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Renal mRNA Expression of Renin, AT_1 Receptor, TGF-β1 and Fibronectin in Obstructive Nephropathy

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The present study was designed to quantify the alterations of renal renin, angiotensin type I receptor (AT_1), TGF-β1, and fibronectin gene expression in rats with unilateral ureteral obstruction (UUO). We also investigated the change of AT_1 density during UUO. Reverse transcription-polymerase chain reaction (RT-PCR) technique and receptor binding assay were used to detect mRNA expression and receptor density, respectively. At one day after UUO, renin mRNA level of the obstructed kidneys was decreased transiently and then subsequently increased to the level of sham kidneys. In the contralateral kidneys of the same rats, on the contrary, renin mRNA level was gradually decreased. Then, at 9 days after UUO, it was significantly lower than that of sham kidneys. The expressions of both AT_1 subtypes, called AT_{1A} and AT_{1B}, mRNAs did not change at any time. UUO led to a significant decrease in AT_1 density in the obstructed kidneys compared with the sham kidneys at 1 and 3 days (66 ± 11.6% ($p<0.05$) and 73 ± 4.0% ($p<0.01$), respectively). Thereafter, AT_1 density was gradually increased and at 9 days it showed a marked elevation in the obstructed kidneys compared to the sham kidneys. In contrast, in the contralateral kidneys AT_1 density was significantly reduced from 3 to 9 days after UUO. The TGF-β1 mRNA level of the obstructed kidneys was unexpectedly decreased at 6 days after UUO. Then, at 9 days it was followed by a significant increase in the obstructed kidneys, whereas it showed an obvious decrease in the contralateral kidneys. In addition, fibronectin mRNA level was also significantly increased in the obstructed kidneys after UUO compared to the sham or the contralateral kidneys of the same rats. These results suggest a differential regulation of renal renin, AT_1 receptor, TGF-β1 and fibronectin mRNA levels at different stages of UUO.

Key Words: Renin, AT_1 receptor, TGF-β1, Fibronectin, RT-PCR, Unilateral ureteral obstruction

INTRODUCTION

The renin-angiotensin system (RAS) has long been considered a circulating endocrine system. In the last decade, however, the concept has evolved that organ-specific or local RAS, with all the components necessary for generating angiotensin II (ANG II), may exist and operate independently or in concert with the systemic RAS (Inagami, 1982; Ingelfinger & Dzau, 1991). Such tissue-specific RASs have been demonstrated, for example, in the brain, heart, testis, and kidney.

Using new ANG II antagonists, two major subtypes of ANG II receptors, namely type I (AT_1) and type 2 (AT_2), have been identified. It is well known that most of the hemodynamic manifestations of ANG II are a result of AT_1 receptor activation (Timmermans et al, 1993; Lee et al, 1995; Yang et al, 1996). Recent studies have reported the preponderance of AT_1 receptors and their distribution in the mammalian kidney. In the rat kidney AT_1 receptors are abundant in proximal tubules, the thick ascending limb of Henle and glomeruli (Sechi et al, 1992; Meister et al, 1993). The human, rabbit, rat and mouse AT_1 receptors are cloned and they share sub-

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stantial homology (Edwards & Aiyar, 1993). Moreover, the genome of rat and mouse contain two subtypes of AT_1 receptor. These two subtypes, called AT_{1A} and AT_{1B}, have highly homologous sequences, and similar binding and functional characteristics (Edwards & Aiyar, 1993).

ANG II has several biological effects in the kidney including vasoconstriction, modulation of tubular reabsorption, and stimulation of renal growth (Wolf & Neilson, 1993). Renal interstitial fibrosis is a common consequence of chronic progressive renal diseases. Fibrogenesis develops as a result of an imbalance between extracellular matrix (ECM) synthesis, matrix deposition and matrix degradation (Kuncio et al, 1991). TGF-β1 has been shown to stimulate the synthesis of ECM proteins, to inhibit matrix degradation through an increase in the activity of protease inhibitors and a decrease in proteases, and to stimulate synthesis of receptors for ECM proteins (Roberts & Sporn, 1988; Kuncio et al, 1991). ANG II has been shown to modulate proliferation of vascular smooth muscle cells by stimulating TGF-β1 synthesis through AT_1 (Gibbons et al, 1992; Stouffer & Owens, 1992). Recently, several groups suggest ANG II may play a role in the progression of tubulointerstitial fibrosis through activation of TGF-β1 (Kuncio et al, 1991; Wolf & Neilson, 1993). In addition, evidence exists that ANG II is partly responsible for the adaptive changes in glomerular hemodynamics after loss of functioning nephrons (Brenner, 1989; Wolf & Neilson, 1991). Some reports denote ANG II receptor antagonist and ACE inhibitors effectively attenuated the progression of tubulointerstitial fibrosis in obstructive nephropathy (Kaneto et al, 1994; Ishidoya et al, 1995). They suggest that the RAS has a major role in the pathogenesis of the tubulointerstitial fibrosis in obstructive nephropathy. Finally, Pimentel (1995) and collaborators suggest that early changes in the regulation of the genes coding for the RAS is a fundamental determinant of the functional manifestations of unilateral ureteral obstruction (UUO). Nevertheless, some discrepancies exist about the regulation of renal RAS and TGF-β1 gene expression at different stages of UUO.

