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Regulation of the renal angiotensin II receptor gene in acute unilateral ureteral obstruction

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Regulation of the renal angiotensin II receptor gene in acute unilateral ureteral obstruction. We have shown that acute (24-hr) unilateral ureteral obstruction (UUO) induces the genes encoding for renin, in juxtaglomerular apparatuses and in tubules, for angiotensin converting enzyme in vascular endothelial cells, and for angiotensinogen in perivascular fat. These molecular changes occur in temporal association to marked reductions in renal blood flow (RBF) and glomerular filtration rate (GFR), suggesting that angiotensin II (Ang II) is at least partly responsible for the renal vasoconstriction. We tested the hypothesis that down-regulation of the Ang II type-1 receptor (AT_1-R) gene occurs in UUO in response to Ang II, by examining the effects of an ACE inhibitor [lisinopril (Li), 5 mg/kg/day] and of the specific nonpeptidic AT_1-R blocker, losartan (Lo) (10 mg/kg/day). UUO or sham operated (which included manipulation but not obstruction of the ureter) rats (S) were studied. Northern blot analysis of the steady state concentration of AT_1-R mRNA corrected for GAPDH mRNA showed a marked decrease in receptor expression (-77%, $N = 4$, $P < 0.01$) in the obstructed kidney (UUO) compared to S; sham diminished gene expression modestly compared to the contralateral kidneys (C) of UUO. *In situ* hybridization for AT_1-R mRNA also showed diminished expression in UUO compared to C kidneys ($N = 4$). Treatment of UUO rats ($N = 4$) with Lo increased AT_1-R mRNA five times above the levels in UUO rats receiving vehicle; the increase induced by Li was 50% that of Lo; S ($N = 4$) and C ($N = 4$) did not change. Losartan, but not vehicle treatment increased RBF (sixfold) and GFR (fivefold) in the UUO kidneys. We conclude that UUO leads to down-regulation of AT_1-R mRNA, and that this effect is mediated by Ang II, which is also responsible for a major component of the renal hemodynamic changes. Some function of UUO or ureteral manipulation, possibly stretch or nerve stimulation, differentially regulates the genes encoding for the renin-angiotensin system in the kidney.

Acute (24 hr) unilateral ureteral obstruction (UUO) leads to a progressive increase in renal vascular resistance (RVR) and a marked decrease in renal blood flow (RBF) and glomerular filtration rate (GFR) [1, 2]. Studies performed in the last decade have demonstrated a role of several potent vasoactive substances in the renal hemodynamic changes of UUO, including angiotensin (Ang) II [3], thromboxane A_2 [4], prostaglandins (PGE_2, PGI_2) [5, 6], bradykinin [7], atrial natriuretic peptide [8], and nitric oxide [9]. A large body of evidence, however, supports the concept that the renin angiotensin system (RAS) is the central modulator of the renal hemodynamic response to UUO [3, 10, 11].

We have recently reported that UUO leads to enhanced expression of RAS genes including those of renin (R), angiotensinogen (Ao) and angiotensin I-converting enzyme (ACE) in the obstructed kidney [11]. Furthermore, losartan, a non-peptidic Ang II type-1 receptor (AT_1-R) blocker, resulted in marked recovery of renal function after release of 24-hours UUO in awake rats [12]. These findings suggest that the RAS plays a fundamental role in UUO and that some component thereof (hemodynamic changes, stretch, pressure, obstruction to flow, etc.) induces transcriptional or post-transcriptional changes in RAS mRNAs. Evidence for increased production of Ang II in the obstructed kidney has been obtained in pigs with acute UUO and in rats with chronic UUO [10, 13]. Under conditions of increased local Ang II production, increased AT_1-R mRNA expression would lead to increased synthesis of receptor molecules and would contribute to increased sensitivity and responsiveness to the vasoconstrictor actions of this peptide, and worsen the decreased renal blood flow in UUO. Nevertheless, the effect of acute UUO on the renal expression of the AT_1-R gene is unknown. The present study was designed to examine the effect of UUO on AT_1-R mRNA and to determine the role of Ang II in the changes, if any, of the AT_1-R gene expression. In addition, we have examined the role Ang II on the renal hemodynamics changes of UUO by testing the effect of losartan.

