

Cellular Response to Renal Hypoxia Is Different in Adolescent and Infant Rats

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ABSTRACT

Immature renal tubules are more tolerant to ischemia than mature renal tubules. Here we compared the developmental pattern for some cellular responses evoked by hypoxia and reoxygenation in renal proximal tubules from 10- and 40-day-old rats. Redistribution of $\text{Na}^+\text{-K}^+\text{-ATPase}$ from the plasma membrane was studied by confocal microscopy techniques in primary cultured renal proximal tubular cells. The developmental expression of $\text{Na}^+\text{-K}^+\text{-ATPase}$, $\mu\text{-calpain}$ and heme oxygenase-1 was measured by RT-PCR techniques in rat renal cortex. In response to hypoxia $\text{Na}^+\text{-K}^+\text{-ATPase}$ redistribution from the plasma membrane was almost 2-fold increased in cells isolated from mature kidneys compared with cells isolated from immature kidneys. Reoxygenation resulted in a complete reestablishment of $\text{Na}^+\text{-K}^+\text{-ATPase}$ in the plasma membrane in the immature but not in the mature cells. The dissociation of $\text{Na}^+\text{-K}^+\text{-ATPase}$ from the plasma membrane was associated with a reduced activity and a reduced expression of $\text{Na}^+\text{-K}^+\text{-ATPase}$ in the mature but not in the immature tubular cells. The expression of

$\mu\text{-calpain}$, a factor shown to induce ischemic injury to proximal tubular cells, was significantly lower in the immature compared with the mature kidney, whereas the expression of heme oxygenase-1, a factor shown to protect from renal ischemic injury, was significantly higher in the immature kidney. The results help to explain the increased tolerance of the immature kidney to injury caused by ischemia and reperfusion. (*Pediatr Res* 55: 485-491, 2004)

Abbreviations

RPT, renal proximal tubular
PCR, PCR
NKA, $\text{Na}^+\text{-K}^+\text{-ATPase}$
HO-1, heme oxygenase-1
HSF, heat shock factor
HSP, heat shock protein
HIF, hypoxia-inducible factor
ARF, acute renal failure

Newborn babies with respiratory distress syndrome seldom develop acute renal failure despite severe hypoxemia and, limited renal perfusion and oxygen delivery to the kidney. It has been postulated that the immature kidney tolerates hypoxia and oxidative stress better than the mature kidney. Experimental studies have shown that immature renal tubules are more tolerant of anoxia with diminished cellular injury, which has been attributed to greater glycolytic capacity and preservation of ATP levels in the immature kidney (1, 2, 3). However, neither preservation of ATP nor enhanced glycolytic activity plays a dominant role in the anoxic tolerance of the developing kidney (4, 5).

Hypoxia and reoxygenation evoke a variety of cellular responses in the renal proximal tubular cell, including a disso-

ciation and redistribution of $\text{Na}^+\text{-K}^+\text{-ATPase}$ from the basolateral plasma membrane, increased intracellular Ca^{2+} level and subsequent activation of Ca^{2+} -activated proteases, *e.g.* calpain and oxidative stress. The present study was performed to analyze the developmental pattern of these cellular responses to hypoxia and reoxygenation, in renal proximal tubules, the part of the nephron less tolerant of hypoxia.

Approximately 70% of renal oxygen consumption is related to $\text{Na}^+\text{-K}^+\text{-ATPase}$ that uses it for vectorial transport of sodium, water, and other solutes across the renal tubular epithelial cell. One well-known event during renal ischemia is the dissociation of proximal tubular $\text{Na}^+\text{-K}^+\text{-ATPase}$ from its cytoskeletal anchorage, followed by a redistribution of $\text{Na}^+\text{-K}^+\text{-ATPase}$ from the basolateral plasma membrane (6, 7, 8). The transient loss of $\text{Na}^+\text{-K}^+\text{-ATPase}$ from its basolateral localization explains the partially reversible clinical sequelae such as renal sodium, glucose, amino acid and water loss. We examined the spatial distribution of $\text{Na}^+\text{-K}^+\text{-ATPase}$ by confocal microscopy techniques in primary cultures of renal proximal tubular (RPT) cells from 10- and 40-day-old rats. The

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results suggest that Na⁺-K⁺-ATPase remained at the plasma membrane to a greater extent in immature compared with mature tubular cells in response to hypoxia.