Thus, the aims of this study were to examine the changes in the renal expression of renin, AT_1 receptor, TGF-β1 and fibronectin at different time intervals after UUO. To evaluate the compensatory response of the contralateral kidneys of the same rats, the changes in the obstructed kidneys were compared with those in the contralateral kidneys as well as those in the sham kidneys. To minimize the inter-experimental variations, this study was performed to examine the effect of UUO on gene expression on the renal RAS components, TGF-β1 and fibronectin from a single set of animals.

METHODS

Experimental protocol

Male Sprague-Dawley rats, weighing 230 to 280 g, were used. Under pentobarbital sodium anesthesia (40 mg/Kg) the left ureter was ligated with 4-0 silk. Sham-operated rats had their ureters manipulated but not ligated. Rats were sacrificed at 1, 3, 6, and 9 days after UUO. Sham-operated rats and unoperated rats were used to obtain "control" kidneys. The kidneys were removed quickly from each rat and frozen in liquid nitrogen, and then kept in deep freezer (-70℃).

Total RNA isolation

Total RNA was isolated according to the method described by Chomczynski et al (1987) with slight modifications. Frozen tissue was ground to fine powder using the pestle and mortar in the presence of liquid nitrogen, and the denaturation solution (4 M guanidium thiocyanate, 25 mM sodium citrate, pH 7, 0.5% sarcosyl, 0.2 M β-mercaptoethanol) was added (10 ml/g tissue). Homogenate was transferred to a new tube and 1/10 volume of 2 M sodium citrate (pH 4.0), 1 volume of water-saturated phenol and 1/5 volume of chloroform-isoamylalcohol (49 : 1) were sequentially added with gentle mixing after the addition of each reagent. The tube was kept on ice for 15 min and was centrifuged at 12,000 rpm for 15 min at 4°C. The upper aqueous phase was transferred to a new tube and reextracted with an equal volume of chloroform- isoamylalcohol (49 : 1). The supernatant was transferred to a new tube and mixed with an 2.5 volume of absolute ethanol, and then placed at −20°C for at least 1hr. RNA was precipitated by centrifugation at 12,000 rpm for 15 min at 4°C. The resulting pellet was washed in 75% ethanol. After drying in the air, RNA pellet was redissolved in formamide and kept at −20°C until needed. The concentration and purity of RNA was determined by measuring the absorbance at 260 nm and 280 nm.

Table 1. Primer sequences for AT_{1A}, AT_{1B}, renin, TGF-β1, fibronectin and GAPDH used in this study

Gene	Primer sequence	Product size	Reference
AT_{1A}			
(S)	GCCTGCAAGTGAAGTGATTT	388 bp	Kitami et al [1992]
(AS)	TTTAACAGTGGCTTTGCTCC		
AT_{1B}			
(S)	GCACACTGGCAATGTAATGC	204 bp	Kitami et al [1992]
(AS)	GTTGAACAGAAGAAGTGACC		
renin			
(S)	AGGCAGTGACCCTCAACATTACCAG	362 bp	Burnham et al [1987]
(AS)	CCAGTATGCACAGGTCATCGTTCCT		
TGF-β1			
(S)	AATACGTCAGACATTCGGGAAGCA	498 bp	Qian et al [1990]
(AS)	ACACATGCCGTCGACATGTAACTG		
fibronectin			
(S)	GTCAGCGTCTATGCTCAGAA	370 bp	Wang et al [1991]
(AS)	CTGGTTGGACTTCAAGTGAGT	102 bp	
GAPDH			
(S)	ATCAAATGGGGTGATGCTGGTGCTG	504 bp	Tso et al [1985]
(AS)	CAGGTTTCTCCAGGCGGCATGTCAG		

Reverse transcription PCR (RT-PCR)