Methods

Male Wistar rats weighing 150 to 200 g (Charles River Laboratories, Wilmington, Massachusetts, USA) were used for these experiments. The rats were housed individually in a room providing 12-hour light and dark cycles and were maintained on standard Purina rat pellets with free access to food and water at all times prior to the experiment. General anesthesia was induced by intraperitoneal injection of ketamine (100 mg/kg body wt) and xylazine(10 mg/kg body wt), and the left ureter was exposed through a small left flank incision and dissected from its retroperitoneal site. Complete occlusion of the ureter was performed by a 4-0 silk square knot ($N = 12$). The abdominal incision was closed and animals were allowed to recover prior to returning to their cages. Eight rats were sham operated: ureter exposed and manipulated (naked-eye) as in the experimental groups but not tied ($N = 4$); in one rat both sides

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Table 1. Renal hemodynamics in awake rats pretreated with vehicle or losartan for 5 days prior to UUO

	ml/min/kg body wt			
	GFR		ERPF	
	Vehicle (N = 4)	Losartan (N = 4)	Vehicle (N = 4)	Losartan (N = 4)
Obstructed kidney	0.63 ± 0.21	3.11 ± 0.20	2.41 ± 0.88	13.18 ± 0.23
P	< 0.001		< 0.001	
Control kidney	8.25 ± 0.21	8.34 ± 0.33	20.07 ± 0.42	20.26 ± 1.0
P	NS		NS	

Data are mean ± SEM. NS = not significant. The P values in the table refer to comparison between losartan and vehicle in either group.
P < 0.001 compared to control kidney of losartan group and to vehicle-treated control kidney.

were dissected (naked eye) while in another the procedure was done under the dissecting microscope and the utmost care taken to not injure the ureter in any way. This was also done bilaterally in another rat that had as its comparison a rat that underwent bilateral free-hand dissection (naked eye). Two others were ipsilateral controls (C) (rats operated, but ureters not manipulated). Four additional groups of four rats each were studied as follows: group A was sham operated; group B was also sham operated and received treatment with losartan, given by gavage (10 mg/kg/day) for five days prior to and including the day of surgery; group C received the converting enzyme inhibitor (CEI) lisinopril (5 mg/kg/day) by gavage for five days including the day of UUO; and group D received losartan as already described for group B before UUO. Four additional UUO rats were treated with losartan as described above and GFR, measured by inulin clearance, and effective renal plasma flow measured by PAH clearance as already described from this laboratory [11]. Another group of rats (N = 4) received an equivalent amount of vehicle by gavage for the same period of time. Losartan was a gift of Dr. Ronald D. Smith, E.I. DuPont Merck Pharmaceutical (Wilmington, Delaware, USA) and lisinopril was a gift of Merck Sharpe and Dohme (West Point, Pennsylvania, USA).

At the end of the experiments (24 hrs) the rats were sacrificed with an anesthetic overdose; the kidneys were immediately removed from all rats except those used for the renal hemodynamic studies and snap-frozen in liquid nitrogen. Frozen kidneys were chiseled into small pieces, which were placed in guanidium isothiocyanate (GTC), and homogenized immediately. Samples were then stored at 4°C until analysis.

RNA extraction and Northern hybridization analysis

Total RNA was extracted by the guanidine thiocyanate method, as previously described [14]. A total of 20 μg RNA was run on 1% agarose gels using formaldehyde. RNA was transferred by capillary blotting using standard procedures and Immobilon-N membranes (charge-modified PVDF transfer membranes, Millipore Corp., Bedford, Massachusetts, USA). Blots were washed in 6× SSC for 5 to 10 minutes, then baked under vacuum for one hour at 80°C.

Table 2. Plasma renin activity measurements

Experimental groups	PRA *ngAI/ml/hr*	N	P value
Control (C)	1.3 ± 0.3	5	
Sham (S)	1.8 ± 0.3 P = 0.03 vs. (S + Lo)	5	NS
Sham + vehicle (S + V)	2.0 ± 0.4 NS vs. (S)	4	NS
Sham + losartan (S + Lo)	13.4 ± 2.8 P = 0.008 vs. (S + V)	4	0.002
UUO + vehicle (UO + V)	3.5 ± 0.8 NS vs. (S + V) & vs. (S)	4	0.04
UUO + losartan (UO + Lo)	77.0 ± 7.0 P = 0.01 vs. (UO + V) & vs. (S + Lo)	7	<0.001
UUO + lisinopril (UO + Li)	78.0 ± 9.0 P = 0.01 vs. (UO + V) & vs. (S + Lo)	7	<0.001

Data are mean ± SEM. The P value column refers to comparisons between the respective groups and control (C).
In the **Methods** section, (S + V), (S + Lo), (UO + V), and (UO + Lo) are referred to as Groups A, B, C, and D, respectively.

Preparation of riboprobes

AT_1 receptor cDNA was originally cloned in the pCDM8 expression vector by Murphy et al [15]. The AT_1 receptor cDNA, a gift of Drs. T.J. Murphy and R. W. Alexander, was subcloned into pGM4 vector, transcribed and labeled using ^{32}P-UTP (Amershan, Arlington Heights, Illinois, USA), specific activity 1200 Ci/mmol. A full-length antisense transcript was used for hybridization.

Labeled riboprobes were prepared using 0.5 to 1 μg linearized plasmid and α-^{32}P(UTP) or ^{35}S(UTP) for Northern blots or *in situ* hybridization, respectively. Purified riboprobes were stored in 70% ethanol at −20°C.

