cases). During this time, UV, $U_{Na}V$, and FE_{Na} also increased by 43 ± 14% ($P < 0.01$), 194 ± 83% ($P < 0.001$), and 111 ± 59% ($P < 0.01$), respectively. These changes were similar in the two kidneys.

As in the other groups, RAP was maintained during Ang II infusion. In the right kidney, Ang II decreased RBF and GFR by 57 ± 9% ($P < 0.01$) and 49 ± 8% ($P < 0.001$), respectively. In addition, UV, $U_{Na}V$, and FE_{Na} decreased by 59 ± 6% ($P < 0.001$), 69 ± 5% ($P < 0.001$), and 35 ± 8% ($P < 0.05$), respectively. In the left kidney, Ang II also reduced RBF and GFR by 47 ± 8% and 38 ± 6%, respectively ($P < 0.01$ in both cases). With regard to the tubular parameters, UV, $U_{Na}V$, and FE_{Na} decreased by 53 ± 7% ($P < 0.001$), 64 ± 5% ($P < 0.001$), and 42 ± 8% ($P < 0.05$), respectively. All of these responses were similar in the two kidneys. In both kidneys, during the recovery period, all of the variables returned to values comparable to those found with captopril alone.

Table 2. Blood pressure and renal hemodynamic and tubular data of UUO/LT group ($N = 9$)

		Basal	Captopril	Angiotensin II	Recovery
BP *mm Hg*		107 ± 1	99 ± 1[b]	98 ± 2	101 ± 2
RBF *mL/min/g*	(R)	3.05 ± 0.36	4.57 ± 0.82[a]	1.84 ± 0.42[f]	3.06 ± 0.61
	(L)	3.36 ± 0.25	4.89 ± 0.54[b]	2.38 ± 0.25[f]	3.15 ± 0.43
GFR *mL/min/g*	(R)	0.56 ± 0.06	0.74 ± 0.09[b]	0.41 ± 0.09[f]	0.63 ± 0.11
	(L)	0.62 ± 0.06	0.83 ± 0.09[b]	0.48 ± 0.04[f]	0.60 ± 0.08
UV *μL/min/g*	(R)	7.74 ± 0.73	10.02 ± 0.87[c]	4.36 ± 0.93[g]	9.96 ± 1.86
	(L)	9.43 ± 1.05	12.88 ± 1.36[b]	6.64 ± 1.63[g]	12.20 ± 2.20
$U_{Na}V$ *μmol/min/g*	(R)	0.73 ± 0.11	1.57 ± 0.16[c]	0.52 ± 0.11[g]	1.75 ± 0.39
	(L)	1.03 ± 0.22	2.08 ± 0.33[c]	0.85 ± 0.21[g]	2.12 ± 0.50
FE_{Na} %	(R)	1.18 ± 0.32	1.78 ± 0.37[c]	1.22 ± 0.27[e]	2.33 ± 0.54
	(L)	1.40 ± 0.37	1.99 ± 0.42[b]	1.19 ± 0.27[e]	2.60 ± 0.55

Data are means ± SEM. Abreviations are: BP, blood pressure; GFR, glomerular filtration rate; RBF, renal blood flow; UV, urine flow rate; $U_{Na}V$, absolute sodium excretion; FE_{Na}, fractional excretion of sodium; R, right kidney; L, left kidney.

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, captopril vs. basal

[e] $P < 0.05$, [f] $P < 0.01$, [g] $P < 0.001$, angiotensin II vs. captopril

DISCUSSION

Ureteral obstruction is one form of obstructive uropathy, a condition that includes any structural or functional change in the urinary tract that impedes normal flow of urine. In humans, ureteral obstruction is associated with pain and renal damage [1, 2]. The extent of the damage is related to the duration of the obstruction and to the degree of the blockade. Longer durations and complete blockade give rise to more severe damage [28, 29]. The renal damage is associated with alterations in glomerular filtration and tubular function, which may or may not be reversible depending on the duration of the obstruction. The effects of ureteral obstruction on renal function in the immediate period following relief of obstruction are well studied. However, the long-term recovery from ureteral obstruction is less well understood [20].

In our study, we chose to use a period of 24 hours of ureteral obstruction as a model of obstructive uropathy because this duration was severe enough to cause a reversible damage to the kidney. In this model, immediately following the creation of the obstruction, there is an increase in RBF, which has been shown to be due to the release of vasodilatory prostaglandins [30]. Subsequently, over the next few hours, RBF progressively falls until it reaches 50% of control values at 24 hours. Over the same time period, GFR falls to around 25% of control values [31]. Following reversal of the obstruction, RBF and GFR gradually increase over the next 14 days to values similar to those recorded in control kidneys [4]. Immediately after reversal, there is an increase in sodium and water excretion [4, 29], which decreases to normal over 14 days [4]. Histologically, there is a decrease in the number of functioning nephrons, suggesting an increase in single-nephron GFR [4]. It has also been noted that the osmolality of the urine remains lower than normal even after 60 days postobstruction, suggesting that there is a persistent functional defect in the distal nephron [4].