The nucleotide sequence of the primers and references are presented in Table 1. Fibronectin mRNA is alternatively spliced to yield several isoforms of fibronectin (Norton & Hynes, 1987). Among several splicing alternates of fibronectin mRNA the EDIIIA region has been shown to be regulated by TGF-β. Thus, the sequence of the fibronectin primers is designed to flank the EDIIIA region of the rat gene (Wang et al, 1991). Total RNA (20 μg) was primed with oligo(dT) primers, and first strand cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase in a 30 μl of reaction volume for 90 min at 37°C. The reaction mixture was heated for 5 min at 95°C to degrade template RNA and enzyme. In brief, 1 or 2 μl of each cDNA was amplified in a total volume of 25 μl containing PCR buffer (10 X polymerase standard buffer plus 5mM $MgCl_2$ and 0.1% formamide), 200 μM of each dNTP, 20 pmole of each primers and 1.25U of Taq DNA polymerase (Promega, USA). PCR was carried out using a Perkin-Elmer Cetus DNA Thermal Cycler as follows: 94°C for 45 sec, 54°C for 45 sec, 72°C for 1 min. PCR conditions are the same for all genes examined in this study except PCR cycles and the amount of template that were carefully selected according to the relative abundance of mRNAs in the kidney. After the end of PCR, one fifth of reaction mixture was separated on a 1.2% of agarose gel containing 0.5 μg/ml of ethidium bromide.

Densitometric analysis

The polaroid film was scanned using an Epson (ES-600) scanner with a resolution of 70 DPI (dot per inch) and 256 gray scale unit. In order to achieve a linear response curve, the brightness and the contrast was finely tuned to the point where the gray scale unit of the background were within 20-40 and that of the darkest band was between 200-240. The resulting image was analysed from NIH-Image analysis program (written by Wayne Rasband, NIH, Bethesda,

MD). Using a selection box, the gray scale unit of various background areas were measured and subtracted from each band. Then, the mean density and total area of the band were measured. The scale of each band was expressed by multiplying the mean density by the total area of the band. The resulting scale was used to quantify each band.

Receptor binding assay

The receptor binding assay was performed according to the method described by Ambuhl et al (1995). The kidney was homogenized in 10 ml ice-cold Tris-HCl buffer (50 mM tris, 1 mM EDTA, 150 mM NaCl, pH 7.2) and centrifuged at 2000 g for 10 min. The pellet was discarded and the supernatant was re-centrifuged at 50,000 g at 4°C for 20 min. The resulting pellet was resuspended in 4ml Tris-HCl buffer, homogenized and assayed for its protein content with the Bradford method. One hundred μg membrane proteins were incubated with 150 pM of the radiolabeled ANG II antagonist ^{125}I-Sar^{1},Ile^{8}-ANG II in the presence or absence of 1 μM cold ANG II, losartan or PD 123319 to determine the nonspecific, total, AT_1 and AT_2 binding, respectively. The membrane proteins were incubated in duplicate with the receptor ligands in 500 ml of incubation medium containing Tris-HCl buffer, 1 mM bacitracin, 10 mM O-phenanthroline and 0.1% bovine serum albumin for 60 min at room temperature. At the end of the incubation procedure, membrane-bound ligand was collected on filter paper (Whatman, GF-B, USA) using a cell harvester (Brandell, USA) and radioactivity was determined with a gamma-counter (Cobra II, Packard, USA).

Statistical analysis

All data were presented as mean±SE. Unpaired *t*-test was used for the comparison of means of different groups. Differences were considered significant if $p < 0.05$.

RESULTS

There was no difference in the body weight, measured before decapitation, of sham-operated and UUO groups at different stages after UUO (data not shown). At 9 days after UUO, obstructed kidneys weighed significantly more than sham kidneys (data not shown). All the parameters estimated in this study did not show any significant changes in sham groups at any time, and moreover they were similar to the basal values of unoperated control groups. Therefore, we delineated concisely all of the results acquired in this study comparing with average values of the whole sham group.