Ischemia/reperfusion leads to oxidative stress. Several endogenous enzymes are proposed to promote or prevent the effects of oxidative stress. We examined the developmental pattern for two such enzymes; calpain which is believed to have a key role in ischemic/reperfusion-induced renal tubular cell injury (9) and heme oxygenase-1 which has been shown to ameliorate renal ischemia and reperfusion injury (10).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (B & K Universal, Sollentuna, Sweden) aged 10 and 40 d, were used in the study. 10-day-old rats developmentally correspond to an infant human kidney and 40-day-old rats, previously shown to have reached full structural maturation of the nephrons (11) correspond to an adolescent human kidney.

Preparation of primary cultured rat renal proximal tubule (RPT) cells. Rat proximal tubule cells were isolated from kidneys of 10- and 40-day-old male Sprague Dawley rats as previously described (12). Cells were cultured in supplemented DMEM (20 mM HEPES/24 mM NaHCO₃/10 μg/mL penicillin/10 μg/mL streptomycin/10% FBS) on glass coverslips or culture dishes for 48 h in 5% CO₂ at 37°C.

Hypoxic chamber. The chamber was custom-made. The chamber was placed on a heater and kept at 37°C. Ready-made mixed gas (0.5% O₂, 5% CO₂, 94.5% N₂) was obtained from AGA Gas AB (Sweden). The gas flow was adjusted to maintain constant outflow. Normoxic cells were exposed to 20% oxygen, corresponding to Po₂ 150 mm Hg, and hypoxic cells were exposed to 0.5% oxygen, corresponding to Po₂ 4 mm Hg.

Ischemia-reperfusion. The abdomen was opened with a midline incision. The left renal artery was completely occluded by a nontraumatic vascular clamp for 15 min. After release of the clamp, revascularization of the kidney was observed. Following 60 min of reperfusion, both kidneys were removed and the right kidney was used as a control. During the development of the mRNA technique, experiments were performed in kidneys not subjected to ischemia. The mRNA expression in these kidneys was not different from those obtained in the contralateral (right) kidney used as a control. Throughout the surgical procedure, the body temperature was maintained at 37°C.

Immunocytochemistry. RPT cells were fixed with methanol for 9 min at -20°C. After washing with PBS, cells were incubated for 10 min in PBS containing 16% normal goat serum. The cells were then incubated during overnight at 4°C with primary monoclonal mouse- anti-Na⁺-K⁺-ATPase α-1 antibody (UPSTATE Biotechnology, NY, U.S.A.). After washing with PBS, the cells were incubated for 1 h at room temperature with secondary goat anti-mouse Alexa546 antibody (Molecular Probes). Negative controls used were cells incubated with secondary antibody in the absence of primary Na⁺-K⁺-ATPase α-subunit antibody.

Confocal microscopy and Fluorescence quantification. The immunolabeled cells were examined using a Leica TCS SP inverted confocal scanning laser microscope using a 40/1.4

numerical aperture objective with excitation at 543 nm and detection at 560–650 nm. Thin optical sections spaced 0.2 μm apart were recorded throughout the cells. Gain and brightness settings were controlled to use the full dynamic range of the detectors. Optical slices taken through the center of the cells were used for the interpretation of the results. The optical sections were obtained from sites where cells revealed the strongest signal in the membrane. From each coverslip between 15 and 30 cells were analyzed.