In our study, the reversal of the obstruction was confirmed by microscopic observation of the passage of urine past the site of obstruction. Although it is possible that minor edema and surgical adhesion, which occur postoperatively, might have caused a partial obstruction. Such an obstruction would have had only a minor effect on the recovery from ureteral obstruction since baseline renal function returned to normal after 14 days, consistent with the observations reported by Bander et al [4].

To test the integrity of the postobstructed kidney, our protocol consisted of stimulating the kidney with a relatively low physiological concentration of Ang II (25 ng/kg/min) [14, 32]. To more clearly resolve the effect of the Ang II infusion on renal function, we blocked the endogenous production of Ang II using captopril prior to the exogenous Ang II infusion. The effectiveness of this blockade was demonstrated by the reduction of the pressor response to Ang I to less than 10% of precaptopril pressor response [25, 32]. In addition, the renal nerves were sectioned to avoid any neural reflex alterations in renal function. Furthermore, the renal artery pressure was servo-controlled to pre-Ang II infusion levels to avoid changes in renal perfusion pressure influencing the renal response to Ang II.

In the sham group, captopril administration caused a prompt fall in RAP and an increase in both RBF and GFR (Table 1), which is consistent with previous reports [33–35]. The increase in RBF in this study was comparable to that reported by Mattson and Roman [33–35], but their increase in GFR was twice that in our study. This difference is probably explained by the relative hypovolemic state of their animals compared with those in the present study, and hypovolemia is known to elevate basal plasma renin activity.

To isolate the intrarenal action of Ang II from renal effects associated with increases in RAP, the aorta, above the level of the kidneys, was constricted to maintain RAP at preinfusion levels. During this infusion pe-

riod, RBF and GFR decreased, as did the urine flow rate and $U_{Na}V$ and FE_{Na} (Figs. 1 and 2). These responses to a low dose of Ang II were similar to those reported by others [33, 36]. The decrease in urine flow rate and $U_{Na}V$ and FE_{Na} suggests that Ang II increased proximal tubular sodium and water reabsorption.

The baseline hemodynamic and tubular parameters in the UUO group were similar to those in the sham group and were consistent with the observations of Bander et al [4]. In the UUO group, captopril caused a fall in RAP (Table 1) similar to that observed in the sham group. In contrast to the sham group, however, captopril did not increase RBF or GFR in the UUO group. Further, when Ang II was infused into the UUO rats, RBF did not change, but a significant increase in GFR occurred. This suggests that Ang II does not contribute appreciably to renal vascular tone in these postobstructed kidneys. One possible explanation for these findings is that the renin-angiotensin system is not active in these postobstructed kidneys. This, however, seems unlikely given the fall in RAP observed in response to captopril, which indicated an active renin-angiotensin system. Alternatively, down-regulation of Ang II type 1 receptors (AT1), the main receptors found in the preglomerular vessels [37], may have occurred in response to UUO. Indeed, Pimentel, Wang, and Martinez-Maldonado have reported that 24 hours following UUO, there was a down-regulation of the AT1 receptor mRNA expression mediated by the increase in intrarenal Ang II levels [38]. In addition, Bander et al observed that RBF returned to baseline only 14 days after the reversal of the obstruction [4]. Thus, it would appear that the AT1 receptors remain down-regulated for at least 14 days. In the current study, Ang II infusion caused no vasoconstriction of the afferent arterioles and vasoconstriction of the efferent arterioles, which led to elevation of glomerular hydrostatic pressure and GFR, indicating that only Ang II receptors on the afferent arterioles remained responsive. Indeed, it has been suggested that AT1 receptors on the efferent arterioles are more sensitive to Ang II than those on the afferent arterioles [14, 39, 40]. It is possible that UUO exaggerates the differential sensitivity of these receptors. Furthermore, a selective down-regulation of the AT1 receptors has been reported in various physiological conditions. Amiri and Garcia observed that activation of the renin-angiotensin system using a low-sodium diet resulted in a down-regulation of Ang II receptors in preglomerular vessels, whereas the glomerular AT1 receptors were not changed [37]. This lack of Ang II responsiveness in the afferent arteriole may be physiologically relevant in that RBF would be better maintained in the postobstruction period.