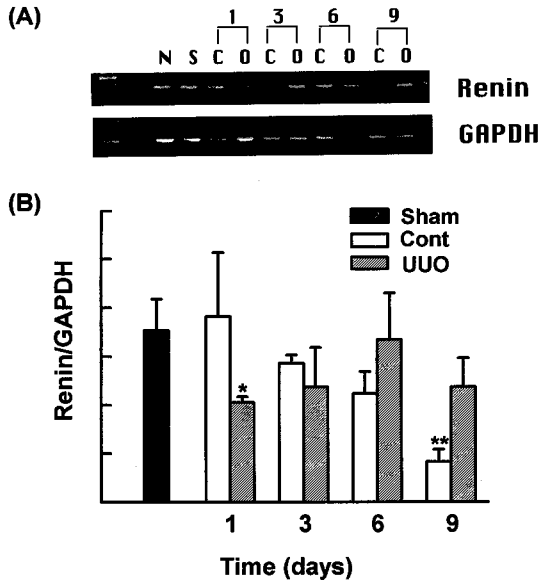

Fig. 1. Ethidium bromide-stained gel of PCR products for renin in the kidneys after unilateral ureteral obstruction (UUO) (A). The abbreviations are: N (unoperated), S (sham-operated), C (contralateral) and O (obstructed). Figures 1, 3, 6 and 9 denote elapsed time (day) after UUO. Densitometric analyses of the PCR products corrected for GAPDH in each rat (B). The values were expressed in arbitrary densitometric unit (A.D.U.). Sham, UUO and Cont denote the sham kidneys, the obstructed kidneys and the contralateral kidneys of the same rats, respectively. *p<0.05, **p<0.01, compared to sham values.

The results of RT-PCR for renin mRNA are shown in Fig. 1. At one day after UUO, the renin mRNA level of the obstructed kidneys was decreased ($p< 0.05$) and then subsequently increased. Finally, it returned to the level of sham kidneys. On the contrary, in the contralateral kidneys of the same rats the mRNA level of renin was progressively decreased. At 9 days, it was significantly lower than that of sham kidneys ($p<0.01$). The mRNA expressions of both AT_{1A} and AT_{1B} receptors (Fig. 2) in the obstructed kidneys did not show any significant changes from those of sham and contralateral kidneys throughout the various stages of UUO. In the kidney, all of the

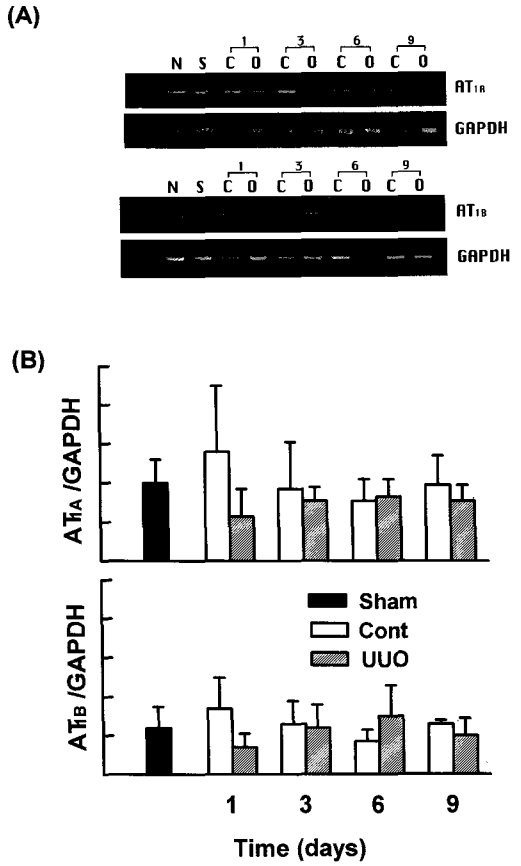

Fig. 2. Ethidium bromide-stained gel of PCR products for AT_{1A} and AT_{1B} in the kidneys after UUO (A). The abbreviations are the same as in Fig. 1. Densitometric analyses of the PCR products corrected for GAPDH in each rat (B). The values were expressed in arbitrary densitometric unit (A.D.U.). Sham, UUO and Cont denote the sham kidneys, the obstructed kidneys and the contralateral kidneys of the same rats, respectively.

specific bindings we demonstrated was AT_1 density. However, AT_2 density was hardly detected. The sequential changes of AT_1 density are shown in Fig. 3. The specific binding means total binding minus nonspecific binding in the presence of ANG II. The AT_1 density of sham kidneys (16±5.5 fmole/mg protein) was similar to that of unoperated control kidneys (16±3.3 fmole/mg protein). At 1 and 3 days after UUO, the AT_1 density was markedly decreased in the obstructed kidneys compared to the sham kidneys (66 ±11.6% ($p<0.05$) and 73±4.0% ($p<0.01$), respectively). Thereafter, it was subsequently increased and at 9 days it showed higher value compared to that of sham kidneys. In contrast, in the contralateral kidneys AT_1 density was significantly reduced from