The confocal fluorescence images were recorded and analyzed using Scion Image public domain Beta 4.0.2 (Scion Corp.). An area in the plasma membrane and an area, without nucleus, in the cytoplasm were outlined. The average of the fluorescence intensity was measured in both areas, and the membrane/cytoplasm ratio was calculated.

Ouabain sensitive ⁸⁶Rb⁺ influx. ⁸⁶Rb⁺ influx, used as an index of K⁺ uptake, was performed as previously described (13). Briefly, RPT cells were washed twice with PBS and preincubated for 30 min at 37°C in a buffer containing (in mM) 100 NaCl, 4.0 KCl, 1.0 CaCl₂, 1.2 MgCl₂, 20 HEPES, 25 NaHCO₃, 1.0 NaH₂PO₄, 1.0 NaH₂PO₄, 10 D-glucose, pH 7.4 in the presence or absence of 1 mM ouabain. Hypoxia and reoxygenation was performed in the hypoxic chamber. Na⁺-K⁺-ATPase-dependent ⁸⁶Rb⁺ influx was calculated as the difference between ⁸⁶Rb⁺ influx in the absence (total influx) and the presence (ouabain-insensitive influx) of ouabain (10⁻³ M). Protein content was determined by the method of Bradford using a kit from Bio-Rad using BSA as a standard. The rate of ⁸⁶Rb⁺ influx was expressed in pmol min⁻¹mg protein⁻¹.

Semiquantitative RT-PCR. Total RNA was extracted from frozen renal cortex or RPT cells using RNeasy Mini Kit (QIAGEN, Germany) and measured with a spectrophotometer (Beckman DU 640, CA, U.S.A.).

Reverse transcription was carried out in a 10 μL reaction volume containing 1 μg total RNA, standard M-MLV RT buffer, 0.4 pM oligo-dT primer, 20 U rRNasin, 80U M-MLV reverse transcriptase (Promega, WI, U.S.A.), 200 μM dNTP (Amersham Pharmacia Biotech, UK), and incubated for 60 min at 42°C. The RT reactions were terminated by inactivation at 95°C for 10 min and stored at 4°C. 10 μL of RT reaction were converted to a 100 μL PCR mixture containing standard PCR Buffer II, 5U AmpliTaq Gold (Applied Biosystems, NJ, U.S.A.), 2.5mM MgCl₂, 200 μM dNTP, 20pM specific primer pairs, internal control primer pairs and sequentially cycled for 25, 30 and 35 cycles (94°C for 30 s, 60°C for 45 s., 72°C for 60 s), starting at 96°C for 8 min and finishing at 72°C for 5 min. To analyze Na⁺-K⁺-ATPase mRNA expression 20 μL from each PCR reaction mixture were separated on a 1.5% agarose gel and stained with ethidium bromide. To analyze μ-calpain 1 and HO-1 mRNA expression PCR products were separated on 4.5% polyacrylamide gel. Digital images were acquired using a Fluor-S MultiImager and analyzed with original software (Quantity One, version 4.2.1, Bio-Rad Laboratories, Hercules, CA, U.S.A.). There was no interference between the specific and internal control primers (data not shown). When the reverse transcriptase was omitted from the RT reaction solution, no product appeared. All SQ RT PCR experiments were made at least three times with three different

RNA preparations. All reaction solutions (except RNA and enzymes) were premixed to eliminate errors during pipetting. DNA molecular weight markers VI (Boehringer-Mannheim) for the polyacrylamide and GeneRuler 100 bp DNA ladder for agarose gels were used for sizing of PCR product.

Primers for PCR (PCR). Primers were designed on the basis of their reported sequences and obtained from CyberGene, Sweden. All primers were selected from different exons to avoid amplification of genomic DNA (Table 2).

Statistics. Values are expressed as mean \pm SEM. The statistical analysis was performed with the *t* test and with analysis of variance (ANOVA) when appropriate. *p* < 0.05 was considered significant.

