BAYLOR RENAL PATHOLOGY LABORATORY

BAYLOR COLLEGE OF MEDICINE, DEPARTMENT OF PATHOLOGY
ONE BAYLOR PLAZA, HOUSTON, TEXAS 77030
TELEPHONE: (713) 798-4657 or (713) 798-3664 • FAX: (713) 798-5838

Luan Truong, M.D.
(713) 790-2459

Roberto Barrios, M.D.
(713) 415-4785 Pager
(713) 798-4661

September 22, 1999

Philip J. Migliore, M.D.
Research Director
The Moran Foundation
Baylor College of Medicine, Houston, Texas 77030

Dear Dr. Migliore:

Please find enclosed the progress report related to the Moran Foundation Research Awards to Luan Truong.

Award #97-0090: Experimental Chronic Obstructive Uropathy. Improvement of Renal Tissue Damage by Inhibiting the Interaction of Selectin and its Carbohydrate Ligand Sialyl Lewis x.

This experiment was already completed. Chronic obstructive uropathy was created in two groups of rats. Animals in one group were first treated with an experimental inhibitor of Sialysis Lewis x obtained from a pharmaceutical company. Evaluating the morphologic parameters of tissue injury, including light microscopy, apoptosis, cell proliferation and inflammatory cell phenotypes, was already completed. Unfortunately, this pharmacologic manipulation did not improve the renal lesions. In fact, it unexpectedly worsened the renal lesions. We are trying to find some way to deal with this awkward outcome. No abstract or manuscript derived from this project is currently available.

Award #97-0091: Activation of the Intrarenal Renin-Angiotensin System Mediates Renal Changes Associated with Unilateral Ureter Ligation. Evidence from a Mouse Strain Genetically Deficient of Angiotensin II Receptor type 1A.

This project was already completed. The results were presented both as *a platform and poster presentation* (indicating exceptional values) at the Meeting of the American Society of ~~Pathology~~ NEPHROLOGY in Philadelphia, ~~November~~ OCTOBER 1998. A copy of the corresponding

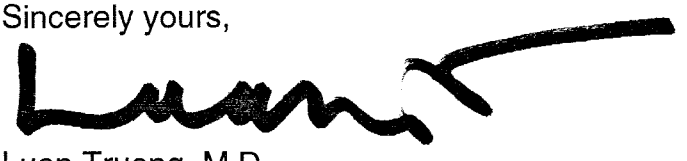
abstract published in the Journal of American Society of Pathology is enclosed. The full-length manuscript is being prepared.

Award #98-0095: Macrophages Mediate Renal Changes in Chronic Obstructive Uropathy. Evidence from a Mouse Strain with Null Mutation for MAC-1, a Macrophage-Specific Integrin.

The experiment in which control mice and mice with null mutation for MAC-1 was already completed. Light microscopic evaluation, macrophage staining, apoptosis and cell proliferation studies were already completed. The results are being quantitated and analyzed. An abstract and a manuscript from this project are being prepared. The preliminary review of the tissue sections alone without the benefit of quantitative data indicates that the MAC1 null mutation confers little protection for the renal damage. This is quite surprising (and quite frustrating).

I am thankful for the generosity of the Moran Foundation and especially for your support. They are instrumental for my research effort focusing on the mechanism of chronic tubulointerstitial injury of the kidney.

Sincerely yours,



Luan Truong, M.D.

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A2291 T525 (PS) M1152 (FC)

GLUCOSE 6-PHOSPHATE DEHYDROGENASE (G6PD) PLAYS AN IMPORTANT ROLE IN CELL DEATH. Wang-Ni Tian,* Leigh D. Braunstein,* Jiongdong Pang,* Mark Rose,* and Robert C. Stanton. *Beth Israel Deaconess Medical Center, Joslin Diabetes Center, and Harvard Medical School, Boston, MA.*