Angiotensin II plays an important role in the tubular handling of sodium and water. Captopril treatment in the sham rats resulted in an increase in sodium and water excretion, whereas administration of exogenous Ang II resulted in an antinatriuresis and antidiuresis. This is consistent with the observations of Barraclough, Jones, and Marsden [36] and Ding et al [41] in normotensive rats. In the UUO animals, captopril caused a decrease in water excretion, with no measurable change in sodium handling. Conversely, Ang II resulted in a natriuresis and diuresis. Much of this response is caused by the increase in GFR and the increased filtered load. However, the fact that there was an increase in FE_{Na} suggests that there was also a decrease in tubular reabsorption in response to Ang II. One possible mechanism for this is that the large increase in GFR results in an increased delivery of Ang II to the luminal Ang II receptors. Stimulation of these receptors has been shown to give rise to a natriuresis and diuresis [42].

In the kidney, ET receptors are located in the afferent and efferent arterioles [43, 44] on the mesangial cells within the glomerulus [45] and on the tubules [44]. The primary action of ET appears to be vasoconstriction resulting in a decrease in RBF [46]. Plasma and tissue levels of ET are elevated in a number of renal pathophysiological disorders such as hypertension, renal failure, renal ischemia [7, 47], and UUO [8].

Endothelin has been implicated in the tissue damage and dysfunction associated with UUO. Indeed, ET blockade using specific anti-ET antibodies attenuated the fall in renal plasma flow and GFR in rats subjected to unilateral reversal of 24-hour bilateral ureteral obstruction [9]. ET synthesis and release are augmented by circulating and intrarenal Ang II [22, 48, 49]. The subsequent increase in circulating ET probably results in intense and prolonged constriction of the efferent arterioles in particular [50], which would serve to maintain some level of filtration.

To establish whether blockade of ET receptors had any direct influence on renal function, we treated a group of sham UUO rats with bosentan. As shown in Table 1, blockade of the ET receptors had no measurable effect on the baseline renal function or on the response to captopril. Similarly, no effect on the response to Ang II was observed (Figs. 1 and 2). These results suggest that under normal conditions, ET blockade does not have any long-lasting effect on the control of renal function.

Following treatment with bosentan, renal responses to captopril and Ang II in the UUO animals were similar to those observed in the sham and the B/sham animals. Clearly, blockade of the ET receptors prevents the renal dysfunction associated with UUO.

It has been demonstrated that UUO increases both Ang II [1, 15–17] and ET [8], bringing about the renal hemodynamic changes described previously in this article. The interrelationship between Ang II and ET is such that each one potentiates the production of the other. This could explain the elevated concentrations of both vasoactive factors even after the UUO has been reversed. In addition, the proinflammatory nature of ET

[47] may promote edema and thus prolong the recovery from UUO. By introducing the mixed ET_A/ET_B receptor antagonist bosentan, this cycle is broken. Therefore, the renal function would have recovered by 14 days after the 24-hour obstruction, resulting in a normal response to both captopril and Ang II. From the results, it is not possible to establish whether the ET_A, ET_B, or both receptors are responsible for the alterations to renal function following UUO. Further studies using selective ET receptor antagonists are needed to address this issue.

In the UUO/LT group studied between 59 and 63 days after relief of the obstruction, the postobstructed left kidney function was compared with the right control kidney in order to record an age-matched comparison. The RAP was higher in this group of animals compared to the sham group (discussed previously in this article). This is most probably caused by the normal age-related changes in arterial pressure and is consistent with the reports of Reckelhoff et al [51]. In terms of renal hemodynamics, basal RBF and GFR in the post-UUO kidneys were not different to those observed in the right control kidney. Furthermore, in response to captopril, both kidneys showed similar increases in RBF and GFR. Similarly, both kidneys responded to Ang II by decreasing RBF and GFR. The renal tubular responses to captopril and Ang II were the same in the control and post-UUO kidneys. These results suggest that the post-UUO kidney had recovered from the obstruction by 60 days, which probably reflects an up-regulation of the Ang II receptors to normal levels, resulting in normal renal responses to captopril and Ang II.

In conclusion, UUO for 24 hours results in an abnormal response of the previously obstructed kidney to captopril and Ang II, two weeks following the release of obstruction. This abnormality seems to involve both hemodynamic and tubular functions. We have also shown that this abnormality is a short-term one with full recovery by 59 to 63 days. Furthermore, we have demonstrated that the altered renal function is prevented by ET-receptor antagonism during the time of obstruction. These findings emphasize the linkage between the renin-angiotensin system and ETs in the pathogenesis of this diseases. Additional experiments are required to describe more fully the role of ET in the renal damage associated with UUO and to determine the therapeutic role, if any, of ET antagonists.

ACKNOWLEDGMENTS

This research was made possible by the financial support of the Lottery Health Board and the Department of Physiology. Bosentan was kindly donated by Hoffman-La Roche Ltd., Switzerland.

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