The study has been approved by the national ethical committee for animal experimentation.

RESULTS

The effect of hypoxia and reoxygenation on Na⁺-K⁺-ATPase distribution was examined by immunostaining and confocal microscopy techniques in primary cultures of renal proximal tubular (RPT) cells from 10- and 40-day-old rats. Na⁺-K⁺-ATPase was visualized by a MAb directed toward the α -subunit. Measurement of cellular redistribution was performed by Image software and the membrane to cytoplasm immunosignal ratio (M/C ratio) for Na⁺-K⁺-ATPase was calculated (Fig. 1A). A decrease in the M/C ratio suggests a redistribution of Na⁺-K⁺-ATPase from the plasma membrane to cytosolic compartments. The Na⁺-K⁺-ATPase signal was mainly present at the plasma membrane in the normoxic (20% O₂) RPT cells from both 10- and 40-day-old rats. After exposure to hypoxia (0.5% O₂) for 15–120 min the M/C ratio decreased in both age groups, suggesting a redistribution of Na⁺-K⁺-ATPase to cytosolic compartments. In cells from 10-day-old rats the M/C ratio decreased to a smaller extent at the 30- and 120-min time point compared with cells from 40-day-old rats (Fig. 1B). After reoxygenation of RPT cells for 30 min Na⁺-K⁺-ATPase partially redistributed back to the plasma membrane in both mature (80 \pm 6%) and immature (91 \pm 4%) cells. After 24 h reoxygenation a complete return to normal distribution was found in the immature cells (102 \pm 3%) while no further recovery compared with 30 min reoxygenation was found in the mature cells (81 \pm 3%) (Fig. 2C).

Na⁺-K⁺-ATPase activity was measured as ouabain sensitive ⁸⁶Rb⁺ influx in RPT cells (Table 1). In agreement with previous observations control Na⁺-K⁺-ATPase activity was significantly higher in RPT cells isolated from 40-day-old rats than 10-day-old rats (14). RPT Na⁺-K⁺-ATPase activity in 40-day-old rats significantly decreased after 30 min exposure to low oxygen (0.5% O₂) and returned to control values after 30 min reoxygenation. No significant effect was found in RPT Na⁺-K⁺-ATPase activity in 10-day-old rats after 30 min exposure to low oxygen. Ouabain-insensitive ⁸⁶Rb⁺ influx was not affected by hypoxia or reoxygenation.

The response of Na⁺-K⁺-ATPase expression to hypoxia and reoxygenation was determined by semiquantitative RT-PCR techniques (Fig. 2), using β -actin as an internal control. Expression of Na⁺-K⁺-ATPase mRNA was significantly reduced