G6PD is the principal source of the main intracellular reductant, NADPH. We have shown that G6PD plays an important role in cell growth by providing NADPH for control of the redox potential (JBC 273:10609,1998). Oxidative stress is known to play an important role in cell death. We hypothesized that G6PD activity is important for cell death by regulating oxidative stress. Increased G6PD activity was achieved by producing cell lines stably overexpressing G6PD. Decreased G6PD activity was achieved by using G6PD inhibitors. Our results show that 1) G6PD inhibitors potentiated H_2O_2-induced cell death, suggesting that cells were more sensitive to the H_2O_2-induced oxidative stress; 2) Overexpression of G6PD rendered cells more resistant to H_2O_2-induced cell death; 3) Serum-deprivation, another stimulator of cell death, was associated with decreased G6PD activity and resulted in elevated reactive oxygen species (ROS); 4) Additions of substrates for G6PD to serum-deprived cells almost completely abrogated the serum deprivation-induced rise in ROS; suggesting that decreased G6PD activity was responsible for the increased ROS in serum deprivation; 5) Inhibition of G6PD led to a decrease in intracellular NADPH content; 6) Consequences of G6PD inhibition were a significant increase in apoptosis, loss of protein thiols (showing increased oxidative stress), and time-dependent degradation of G6PD. Lastly G6PD inhibitors altered MAP kinase phosphorylation in a manner similar to H_2O_2 and and other oxidants. Taken together, we conclude that G6PD plays a critical role in cell death by affecting the redox potential.

A2292 M604 (PS)

MECHANISM OF CHRONIC OBSTRUCTIVE UROPATHY (COU): INCREASED EXPRESSION OF APOPTOSIS- RELATED MOLECULES. Luan Truong, Yeong-Jin Choi,* Leonardo Mendoza,* C. Wayne Smith,* Christie Ballantyne,* Takehiko Koji,* David-Sheik Hamad, Wadi Suki. *Depts of Pathology, Medicine, and Pediatrics, Baylor Coll of Med and the Methodist Hosp, and Dept of Cell Biology, Nagasaki University, Japan.*

We have demonstrated that apoptosis involving predominantly renal tubular cells occurs during the course of COU and may be pathogenetically relevant. The mechanism of tubular cell apoptosis is, however, poorly understood. Mice with COU created by left ureter ligation were sacrificed in group of three at day 3,6,10,20.30, 45 and 60. Control, ligated and contralateral kidneys were harvested. Apoptotic renal tubular cells were detected by in situ, end-labelling of fragmented DNA and were quantitated. The expresion of several apoptosis-related molecules was evaluated. Immunohistochemistry and Northern hybridization were used for p53, Fas, and Fas ligand. Ribonuclease protection assay with specific antisense RNA probes was used for FLICE, FADD, FAP, FAL, TNFR, TRADD, RIP. We confirmed the presence of tubular cell apoptosis, which appeared as early as day 3 after ureteric ligation and peaked at day 20. mRNAs of several apoptosis-related molecules were noted in control and contralateral kidneys. However, the ligated kidneys displayed a marked increase in mRNA levels of p53, Fas, and Fas ligand. By immunostaining, p53 was observed in tubular cells nuclei of dilated tubules, whereas Fas and Fas ligand were localized to tubular cell cytoplasm. MRNAs for TNFR and FLICE were increased but mRNAs for FADD, FAP, TRADD, and RIP remained comparable with those of control kidneys. We conclude that several apoptosis promoters including p53, Fas, Fas ligand, TNFR and FLICE are induced in tubular cells in obstructed kidney and may play a pathogenetic role in this condition.

A2293 S523 (PS)

ROLE OF "POCKET PROTEINS" ON MATRIX-MEDIATED CELL CYCLE ARREST IN GLOMERULAR MESANGIAL CELLS. N. Tsuboi,* H. Yoshida, S. Iwase,* T. Kawamura, Y. Kawaguchi,* H. Yamada,* and T. Hosoya.* *Jikei University School of Medicine, Tokyo, Japan.*

Mesangial cells (MCs) show proliferative and de-differentiated phenotypes when cultured on a plastic substratum or plastic coated with monomer extracellular matrix (ECM). In contrast, when cultured on polymerized ECM, MCs exhibit quiescent and differentiated phenotypes. To examine mechanisms involved, we highlighted the role of E2Fs and their inactivators, pRb and p130. E2Fs are major transcription factors which play a key role in the G1-S transition of the cell cycle, and the "pocket proteins" pRb and p130 are known to bind and inactivate E2Fs. Human MCs were cultured on polymerized type I collagen (polymerized ECM) or monomer type I collagen (monomer ECM) in the presence of 10% FBS. Monomer ECM allowed for MCs proliferation. In contrast, the cell cycle of MCs cultured on polymerized ECM arrested in G0/G1 phase. The expression of E2F-regulated genes, E2F-1, cyclin A and cdc2, were down-regulated in MCs cultured on polymerized ECM, but not in those on monomer ECM. The expression of E2F-4 gene, which is not regulated by E2F, did not alter in MCs cultured on either monomer ECM or polymerized ECM. Electrophoretic mobility-shift assays detected increased protein complexes which specifically bind to E2F consensus sequence in MCs cultured on polymerized ECM. Supershift assays demonstrated that the binding of the protein complexes was inhibited by anti-pRb and anti-p130 antibodies, suggesting that these protein complexes contain pRb and p130. Finally, polymerized ECM translated pRb and p130 into dephosphorelated forms, indicating polymerized ECM activated pRb and p130. In MCs cultured on ECM monomer, the activation of pRb and p130 was not detected. These results indicate that, in human MCs, polymerized ECM inhibits G1-S transition of the cell cycle, and that pRb and p130 may play an important role in this process through intervention in the function of E2F-regulated genes.