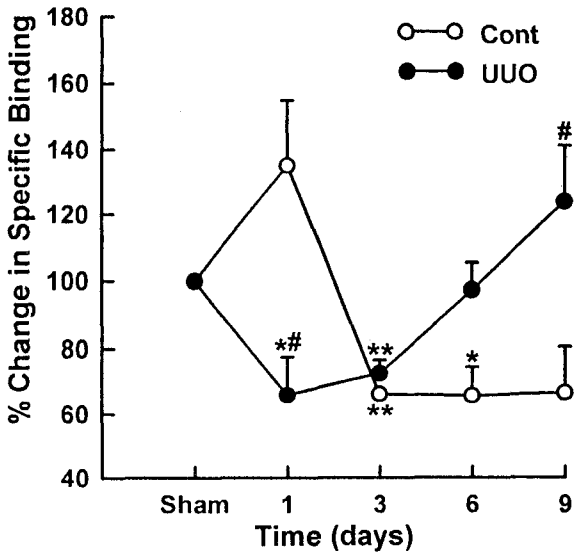

Fig. 3. Mean (±SE) changes in renal AT_1-specific density after UUO. Data represent a percentage compared to sham values. Sham, UUO and Cont denote the sham kidneys, the obstructed kidneys and the contralateral kidneys of the same rats, respectively. *p<0.05, **p<0.01, compared to sham values; # p<0.05, UUO vs. contralateral kidneys.

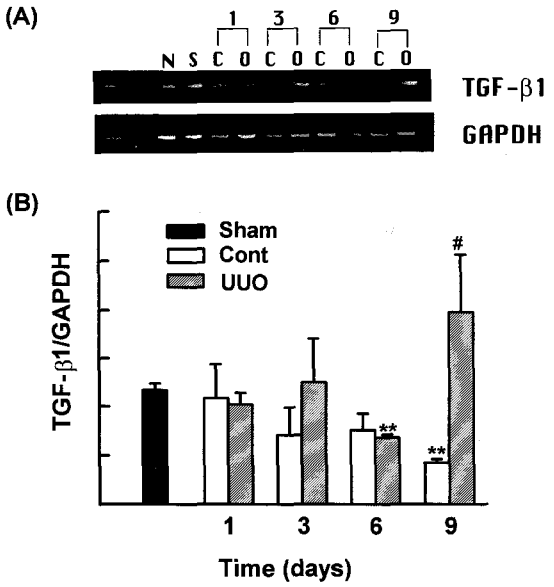

Fig. 4. Ethidium bromide-stained gel of PCR products for TGF-β1 in the kidneys after UUO (A). For abbreviations, see Fig. 1. Densitometric analyses of the PCR products corrected for GAPDH in each rat (B). The values were expressed in arbitrary densitometric unit (A.D.U.). Sham, UUO and Cont denote the sham kidneys, the obstructed kidneys and the contralateral kidneys of the same rats, respectively. **p<0.01, compared to sham values; # p<0.05, UUO vs. contralateral kidneys.

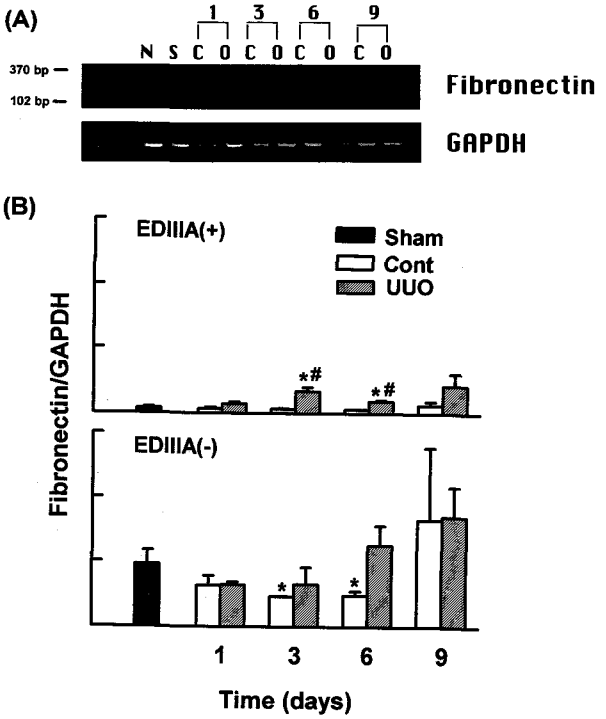

Fig. 5. Ethidium bromide-stained gel of PCR products for fibronectin in the kidneys after UUO (A). The abbreviations are the same as in Fig. 1. Densitometric analyses of the PCR products corrected for GAPDH in each rat (B). The values were expressed in arbitrary densitometric unit (A.D.U.). PCR was carried out using the primers flanking the EDIIIA region of fibronectin gene. EDIIIA+ and EDIIIA- represent the 370 bp and 102 bp bands, respectively. Sham, UUO and Cont denote the sham kidneys, the obstructed kidneys and the contralateral kidneys of the same rats, respectively. *p<0.05, compared to sham values; # p<0.05, UUO vs. contralateral kidneys.