Prehybridization and hybridization

Blots were prehybridized for six hours at 60°C with hybridization buffer [50% formamide, 5× SSC (1× SSC:0.15 M NaCL, 0.015 M Na citrate, pH 7), 8× Denhardt's solution, 1 mM EDTA, 0.1% sodium pyrophosphate, and 0.5% SDS, 200 μg/ml denatured salmon sperm DNA]. A mixture of 0.5 to 1.0 × 10^7 CPM probe in 0.2 ml hybridization buffer was heated to 85°C for five minutes, then added to 4 ml hybridization buffer and 1 ml 50% dextran sulfate and thoroughly mixed. Hybridization was carried out overnight (16 to 18 hr) at 60°C. Membranes were washed for 30 minutes first in 2× SSC + 0.2% SDS at 65°C and finally in 0.2× SSC + 0.2% SDS at 70°C. Dry blots were exposed overnight with intensifying screen on Kodak film xRP-5.

Membrane reprobing

For reprobing with GAPDH cRNA, membranes that had been previously hybridized were washed in 0.1 N Na OH for 30 minutes at room temperature. A Geiger counter check was carried out until there was no detectable radioactivity. Then the

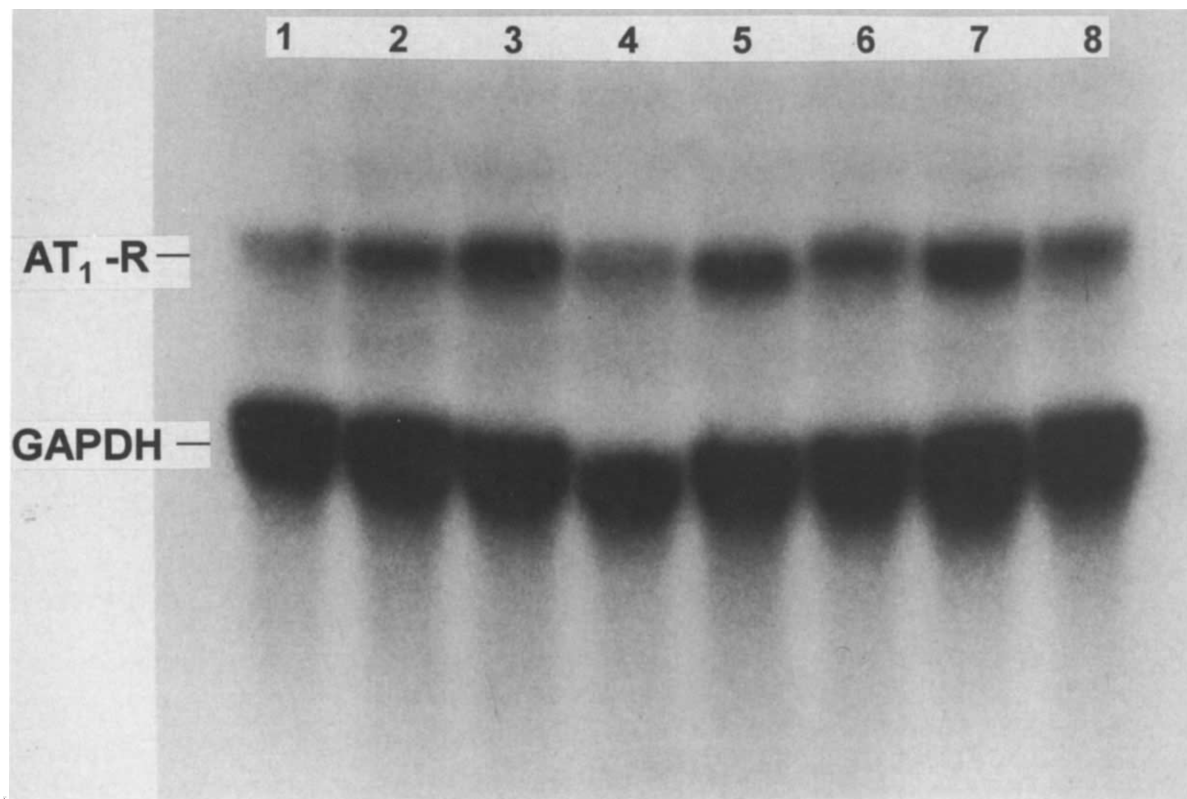

Fig. 1. *Representative autoradiograph of Northern blot (20 μg RNA/lane).* Blots were hybridized with AT_1-R cRNA and GAPDH cRNA (lower bands). Lane 1: RNA isolated from a sham kidney whose ureter was grossly dissected (dissection and ligature threading without tying); Lane 2: RNA isolated from a sham kidney in which microscopic dissection was used to dissect the ureter, otherwise done as described above. The least "traumatic" dissection led to less down-regulation of the message. Lanes 3, 5, and 7 are the control (right) kidney, while lanes 4, 6, and 8 are the obstructed (left) kidney of three (each on a different rat) UUO experiments.

membrane was neutralized by immersion in 0.02 M Tris-HCl, pH 8 + 0.1× SSC + 0.5% SDS for 45 to 60 minutes. The membranes were then ready to be reprobed. Prehybridization and hybridization for GAPDH mRNA were performed as described above.