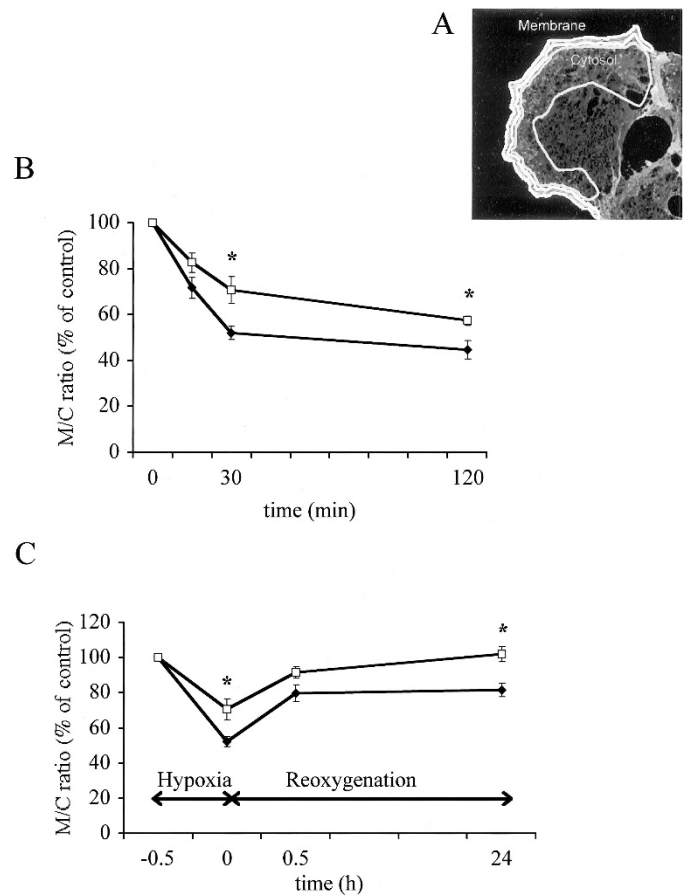


Figure 1. Semi quantitative measurements of Na⁺-K⁺-ATPase redistribution in primary cultures of RPT cells. Na⁺-K⁺-ATPase was labeled with immunohistochemistry techniques and visualized by confocal microscopy. (A) A region in the plasma membrane was selected and the Na⁺-K⁺-ATPase fluorescence intensity was measured. This process was repeated for an adjacent cytoplasmic area. The membrane/cytoplasm (M/C) ratio was then calculated. (B) Hypoxia 0–120 min. Unfilled squares represent RPT cells from 10-day-old rats, filled squares represent RPT cells from 40-day-old rats. (C) Hypoxia for 30 min followed by reoxygenation for 30 min and 24 h. Unfilled squares represent RPT cells from 10-day-old rats, filled squares represent RPT cells from 40-day-old rats. The graphs show Na⁺-K⁺-ATPase signal M/C ratio and represents the mean \pm SEM of at least five separate experiments. **p* < 0.05 vs 40-day-old rats.

in RPT cells from 40-day-old rats after 30 min of hypoxia and recovered completely to normal values after 1 h reoxygenation. In contrast, RPT cells from 10-day-old rats showed no change in mRNA for Na⁺-K⁺-ATPase in response to hypoxia.

A variety of factors are involved in the effects of ischemia/reperfusion in renal proximal tubular cells. Some of these factors mediate the injury associated with ischemia/reperfusion, whereas others protect the cells from such injury. We examined the developmental expression of two such factors, μ -calpain and heme oxygenase-1 (HO-1) by RT-PCR techniques. RNA was extracted from frozen rat renal cortex. In studies on the effect of ischemia/reperfusion, the left renal artery was occluded by a clamp followed by reperfusion. The right unclamped kidney was used as a normoxic control. μ -calpain mRNA significantly decreased in immature renal cortex compared with control value (40 \pm 15%) after 15 min of renal ischemia followed by 60 min reperfusion. In mature