3 to 9 days after UUO.

Expression of TGF-β1 mRNA (Fig. 4) did not show any significant changes at 1 and 3 days after UUO. The amount of TGF-β1 mRNA in the obstructed kidneys was unexpectedly decreased at 6 days compared with the sham kidneys. At 9 days, TGF-β1 mRNA expression was significantly increased in the obstructed kidneys, whereas it was markedly decreased in the contralateral kidneys. To determine whether the changed TGF-β1 contributed to the modulation of ECM gene expression, we carried out PCR to detect the mRNA expression of fibronectin at different stages of UUO (Fig. 5). The predicted size for EDIIIA+ and EDIIIA- was 370 bp and 102 bp, respectively. The EDIIIA+ message in the obstructed kidneys relative to the sham or the contralateral kidneys was markedly increased throughout the various stages of UUO, especially, at 3 ($p< 0.05$) and 6 days ($p<0.05$). In addition, the amount of the EDIIIA- message was also increased to some extent at 6 days after UUO. However, in the contralateral kidneys it was significantly decreased compared to the sham kidneys.

DISCUSSION

In the present study, we used RT-PCR to detect mRNAs for renin, AT_1 receptor, TGF-β1 and fibronectin in the obstructed kidneys. In preliminary experiments we determined parameters to obtain a correlation between serially diluted PCR products and their densitometric signals. Although the exact amount of amplified DNA cannot be determined in an ethidium bromide stained gel, we believe this technique is useful to measure relative mRNA levels under optimized conditions from small amounts of kidney tissue.

At one day after UUO, in the obstructed kidneys we found the level of renin mRNA was transiently decreased to some extent following the gradual return to the level of sham kidneys. In the contralateral kidneys, however, the mRNA expression of renin was decreased and at 9 days it was significantly lower than that of sham kidneys. On the contrary, El-Dahr (1993) and collaborators showed that renal renin mRNA in the obstructed kidneys was increased obviously in the chronic UUO (at 1 and 5 weeks after obstruction). Because limited data are available, this discrepancy is difficult presently to explain. Further studies are required to elucidate it. The concomitant reduction of renal renin mRNA and AT_1 density at early stages of UUO suggests a negative feedback between renin gene and ANG II. Pimentel et al (1995) also suggested a negative feedback between the renin gene and ANG II in the UUO. They demonstrated that the renin mRNA fell toward its basal values between 2 and 24 hours after obstruction, a time of the most profound down-regulation of the AT_1 receptor mRNA. Even though we did not detect any significant elevation of renin mRNA expression after obstruction, this result did not exclude the pathogenic role of the RAS on the progression of renal damage after UUO. One possible explanation for this is the renal renin content might be increased after UUO following up-regulation of renin mRNA. Pimentel et al (1995) found that the renin

mRNA and renal renin content in the obstructed kidneys were increased significantly during the first 24 hours of UUO. Since we did not measure the renal renin content because limited tissues were available, further studies are required to elucidate this possibility.

ANG II may be an important modulator of glomerular filtration and function. Several lines of evidence suggest that reduced ANG II action may account, at least in part, for the changes in glomerular dynamics. However, molecular mechanisms of ANG II actions on the renal hypertrophy and hyperplasia have not been well elucidated. It is well known that most of the hemodynamic manifestations of ANG II are a result of AT_1 receptor activation (Timmermans et al, 1993; Lee et al, 1995; Yang et al, 1996). Recent studies have reported the preponderance of AT_1 receptors and their distribution in the mammalian kidney (Sechi et al, 1992; Meister et al, 1993). Therefore, we postulated ANG II might stimulate tubulointerstitial fibrosis through, at least in part, modulation of renal AT_1 receptor number and/or affinity and its gene expression. We demonstrated that UUO led to the prolonged decrease of AT_1 density. At present, there is no evidence that could explain how ANG II might participate in decreasing mRNA levels of AT_1 subtypes and/or persistently decreasing AT_1 density in obstructive kidneys. We postulated that the receptor preoccupancy or the homologous down-regulation of the receptor sites by ANG II is one of the possible mechanism of reduction of AT_1 density in UUO. Some data (Wilkes, 1987) suggest that ANG II may play a role in the regulation of AT_1 subtype mRNA expressions through multiple pathways and finally other mechanisms may also regulate AT_1 subtype mRNAs expression during UUO. Thus, it remains to be explored whether gene expression of AT_1 subtypes is modulated after the increase in ANG II during UUO. As described, AT_1 subtype mRNA levels appear stable during chronic UUO, whereas AT_1 density decreased after 1 and 3 days and then increased at 9 days after UUO. This discrepancy between the level of AT_1 subtype mRNAs expression and the AT_1 density may be due to changes in translational efficiency, transcript degradation, or increased recycling of AT_1 subtype receptors.