A2294 T523 (PS)

THE EFFECTS OF CHRONIC CYCLOSPORINE REDUCTION ON APOPTOSIS AND APOPTOSIS-RELATED GENE EXPRESSION IN HUMAN RENAL BIOPSIES WITH CHRONIC ALLOGRAFT NEPHROPATHY. Ming Wei,* Hong Song,* Koichi Seta,* John C. Papadimitriou,* Cinthia Drachenberg,* Matthew R. Weir. *University of Maryland, Baltimore, MD.*

Enhanced production of renal cell apoptosis may contribute to the cellular fibrosis and cyclosporine (CsA)-induced nephropathy. p53 and p21-WAF genes are effectors of apoptosis. The current study was designed to determine the amount of apoptosis and p53, p21-WAF gene expression in human renal biopsies from five patients with chronic allograft nephropathy and no acute rejection before and after a 50% reduction in CsA blood levels (200 to 100 ng/ml). Interval between biopsies was 13.7±2 months. Apoptosis was determined by TUNEL staining. The p53, p21-WAF protein expression was determined by immunohistochemical staining (IHCS). Positive TUNEL, p53 and p21-WAF staining was found in glomeruli and tubulointerstitium. Both TUNEL (%) and IHCS staining score (0, no staining; 4, maximum) for p53 and p21-WAF are shown in the Table.

	TUNEL	p53	p21-WAF
High CsA Level	22 ± 3	3.4 ± 0.3	3.6 ± 0.2
Low CsA Level	8 ± 4*	1.1 ± 0.2*	0.7 ± 0.1*

Mean ± SE. *$p < 0.05$ vs high CsA level.

These data demonstrated that chronic reduction of CsA blood level is associated with decreased renal cell apoptosis and p53 and p21-WAF protein levels. These data suggest that renal cell apoptosis and apoptosis-related gene activation may play an important pathophysiological role in chronic allograft nephropathy, and that CsA reduction may reduce the progression of this disease.

A2295 M601 (PS) T1150 (FC)

ANGIOTENSIN II (A II) STIMULATED EXPRESSION OF THE CYCLIN-DEPENDENT KINASE (CDK) INHIBITOR $p27^{KIP1}$ AND INDUCTION OF HYPERTROPHY IN PROXIMAL TUBULAR CELLS DEPEND ON OXYGEN RADICALS. G. Wolf, T.Hannken, R.A.K. Stahl. *University of Hamburg, Germany.*

We have previously demonstrated that A II stimulates hypertrophy of cultured proximal tubular cells (MCT and LLC-PK_1 cells). We have further shown that this hypertrophy depends on the induction of $p27^{Kip1}$. The present study was undertaken to test the hypothesis that reactive oxygen species such as $\bullet O_2^-$ may be involved in the intracellular signal transduction of the ANG II-mediated hypertrophy. A single dose of ANG II (10^{-7} M) significantly increased $\bullet O_2^-$ production in MCT and LLC-PK_1 cells when assessed by lucigenin chemiluminiscence. This response was abolished by the AT_1-receptor blocker losartan (10^{-6} M) but not by an AT_2-receptor antagonist. Furthermore, ANG II-stimulated $\bullet O_2^-$ production was inhibited in the presence of diphenylene iodinium (DIP), an inhibitor of flavoproteins suggesting involvement of a membrane NADH/NADPH oxidase. ANG II induced an increase in mRNA expression of $p22^{phox}$, a subunit of the NADH/NADPH oxidase. ANG II-mediated hypertrophy, measured by increases in de novo protein synthesis, was completely abolished in the presence of DIP or the same concentration of the antioxidants N- acetylcysteine (NAC) or Tiron. Moreover, ANG II-stimulated $p27^{Kip1}$ expression was blunted in the presence of DIP or NAC in MCT and LLC-PK_1 cells suggesting involvement of $\bullet O_2^-$. Transient transfection of MCT cells with $p22^{phox}$ antisense, but not missense, oligonucleotides abolished ANG II-stimulated $p27^{Kip1}$ expression and associated hypertrophy. Generation of reactive oxygen species by xanthine supplementation also increased $p27^{Kip1}$ expression in both cell lines. Our data show for the first time that ANG II-stimulated $\bullet O_2^-$ interacts with cell cycle regulation proteins such as $p27^{Kip1}$. Membrane NADH/NADPH oxidase plays an important role in this process and is necessary for ANG II-induced hypertrophy of tubular cells.