Densitometry analysis and mRNA quantification

The autoradiographs were scanned using the Image Quant™ computing densitometer (Molecular Dynamics, Sunnyvale, California, USA). Results are expressed as relative density. We used the classical approach of housekeeping gene analysis and ethidium bromide stain to ascertain equal loading and specificity of response. We have previously shown that UUO does not alter GAPDH mRNA expresssion [11]. Relative densitometry was determined by the signal density of each RNA sample hybridized to the AT_1-R riboprobe divided by that hybridized to the GAPDH probe.

In situ hybridization

The AT_1 receptor probe was a full-length antisense cRNA labeled with ^{35}S-UTP (Amersham), specific activity 1200 Ci/mmol. The experiments were controlled by hybridizing serial sections with the same cRNA probe transcribed in the sense orientation. *In situ* hybridization was performed as previously described [16, 17]. Briefly, cryosections were prepared with paraformaldehyde, proteinase K (Sigma Chemical, St. Louis, Missouri, USA), and prehybridized in 100 ml hybridization buffer (50% formamide, 0.3 M NaCL, 20 mM Tris pH 8.0, 5 mM EDTA, 0.02% polyvinylpyrrolidine, 0.02% Ficoll, 0.02% bovine serum albumin, 10% dextran sulfate, and 10 mM dithiothreitol) at 42°C. The hybridizations were performed using 600,000 cpm of ^{35}S-riboprobe at 55°C. After hybridization, the sections were washed with 2× SSC (1× SSC = 150 mM NaCl, 15 mM Na citrate, pH 7.0) with 10 mM β-mercaptoethanol and 1 mM EDTA, treated with RNase (Sigma), again washed in the same buffer, followed by a high stringency wash in 0.1× SSC with 10 mM β-mercaptoethanol and 1 mM EDTA, at 52°C. The slides were then washed in 0.5× SSC and dehydrated in graded alcohols containing 0.3 M NH_4Ac, dipped in Kodak NTB2 nuclear emulsion, dried in the dark at 4°C with desiccant for two to eight weeks, and developed at 15°C. Slides were counterstained with hematoxylin and eosin.

Analytical procedures

Plasma renin activity (PRA) was measured by determinig the amount of angiotensin I generated during one hour of incubation at 37°C in the presence of ACE inhibitors and angiotensinases by radioimmunoassay as previously reported [18].

Statistical methods

Values are expressed as mean ± SEM. One way analysis of variance (ANOVA) was used for comparisons between groups.