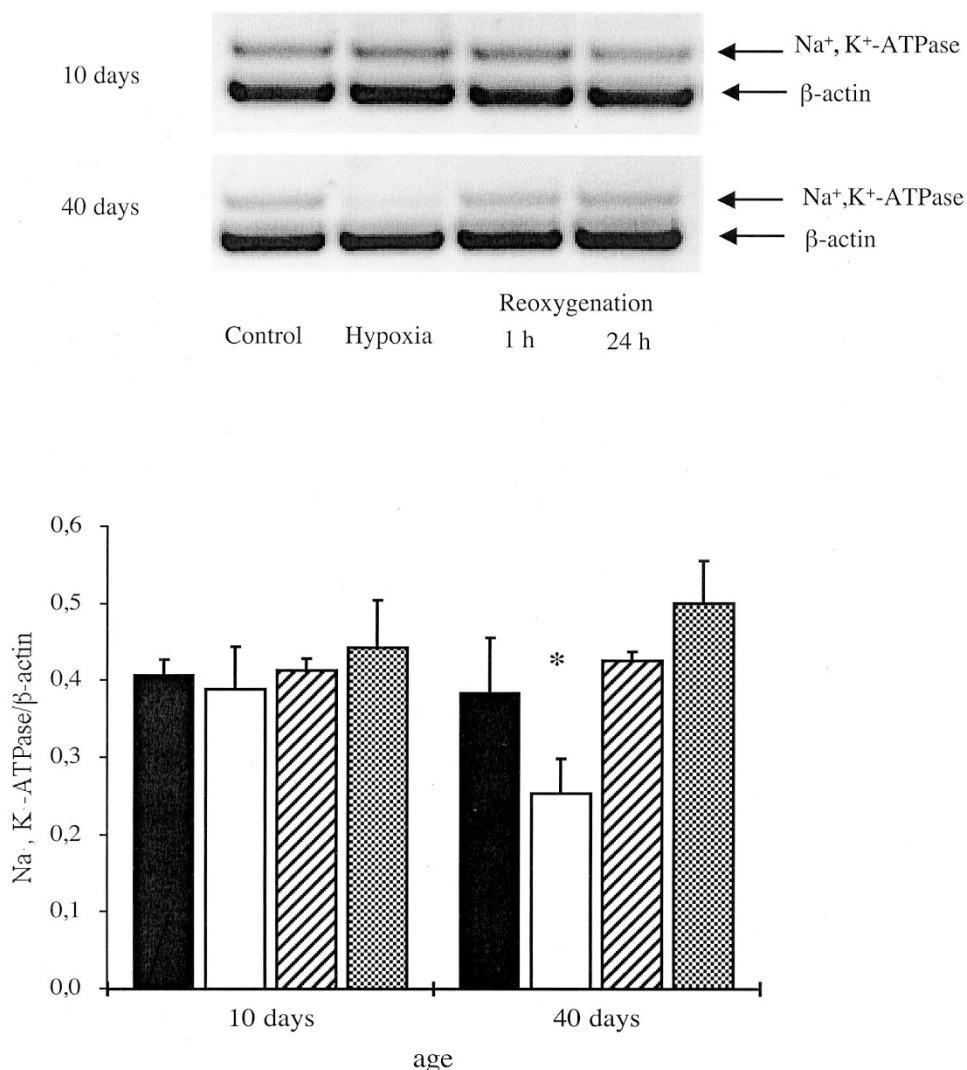


Figure 2. Expression of mRNA for $\text{Na}^+\text{-K}^+\text{-ATPase}$ in response to hypoxia/reoxygenation. (A) Representative blot of $\text{Na}^+\text{-K}^+\text{-ATPase}$ in RPT cells from 10- and 40-day-old rats subjected to hypoxia and reoxygenation. (B) Filled bars represent normoxic control, unfilled bars represent hypoxia 30 min, hatched bars represent hypoxia 30 min followed by reoxygenation 1 h and crosshatched bars represent hypoxia 30 min followed by reoxygenation 24 h. Values are the ratios $\text{Na}^+\text{-K}^+\text{-ATPase}/\beta\text{-actin}$ and represent the mean \pm SEM of at least five separate experiments. * $p < 0.05$ vs control.

Table 1. Effect of hypoxia and reoxygenation on $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in primary cultured RPT cells

	Control	Hypoxia 30 min	Reoxygenation 30 min
10 day old rats			
Total Rb influx	14.83 \pm 1.11*	14.69 \pm 1.68	13.41 \pm 2.74
Ouabain sensitive	6.44 \pm 0.66*	7.01 \pm 0.77	6.69 \pm 0.91
40 day old rats			
Total Rb influx	25.86 \pm 0.7	21.24 \pm 0.73*	25.44 \pm 0.74
Ouabain sensitive	18.76 \pm 0.75	14.84 \pm 0.18*	18.22 \pm 0.64

Data are means \pm SEM and are expressed as $\text{pmol min}^{-1} \text{mg protein}^{-1}$. Ouabain sensitive Rb influx is calculated from total Rb influx-ouabain insensitive Rb influx, and represents the activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$.