We showed that the expression of TGF-β1 mRNA in the obstructed kidneys was gradually increased after UUO, even though unexpectedly decreased at 6 days. At 9 days, it reached a significantly higher level than that in sham kidneys. Nagel and Bulger (1978) reported that in rabbits with UUO the interstitial space was widened at seven days after obstruction; there were increased collagen fibers and numerous fibroblasts detected at this time. These pathologic findings indicate that the process of renal interstitial fibrosis after ureteral ligation may be initiated at seven days or earlier after the onset of obstruction. Khalil et al (1989) showed in a rat model of bleomycin-induced pulmonary fibrosis that the peak level of TGF-β1 which occurred at seven days after bleomycin administration, preceded the maximum collagen synthesis by seven days. Majesky et al (1991) have demonstrated that during vascular injury the increase in mRNA for TGF-β1 preceded the increase of mRNAs for collagen and fibronectin and the thickening of the vascular wall.

We demonstrated that the expression of the EDIIIA region of fibronection mRNA was increased in the obstructed kidney at 3 and 6 days after UUO. Fibronectin mRNA is alternatively spliced to yield several isoforms of fibronectin (Norton & Hynes, 1987) Among several splicing alternates of fibronectin mRNA the EDIIIA region has been shown to be regulated by TGF-β1 (Wang et al, 1991). Thus, it is conceivable that the increased expression of TGF-β1 mRNA in the obstructed kidney may contribute, at least in part, in an increase in ECM protein synthesis in the renal cortex during UUO.

In this study we have not identified the mechanisms by which TGF-β1 mRNA is increased during UUO. We postulated the increased renal RAS stimulated the expression of TGF-β1 mRNA. However, we found unexpectedly the expression of renal RAS component mRNAs after UUO were nearly the same as that of sham-operated rats and they even showed a transient decrease. Nevertheless, this result does not nullify the relationship between the RAS and TGF-β1 because the significantly increased AT_1 density might reflect indirectly the increased action of ANG II at 9 days after UUO. Kaneto et al (1993) stated that ANG II may have a role in stimulating expression of TGF-β1 mRNA in UUO and in increasing the number of active forms of TGF-β1 in this setting. They showed that an ACE inhibitor significantly blunted the increase of TGF-β1 mRNA present in the obstructed kidney after UUO. However, other mecanisms may also regulate TGF-β1 mRNA expression during obstruction because they showed only the increased levels of TGF-β1 mRNA were suppressed by enalapril

to the higher level seen in kidneys of untreated control rats. At present, however, limited data are available, and further studies are required to evaluate clear relationship between local RAS and TGF-β1.

In summary, UUO may lead to the differential regulation of renal renin, AT_1 receptor, TGF-β1 and fibronectin mRNA levels and AT_1 density at different stages of UUO. Further studies are required to elucidate the exact relationship between RAS and the TGF-β1 in the progression of obstructive nephropathy.

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REFERENCES

Ambuhl P, Gyurko R, Phillips MI. A decrease in angiotensin receptor binding in rat brain nuclei by antisense oligonucleotides to the angiotensin AT1 receptor. *Regul Pept* 59: 171−182, 1995

Brenner BM. Chronic renal failure: a disorder of adaptation. *Perspect Biol Med* 32: 434−444, 1989

Burrnham CE, Hawelu-Johnson CL, Frank BM, Lynch KM. Molecular cloning of rat renin cDNA and its gene. *Proc Natl Acad Sci USA* 84: 5605−5609, 1987

Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal biochem* 162: 156−159, 1987

Edwards RM, Aiyar N. Angiotensin II receptor subtypes in the kidney. *J Am Soc Nephrol* 3: 1643-1652, 1993

El-Dahr SS, Gee J, Dipp S, Hanss BG, Vari R, Chao J. Upregulation of renin-angiotensin system and downregulation of kallikrein in obstructive nephropathy. *Am J Physiol* 264: F874−F881, 1993

Gibbons GH, Pratt RE, Dzau VJ. Vascular smooth muscle cell hypertrophy vs. hyperplasia. Autocrine transforming growth factor-β1 expression determines growth response to angiotensin II. *J Clin Invest* 90: 456−461, 1992

Inagami T. Renin in the brain and neuroblastoma cells: an endogenous and intracellular system. *Neuroendocrinology* 35: 475−483, 1982

Ingelfinger JR, Dzau VJ. Molecular biology of renal injury: emphasis on the role of the renin-angiotensin system. *J Am soc Nephrol* 2 (suppl 1): S9−S20, 1991