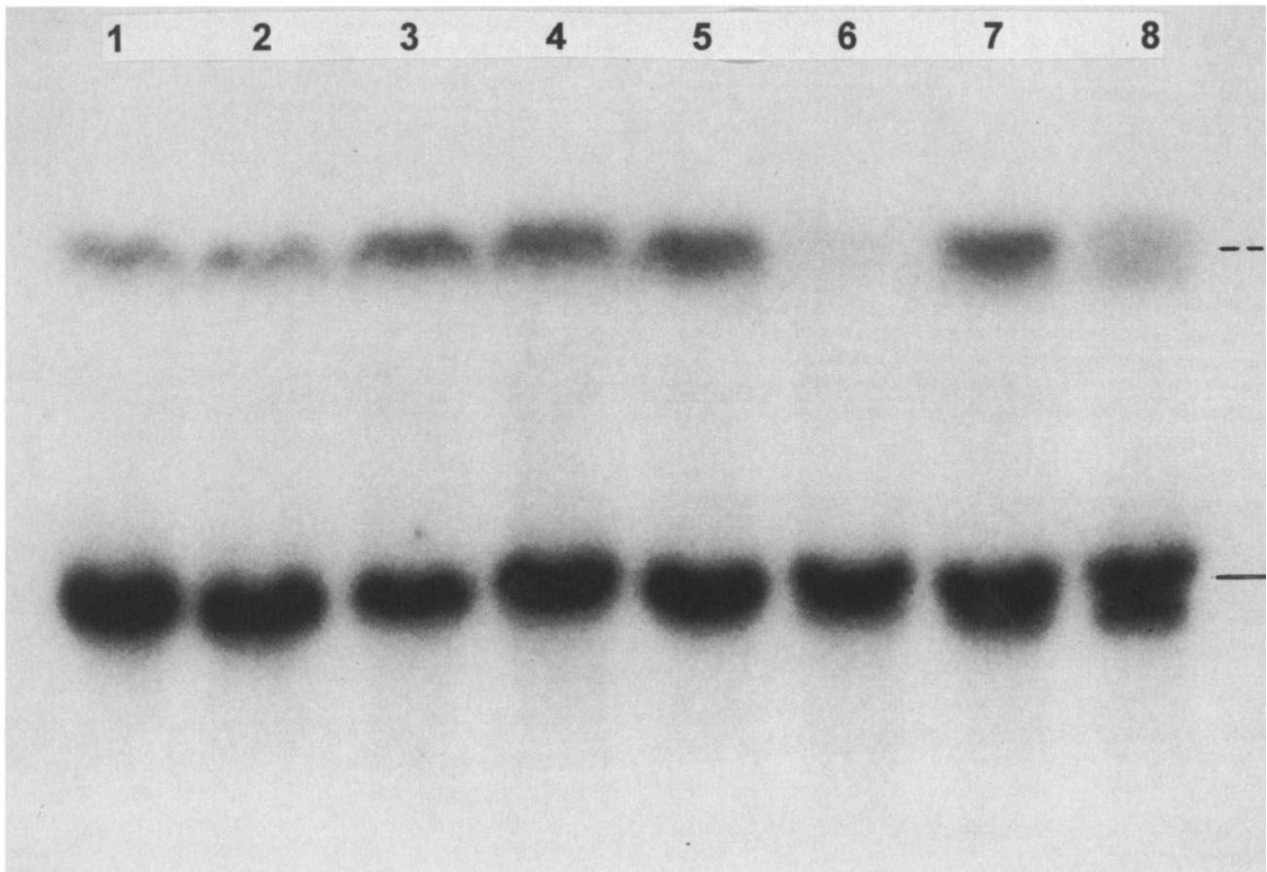

Fig. 2. *Conditions and gel set-up as in Fig. 1.* The upper broken line represents AT_1-R mRNA, the lower solid line GAPDH mRNA. The first two lanes are right and left kidney of grossly bilaterally dissected ureters; the intensity of the bands is equal on both sides, but less than those of the next two lanes (3 and 4) which are equal to each other and represent RNA from each kidney of one rat after careful (microscopic surgery) dissection (shams; see text for further detail). These are indistinguishable from the right kidney of the UUO (lanes 5 and 7). Lanes 6 and 8 are the obstructed kidney (left). It is amply clear that UUO led to down-regulation of the receptor.

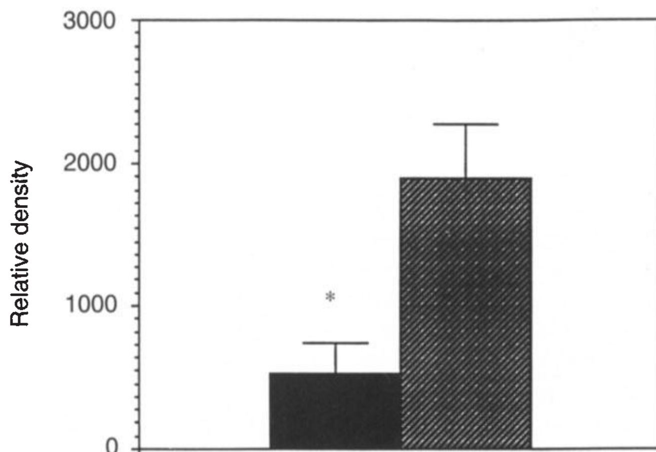

Fig. 3. *Relative densitometry of Northern blots of RNA from obstructed (left, ■) and control (right, ▨) kidneys from 8 rats hybridized with AT_1 cRNA.* The AT_1-R signals have been factored by that of GAPDH mRNA to which an arbitrary value of one was assigned. AT_1-R in the obstructed kidney was down-regulated by ~75% ($P < 0.01$).

When each animal was used as its own control (left vs. right kidney) significance was obtained by paired *t*-test. A value of $P < 0.05$ was accepted as significant.

Results

Hemodynamics and plasma renin activity (PRA)

Table 1 shows the results of losartan treatment on effective renal plasma flow (RPF) and GFR in each kidney of the rats studied ($N = 4$). ERPF and GFR improved ~fivefold from their lowest values. GFR in the obstructed kidney rose from a value of 8% to 37% of the control kidney GFR. ERPF in the obstructed kidney rose from 12% to a value of 66% of the control kidney ERPF. Values of GFR and ERPF before and after losartan were significantly different; both values remained significantly different from control even after losartan treatment. These results confirm our previous studies using losartan in UUO [12]. We have also already reported similar results with lisinopril [19]. Blood pressure fell to similar levels in rats treated with losartan or lisinopril (80 vs. 88 mm Hg; P = NS), indicating that the doses of these drugs were comparable in both groups.