* $p < 0.05$ vs. normoxic control RPT cells from 40-day-old rat.

renal cortex $\mu\text{-calpain}$ mRNA significantly increased to 120 \pm 5% of the control value (Fig. 3). GADPH was used as an internal control.

HO-1 is known to ameliorate oxidative stress induced damage to the proximal tubular cell. RT-PCR showed a significant

increase in mRNA for HO-1 in renal cortex from 10 day compared with renal cortex from 40-day-old rats (Fig. 4).

DISCUSSION

This study describes developmental changes of three cellular responses, all proposed to be involved in ischemia/reperfusion-induced injury to the renal tubular epithelial cells.

The proper insertion of $\text{Na}^+\text{-K}^+\text{-ATPase}$ into the plasma membrane is essential for the reabsorptive capacity of the renal proximal tubular epithelial cells. Here we demonstrate that hypoxic immature RPT cells have a greater capacity to preserve the plasma membrane localization of $\text{Na}^+\text{-K}^+\text{-ATPase}$ and to maintain its pump function than mature RPT cells. Furthermore, the immature kidney has a more favorable ratio between factors known to cause ischemic/reperfusion-induced cell damage and factors known to ameliorate such damage.

It is likely that these cellular responses contribute to the increased tolerance to ischemia and oxidative stress observed in the immature kidney.

Table 2.

Name	Structure of primer	GeneBank accession number	Size of PCR product
μ -calpain U	CAATGCTTCTCGGGCACAATC	NM 019152	401 bp
μ -calpain L	CGTCTCGATCCATGAGGTTCCAC		
HO-1 U	CGTAAAGCGTCTCCACGAGGTG	NM 012580.1	498 bp
HO-1 L	GCCAACAGGAAACTGAGTGTGAGG		
NKA α 1 U	ATGATTGACCCTCCTCGAGCTGCT	X04297-1	775 bp
NKA α 1 L	GGCCTGGATCATACCGATCTGT		
β -actin U	TGAACCCTAAGGCCAACCGT	V01217	635 bp
β -actin L	GCTAGGAGCCAGGGCAGTA		
GAPDH U	TCCGCCCTTCCGCTGAT	AF 106860	340 bp
GAPDH L	CACGGAAGGCCATGCCAGTGA		

NKA = Na⁺-K⁺-ATPase

Renal ischemia causes a rapid fall in cellular ATP, followed by a disruption of the cortical actin cytoskeleton and subsequently a rapid redistribution of actin-associated Na⁺-K⁺-ATPase away from the basolateral plasma membrane (6, 7). This redistribution of Na⁺-K⁺-ATPase occurs in all segments of the proximal tubules but is not observed in the distal tubules (7). Functionally, ischemia-induced redistribution of Na⁺-K⁺-ATPase is in part responsible for reduced renal tubular sodium and glucose reabsorption. With recovery, proximal tubule cells undergo remodeling allowing normal cellular function to return.

We compared Na⁺-K⁺-ATPase distribution in mature and immature proximal tubular cells by immunostaining and confocal microscopy techniques. Na⁺-K⁺-ATPase dissociated from the plasma membrane in both the mature and immature proximal tubular cell after short (15 and 30 min) and prolonged (120 min) hypoxia. In comparison with immature cells, Na⁺-K⁺-ATPase redistribution from the plasma membrane was almost 2-fold higher in mature cells during hypoxia. Duration-dependent reestablishment of Na⁺-K⁺-ATPase in the plasma