A2296 T516 (PS)

ROLE OF HEPATOCYTE GROWTH FACTOR (HGF) IN THE MAINTENANCE OF RENAL STRUCTURE UNDER ISCHEMIA. K Yamamoto,* N Tomita,* R Morishita, H Matsushita,* S Hayashi,* J Higaki,* T Ogihara.* *Department of Geriatric Medicine, Osaka University, Medical School, Japan.*

Mesangial cells are known to secrete various vasoactive substances which may control endothelial and epithelial cell growth. Therefore, apparently cell-cell interactions among these cells are important in the control of renal function. However, the exact mechanisms of maintaining the cell-cell interactions are not yet understood. We have focused on the role of hepatocyte growth factor (HGF) in the regulation of cell-cell interactions, since we have previously reported that HGF exclusively stimulate endothelial and epithelial cells without the replication of mesangial cells. To further investigate the role of HGF in renal injury, we examined 1) the effects of HGF on epithelial injury induced by hypoxia, and 2) the role of local HGF production in the maintenance of renal structure under hypoxic condition. Renal tubular epithelial cells (RTEC) and mesangial cells were maintained under normoxic and hypoxic conditions for 2 and 4 days. Local HGF secretion from these cells was measured by ELISA. In addition, we examined the number of RTEC and mesangial cells exposed to hypoxia after addition of recombinant human HGF (rHGF). Apoptotic changes in epithelial cells were assessed by nuclear morphology and DNA fragmentation assay. Addition of recombinant HGF (rHGF) stimulated the growth of

A3010 S781 (PS) M1116 (FC)

ATTENUATION OF THE RENAL LESIONS ASSOCIATED WITH OBSTRUCTIVE UROPATHY (OU) IN MICE WITH NULL MUTATION FOR ANGIOTENSIN II TYPE 1A (AT1A) RECEPTOR. Luan Truong, Yeong-Jin Choi,* Vinh Nguyen,* David-Sheik Hamad, Iekuni Ichikawa, Wadi Suki. *Baylor College of Medicine and the Methodist Hospital, Houston, Tx, and Vanderbilt University, Nashville, Tn.*

Activation of the local renin-angiotensin system leading to an elevated renal level of angiotensin II (Ang II) is suggested as one of the contributing factor for the pathogenesis of OU. Among the Ang II receptors (AT1A, AT1B, and ATII), the ATIA receptor is known to mediate most of the functions of Ang II. To test whether null mutation of AT1A receptor improves the renal lesions in OU, left ureteric ligation was created in normal mice and in mice with null mutation for AT1A receptor. Mice were sacrificed in groups of three at day 0, 3, 6, 15, 20, 30, and 45. The following parameters were used to compare the renal changes in normal and AT1A null mice: dry kidney weight; morphometric analysis for tubular atrophy, interstitial fibrosis and interstitial inflammatory cells; renal cell apoptosis by in situ end- labelling of fragmented DNA; renal cell proliferation by immunostaining for brdU. We found that renal changes were variably attenuated in mutated mice, with the highest magnitude noted at day 15. At this time point, the ligated kidneys of mutated mice, as compared with ligated kidneys of normal mice, had higher dry weight (139%), less tubular atrophy (83%), less interstititial fibrosis (72%), less tubular cell apoptosis (31%), and less interstititial inflammation (63%). In conclusion, this genetically engineered mouse model provides strong evidence that stimulation of AT1A receptor is responsible for many renal changes associated with OU. In addition, the marked attenuation of tubular cell apoptosis in this model implies Ang II as a direct promoter of tubular cell apoptosis.