Changes in PRA (Table 2) were not significant when shams were compared to control rats. UUO increased PRA as compared to control animals ($P = 0.04$); as expected, interruption of the feedback between Ang II and renin secretion by losartan and lisinopril markedly increased PRA in sham (not shown for Li) and UUO rats. In sham rats, losartan increased PRA eight times over the sham; moreover, PRA was 45 times greater in

Table 3. Densitometric analysis of AT_1-R mRNA autoradiogram signals

Experimental groups ($N = 4$)	Relative density		L/R	P values		
	Left	Right		L vs. R	L vs. C	R vs. C
Control (C)	904 ± 119	866 ± 238	1.0	NS		
Sahm (S)	535 ± 61	889 ± 77	0.6	0.01	0.01	NS
Sham + losartan (S + Lo)	1112 ± 157 $P = 0.01$ vs. S	879 ± 102[b]	1.3	NS	NS	NS
UUO (UO) + V	210 ± 95 $P = 0.04$ vs. S	903 ± 216[b]	0.2	0.03	0.01	NS
UUO + losartan (UO + Lo)	1058 ± 140[a] $P = 0.01$ vs. UO	922 ± 37[b]	1.1	NS	NS	NS
UUO + lisinopril (UO + Li)	523 ± 43[a] $P = 0.02$ vs. UO $P = 0.01$ vs. UO + Lo	753 ± 69[b]	0.7	0.04	0.02	NS

Data are mean ± SEM. Abbreviations are: L, left kidney; R, right kidney.
[a] NS vs. (S + Lo)
[b] NS vs. S

losartan-treated UUO rats than the average PRA value of all sham and control rats. PRA parallels the renin secretory rate, which is partly dependent of RPF; therefore, since ERPF rose only fivefold, the marked increase in PRA in losartan treated UUO rats indicates a major intrarenal effect of the inhibitor to reverse the renin secretion suppressing action of Ang II.

Northern analysis and mRNA quantification

Compared to the contralateral kidney and control ipsilateral kidneys (as described above), the receptor mRNA signal was markedly decreased in the obstructed kidney (Figs. 1 and 2). Densitometry analysis (Fig. 3) revealed the signal to be significantly lower (−77%) in UUO than in controls. There was no statistical difference in receptor mRNA expression between the contralateral and ipsilateral control kidneys (Table 3). Sham operation, done by free-hand (naked eye) dissection of the left ureter from its retroperitroneal bed, consistently led to a decrease in the expression of the AT_1-R mRNA in the ipsilateral kidney compared to the contralateral kidney and to control ipsilateral kidneys (operated rats whose left ureter was untouched; Table 3). AT_1-R mRNA expression was decreased by ~40% in the sham kidney compared to the contralateral or ipsilateral controls. The difference between sham and contralateral and ipsilateral controls was significant and accounted for 25 to 30% of the reduction in steady state AT_1-R mRNA concentration. When free-hand unilateral and bilateral dissection was compared to dissecting microscope-prepared shams (Figs. 1 and 2) free-hand dissection led to reduced expression of the message while, when carefull ureteral dissection was performed the signal from that kidney could not be distinguished from untouched kidneys.

The most striking finding in our experiments is the fact that both lisinopril and losartan caused a marked increase in the expression of AT_1-R mRNA in the kidney with complete ureteral obstruction as well as in the kidneys whose ureters were manipulated (Table 3). By contrast, losartan did not change the receptor expression in control and contralateral kidneys. The effects of lisinopril were only examined in UUO, but the converting enzyme inhibitor consistently led to no change in the contralateral kidney as compared to controls. Conversely, it doubled—to normal levels—the AT_1-R mRNA concentration in the obstructed kidney. The effect of losartan was more dramatic than that of lisinopril. In all instances, losartan Increased steady state AT_1-R mRNA concentrations from two to five times the values in the untreated sham and the untreated UUO rats. Moreover, losartan raised receptor mRNA values two to three times higher than lisinopril.

In situ hybridization studies

In situ hybridization showed localization of AT_1-R mRNA signals in glomeruli and tubules that were more prominent in contralateral compared to obstructed kidneys. An obstructed kidney exhibiting sparse signals in both tubules and glomeruli is shown in Figure 4. Tissue sections from control and sham kidneys did not differ from each other nor from those of the contralateral kidneys of rats with UUO (data not shown). While it is not possible to precisely quantitate the degree of hybridization signal, these data are in general agreement with the clear-cut down-regulation of AT_1-R mRNA in obstructed kidneys without change in expression of the receptor gene in the contralateral kidney observed by Northern analysis. Sense AT_1-R cRNA did not lead to hybridization signals in any of the tissues tested (data not shown).