Ishidoya S, Morrissey J, McCracken R, Reyes A, Klahr S. Angiotensin II receptor antagonist ameliorates renal tubulointerstitial fibrosis caused by unilateral ureteral obstruction. *Kidney Int* 47: 1285−1294, 1995

Kaneto H, Morrissey J, Klahr S. Increased expression of TGF-β1 mRNA in the obstructed kidney of rats with unilateral ureteral ligation. *Kidney Int* 44: 313−321, 1993

Kaneto H, Morrissey J, McCracken R, Reyes A, Klahr S. Enalapril reduces collagen type IV synthesis and expansion of the interstitium in the obstructed kidney of the rat. *Kidney Int* 45: 1637−1647, 1994

Khalil N, Bereznay O, Sporn M, Greenberg AH. Macrophage production of transforming growth factor β and fibroblast collagen synthesis in chronic pulmonary inflammation. *J Exp Med* 170: 727−737, 1989

Kitami Y, Okura T, Marumoto K, Wakamiya P, Hiwada K. Differential gene expression and regulation of type-1 angiotensin receptor subtypes in the rat. *Biochem Biophys Res Commun* 188: 446−452, 1992

Kuncio GS, Neilson EG, Haverty T. Mechanisms of tubulointerstitial fibrosis. *Kidney Int* 39: 550−556, 1991

Lee WJ, Yang EK, Ahn DK, Park YY, Park JS, Kim HJ. Central ANG II-receptor antagonists impair cardiovascular and vasopressin response to hemorrhage in rats. *Am J Physiol* 268: R1500−1506, 1995

Majesky MW, lindner V, Twardzik DR, Schwartz SM, Reidy MA. Production of transforming growth factor β1 during repair of arterial injury. *J Clin Invest* 88: 904−910, 1991

Meister B, Lippoldt A, Bunnemann B, Inagami T, Ganten D, Fuxe K. Cellular expression of angiotensin type-1 receptor mRNA in the kidney. *Kidney Int* 44: 331−336, 1993

Nagel RB, Bulger RE. Unilateral obstructive nephropathy in the rabbit. II. Late morphologic changes. *Lab Invest* 38: 270−278, 1978

Norton PA, Hynes RO. Alternative splicing of chicken fibronectin in embryos and in normal and transformed cells. *Mol Cell Biol* 7: 4297−4307, 1987

Pimentel JL Jr, Montero A, Wang S, Yosipiv I, El-Dahr S, Martinez-Maldonado M. Sequential changes in renal expression of renin-angiotensin system genes in acute unilateral ureteral obstruction. *Kidney Int* 48: 1247−1253, 1995

Qian SW, Konddaiah P, Roberts AB, Sporn MB. cDNA cloning by PCR of rat transforming growth factor β-1. *Nucleic Acids Res* 18: 3059, 1990

Roberts AB, Sporn MB. Transforming growth factor β. *Adv Cancer Res* 51: 107−145, 1988

Sechi LA, Grady EF, Griffin CA, Kalinyak JE, Schambelan M. Distribution of angiotensin II receptor subtypes in rat and human Kidney. *Am J Physiol* 262: F236−F240, 1992

Stouffer GA, Owens GK. Angiotensin II-induced mitogenesis of spontaneously hypertensive rat derived cultured smooth muscle cells is dependent on autocrine production of transforming growth factor-β1. *Cir Res* 70: 820−828, 1992

Timmermans PB, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JM, Smith RD. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev* 45: 205−251, 1993

Tso JY, Sun XH, Kao TH, Reece KS, Wu R. Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: Genomic complexity and molecular evolution of the gene. *Nucleic Acids Res* 13: 2485−2502, 1985

Wilkes BM. Reduced glomerular angiotensin II receptor density in diabetes mellitus in the rat: Time course and mechanism. *Endocrinology* 120: 1291−1298, 1987

Wolf G, Neilson EG. Molecular mechanisms of tubulointerstitial hypertrophy and hyperplasia. *Kidney Int* 39: 401−420, 1991

Wolf G, Neilson EG. Angiotensin II as a renal growth factor. *J Am Soc Nephrol* 3: 1531−1540, 1993

Wang A, Cohen DS, Palmer E, Sheppard D. Polirized regulation of fibronectin secretion and alternative splicing by transforming growth factor b. *J Biol Chem* 266: 15598−15601, 1991

Yang EK, Lee WJ, Park YY, Ahn DK, Park JS, Kim HJ. Effects of chronic central administration of losartan on the cardiovascular and hormonal responses to hemorrhage in conscious rats. *Regul Pept* 67: 107−113, 1996