A3011 S759 (PS) M1123 (FC)

ROLE OF TYROSINE PHOSPHORYLATION IN THE REASSEMBLY OF OCCLUDIN AND OTHER TIGHT JUNCTION PROTEINS. Tatsuo Tsukamoto,* Sanjay K. Nigam. *Renal/Medicine, Brigham & Women's Hospital/ Harvard Medical School, Boston, MA.*

Ischemia and/or ATP depletion causes a number of lesions in epithelial cells including mispolarization of some membrane proteins, perturbation of the actin cytoskeleton, and disruption of the permeability barrier, resulted in loss of function of the tight junction (TJ)[ASN abstract 1997, A2779/JBC 272, 16133, 1997]. We now show an involvement of tyrosine kinase(s) in the functional reassembly process of the TJ after ATP depletion. The recovery of transepithelial electrical resistance as well as re-establishment of impermeability to [^{3}H] mannitol in ATP repleted MDCK monolayers was markedly inhibited by the tyrosine kinase inhibitor, genistein. Indirect immunofluorescence indicated redistribution of occludin, a membrane component of the TJ, to an intracellular pool after ATP depletion which reversed after ATP repletion; this reversal process was inhibited by genistein. Examination of the Triton X-100 solubilities of occludin and non-membrane TJ proteins, ZO-1 and ZO-2, revealed a shift of occludin as well as ZO-1 and ZO-2 into an insoluble pool following ATP depletion. These changes reversed after ATP repletion and the movement of insoluble TJ proteins back into the soluble pool was again via a genistein-sensitive mechanism. In ^{32}P labeled cells, dephosphorylation of all studied TJ proteins was observed during ATP depletion, followed by rephosphorylation during ATP repletion; the rephosphorylation of occludin was inhibited by genistein. During the ATP repletion phase, tyrosine phosphorylation of occludin as well as ZO-2, p130/ZO-3 and several other bands (though not ZO-1) was evident; this tyrosine phosphorylation was completely inhibited by genistein. These results provide insights into the molecular mechanism of tight polarized epithelial cell recovery from ischemia.

A3012 T728 (PS)

MECHANISM OF MITOCHONDRIAL DYSFUNCTION IN PROXIMAL TUBULES DURING HYPOXIA/REOXYGENATION. M.A. Venkatachalam, I. Nissim, and J.M. Weinberg. *UTHSC, San Antonio, Texas, University of Penn., Philadelphia, PA and Univ. of Michigan, Ann Arbor, MI.*

Glycine-protected isolated rabbit proximal tubules (PT) develop a severe functional deficit of their mitochondria (M) during hypoxia/reoxygenation (H/R) that is ameliorated by inhibitors of the mitochondrial permeability transition (MPT) (KI 52:140, 1997). Ultrastructurally, M of control PT have a normal "orthodox" configuration. We now show that hypoxia (H) rapidly induces a condensed configuration of M, lasting 60 min. During reoxygenation (R) under conditions where function recovers, M resume an orthodox configuration. But, when function does not recover, M remain condensed. During up to 60 min H/R they do not swell as would be seen with a fully developed MPT. As indicated by loss of preloaded tetramethylrhodamine from M in PT examined by confocal microscopy, M are de-energized at the end of 60 min H, but then recover membrane potential ($\Delta\Psi$) during R despite occurrence of the functional deficit. However, $\Delta\Psi$ in the affected cells is substantially reduced. To better quantitate this behavior we used the dye, JC-1, which allows for ratios between red (energized) and green (deenergized) emissions. Red/green fluorescence ratios were 2.1±0.06 (controls), 0.11±0.01* (completely de-energized with uncoupler), and 0.92±0.03*# (60 min H+60 min R), n=12-79, significantly different from *control, #uncoupler. Measurements of respiratory function with site specific substrates in digitonin-permeabilized PT showed marked inhibition of the respiratory chain at Site I, moderate inhibition at Site II, and intact function at Site III, which indicates normal retention of cytochrome c. Taken together with our earlier observations on benefit from MPT inhibitors, these data suggest that a sustained, incomplete form of the MPT with predominant electron transport inhibition at Site I develops in PT during H/R.

A3013 T710 (PS)

REGENERATION OF THE REMNANT KIDNEY (RK) AFTER ISCHEMIA/ REPERFUSION (I/R) INJURY. Sven R. Vercauteren,* Dirk K. Ysebaert,* Kathleen E. De Greef* & Marc E. De Broe. *Dept.Nephrol. and Exp.Surg., Univ. Antwerp, Belgium*

Older patients with fibrotic remodelling of the aging kidney due to nephron loss or renal disease, are at risk to develop acute renal failure.