Discussion

The present study shows that 24-hour UUO is associated with down-regulation of AT_1-R gene expression when the ureter is obstructed or manipulated without careful care not to traumatize the area dissected. Careful dissection (under a microscope) of the ureter did not down-regulate the message, which was also unaltered in control or contralateral kidneys. Suppression of AT_1-R mRNA expression in kidneys with either manipulated (grossly dissected) or obstructed kidneys suggests that mechanical, humoral, or perhaps neural stimuli arising from the ureter may operate in the regulation of kidney AT_1-R mRNA. The fact, however, that obstruction resulted in a greater inhibition of steady state mRNA than ureteral manipulation alone, and that the difference in this measurement between kidneys with manipulated and obstructed ureters was significant, is indirect evidence that these two stimuli are capable, possibly by different mechanisms, of suppressing gene transcription. While mechanical changes such as alterations in urine flow patterns, increased pressure and/or stretch of the ureter or of the intrarenal tubule system, may play a role in the regulation of AT_1-R

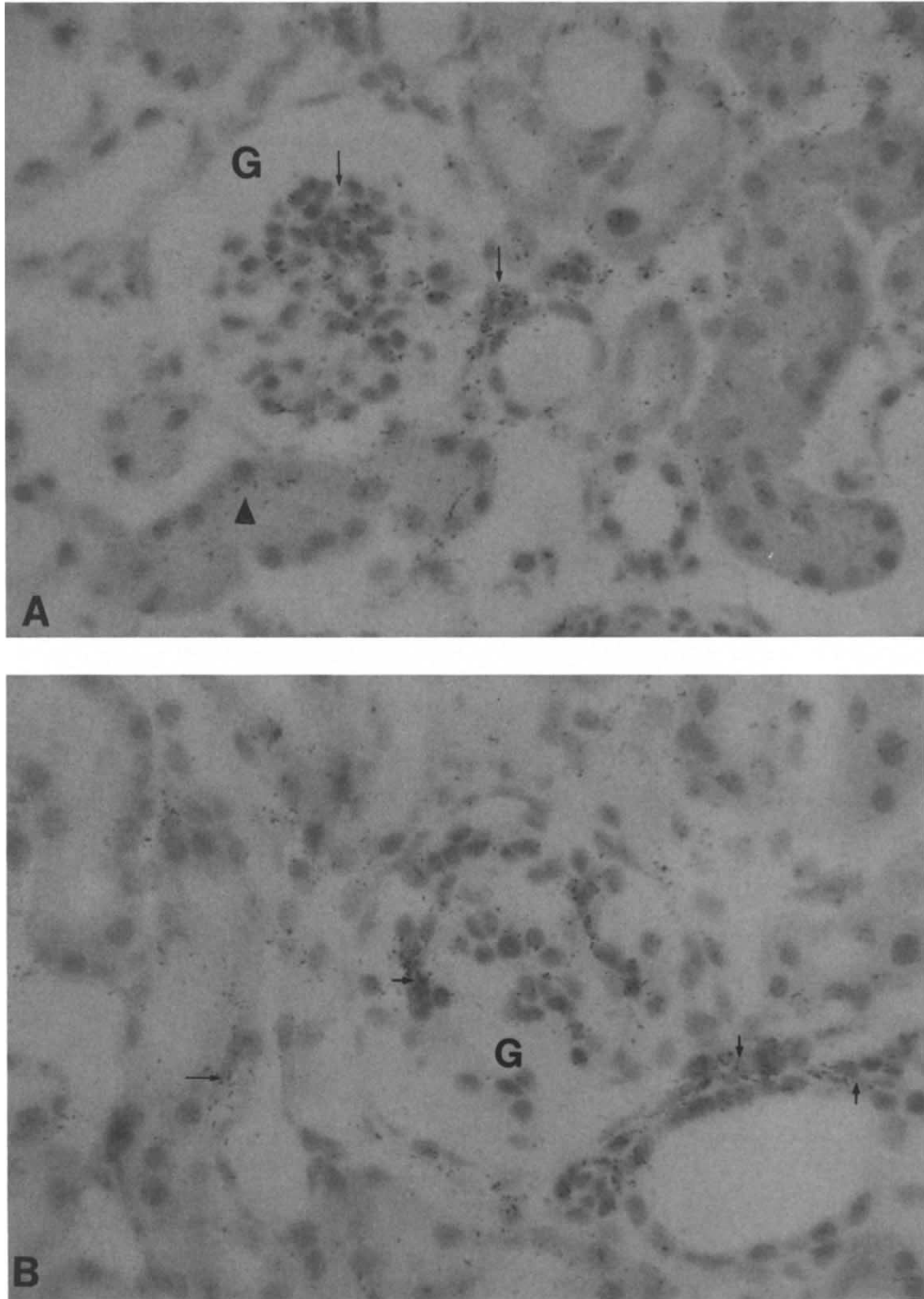

Fig. 4. *In situ hybridization with a whole length AT_1 cRNA of control (right;* **A**) *and UUO (left;* **B**) *kidney section.* The arrowhead (A) points to tubule hybridization which is reduced in B, although some areas in the latter show clumps of signal. Vertical arrows indicate glomerular and macula densa signals. In B (UUO), the sparse nature of the receptor mRNA can be noted; the abundance of the message was calculated to be twice as high in the contralateral kidney. The long horizontal arrow points to message in tubules. The short horizontal arrow points to AT_1 mRNA expression in the mesangium. The two vertical arrows indicate gene expression along the afferent arteriole and the macula densa (magnification ×250).

mRNA expression, the results obtained in animals treated with losartan or lisinopril suggest that, whatever the initiating step might be, a hormonal pathway or mechanism is involved. Moreover, it is clear from our results with losartan and the CEI lisinopril that Ang II is a critical hormone in the renal regulation of AT_1-R mRNA during obstruction and/or manipulation of the ureter. It is of interest that Paxton et al [20] found marked AT_1-R immunoreactivity in the smooth muscle cells of the ureter and renal pelvis of both Wistar and Sprague-Dawley rats, suggesting a role for Ang II in the contractility of these structures. It is conceivable that the presence of these Ang II responsive cells may be related to the changes in receptor gene expression in the kidney after manipulation or obstruction of the ureter, the nature of which remains to be uncovered. Kopp and her associates [21, 22] have shown that acute UUO, albeit of short duration (30 to 40 min), increases ipsilateral afferent renal nerve activity and decreases contralateral efferent renal nerve activity. The sensory receptors activated by these interventions have been localized to the renal pelvis [21]. Furthermore, inhibitory reno-renal reflexes in response to mechanical and chemical activation of these receptors are prostaglandin-mediated [22]. It is well known that a close interrelationship exists among prostaglandins, renin secretion and thus the response to Ang II in UUO [23, 24]. The full significance, however, of the renal nerves and of mechanical alterations in ureteral and pelvic functions in the regulation of the renal expression of AT_1-R mRNA remains to be clarified. Nevertheless, in view of the fact that ureteral manipulation reduced ipsilateral renal AT_1-R gene expression, we suggest the possibility that afferent renal nerve activity serves a role in the regulation of this and perhaps other genes in the RAS cascade. Inasmuch as 24-hour UUO may be compared to 30 minute UUO, and in view of the lack of change in AT_1-R mRNA, diminished efferent renal nerve activity in the contralateral kidney does not seem to affect regulation of this gene.

A recent study by El-Dahr and collaborators [10] has shown that chronic UUO increases ipsilateral renin mRNA, but leads to down-regulation of kallikrein gene expression, increased plasma ACE-kininase II and reduced immunoreactive kallikrein content in the ipsilateral kidney. In addition, we have shown an increase in both renin and ACE mRNAs expression in acute UUO [11]. These results suggest a role for bradykinin in the intrarenal regulation of RAS genes. Lisinopril, which by inhibition of ACE-kininase II should increase local bradykinin production, was less effective in restoring AT_1-R mRNA than losartan, which does not alter bradykinin production [25]. This may be indicative of an AT_1-R gene suppressive effect of bradykinin and further suggests the possibility of at least a permissive role of the kallikrein system in the intrarenal regulation of the RAS.

Reduced steady state levels of AT_1-R mRNA in acute UUO would lead to decreased transcription rate, diminished synthesis of receptor molecules, and diminished response to locally generated Ang II. Our findings of a reduced renal receptor mRNA concentration are in keeping with the elevated plasma renin activity (and presumably Ang II levels) in the early phases of UUO since Ang II would lead to down-regulation of its receptor in the kidney [26, 27]. Receptor down-regulation would be potentiated by the increased intrarenal Ang II production which has been shown to occur in response to UUO [10, 13]. The restoration and enhancement of receptor gene expression, the partial but significant hemodynamic recovery and the dramatic increase in PRA induced by both losartan and lisinopril treatment in UUO, are in keeping with the proposal of the principal role of Ang II in down-regulation of receptor gene expression and receptor synthesis.

In a previous study, we found that UUO leads to an early rise in plasma renin activity and to marked elevations in the steady state concentration of renin mRNA as determined by Northern analysis [11]. In addition, it was shown that vascular endothelium and perivascular fat exhibited increased expression of ACE and angiotensinogen mRNA, respectively. Thus the crucial first 24 hours of obstruction are accompanied by increased expression of the mRNAs for renin, angiotensinogen, angiotensin converting enzyme and, possibly, increased angiotensin II production which eventually down-regulates the receptor mRNA. These findings indicate that the intrarenal RAS is regulated by some component of the obstructive process and, in view of the response to losartan, that Ang II contributes in a major way to the hemodynamic alterations observed.

In summary, we conclude that acute (24 hr) UUO leads to increased intrarenal production of Ang II which is responsible for the hemodynamic changes and down-regulation of the AT_1-R mRNA in the obstructed kidney. The increased Ang II synthesis results from enhanced expression of renin, angiotensinogen and angiotensin I-converting enzyme mRNAs in UUO [11]. Some function of UUO, possibly stretch or pressure, differentially regulates the genes encoding for the renin-angiotensin system.

Acknowledgments

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