We were interested if such a kidney with structural and functional changes, in the absence of concomittant factors, is indeed more vulnerable and has a reduced regenerating capacity after an acute ischemic insult. Lew rats were randomly assigned to undergo 5/6 (RK), 3/6 (1K) or no nephrectomy at all (2K), and to a control group. After 10 weeks, the first 3 groups were subjected to 60 min of renal ischemia.

S-creat (mg/dl) had the following course: (* p<0.05 versus RK)

	RK	1K	2K	Control	
day 0	1.03 ± 0.15	0.63 ± 0.05 *	0.50 ± 0.00 *	0.53 ± 0.05 *	
day 1	3.00 ± 0.26	3.70 ± 0.80	1.58 ± 0.64 *		
day 6	1.98 ± 0.75	0.85 ± 0.13 *	0.75 ± 0.10 *		
day 15	1.60 ± 0.87	0.73 ± 0.05 *	0.53 ± 0.05 *		
day 35	1.25 ± 0.30	0.68 ± 0.09 *	0.58 ± 0.05 *		

All rats had polyuria and proteinuria, with the highest levels in RK. Morphological analysis (PAS staining) showed severe acute tubular necrosis (ATN) in the 3 groups, most pronounced in 1K. In RK, the number of apparent unaffected tubules in the OSOM was comparable with this in 2K, but significantly higher (p<0.05) than in 1K at day1 and 3 (resp. 43/45%, 17/16%, 35/54% at day1/day3 in RK, 1K, 2K). Proliferation (PCNA+cells) was max. at day3, especially in 1K. At day10, regeneration was nearly complete in the 3 groups. Infiltration with white blood cells (WBC) (OX-1+cells) was already present at day0 in RK and did not significantly rise (range 316-555 cells/mm^2), whereas in 1K and 2K infiltration was nearly absent at day0, but rose sharply and reached a maximum at day10 (901 and 668 cells/mm^2;*). These WBC consisted mainly of monocytes (ED1+) at day6 and CD4 cells (W3/25+) at day10.

The RK with chronic renal dysfunction and interstitial fibrosis, develops the same degree of ATN as a normal kidney after I/R, and its regenerative capacity seems to be unimpaired. In contrast, the number of infiltrating WBC in a normal kidney after injury, exceeds the number in the RK, in which the basal infiltration does not increase.

A3014 T726 (PS)

THE ROLE OF DOWNREGULATED ROMK mRNA IN THE PATHOPHYSIOLOGY OF HYPERKALEMIA OF ACUTE TUBULAR NECROSIS (ATN). H. Wald,* L. Shustin,* M.M. Popovtzer. *Hadassah University Hospital, Jerusalem, Israel.*

ATN is associated with hyperkalemia. Recently we have shown that CHIF, a gene implicated in K^+ homeostasis, was downregulated in the renal medulla in ATN (*Amer J Kid Dis*, in press). ROMK is a gene encoding inwardly rectifying ATP regulated K^+ channels (*Nature* 361:31-38, 1993). It is suggested that ROMK represents the low conductance secretory K^+ channels in the TAL and CD. To further characterize the impaired K^+ handling in ATN, ROMK mRNA expression in kidney and colon was studied in 3 groups (g) of rats with glycerol-induced ATN: 1) control (c), 2) moderate ATN, and 3) severe ATN. Serum creatinine was in 1) 48.5±1.8 μM, in 2) 207.5±7.8 and in 3) 359±13.6. In 3) significant hyperkalemia (p<0.01 vs. c) was noted. The expression of ROMK mRNA in relative units (% of c) was in moderate ATN in cortex and medulla 50.9±16.8 (p<0.02) and 36.9±1.1 (p<0.001), and in severe ATN 37.6±8 (p<0.001) and 21±0.5 (p<0.001) respectively. ROMK downregulation correlated highly with serum creatinine in the cortex R=-0.9535 (p<0.01) and in the medulla R=0.9497 (p<0.01). These results show that 1) both in moderate and severe ATN ROMK mRNA is reduced in the kidney cortex and medulla, 2) the downregulation of ROMK is in direct relationship to the severity of ATN, and 3) ROMK is not expressed in the colon neither in intact rats or rats with ATN. These results suggest that the hyperkalemia in severe ATN may stem at least in part from the downregulation of ROMK mRNA in kidney cortex and medulla.