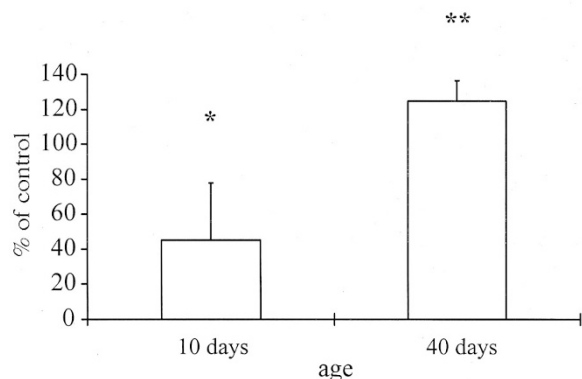
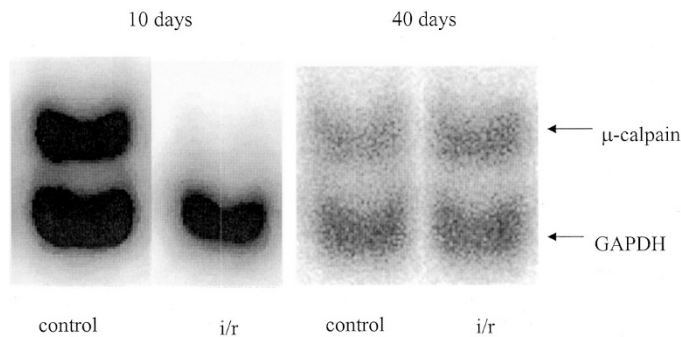


Figure 3. Expression of mRNA for μ -calpain in response to ischemia/reperfusion. (A) Representative blots of μ -calpain in renal cortex subjected to ischemia/reperfusion (i/r). (B) Effect of i/r as a percent of normoxic control; mean \pm SEM of at least five separate experiments. * $p < 0.05$ vs normoxic control kidney from 10-day-old rats ** $p < 0.05$ vs normoxic control kidney from 40-day-old rats.

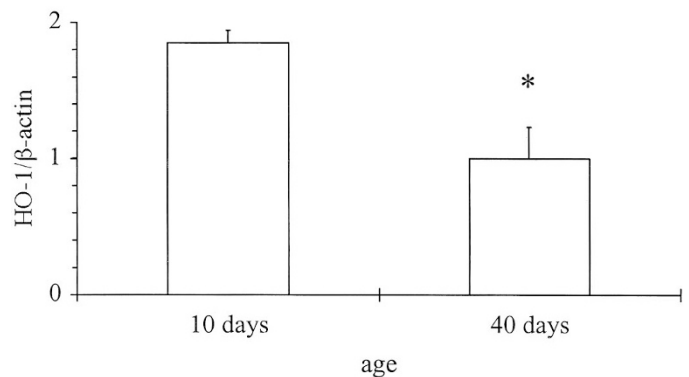
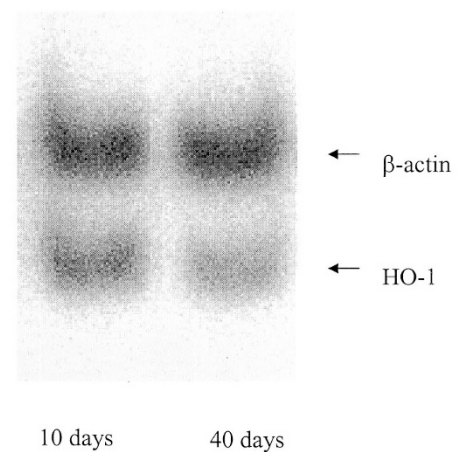


Figure 4. Expression of mRNA for heme oxygenase-1 (HO-1) in renal cortex from immature and mature rats (A) Representative blots of HO-1 in renal cortex. (B) Values are the ratios HO-1/ β -actin and represent the mean \pm SEM of at least five separate experiments. * $p < 0.05$ vs immature renal cortex.

membrane occurred in both mature and immature cells during reoxygenation. In contrast to the mature cell a complete recovery was observed after 24 h of reoxygenation in immature cells.

Hypoxia is associated with decreased activity of Na⁺-K⁺-ATPase and a down-regulation of its α and β subunits in mature renal tubular cells (15, 16). The dissociation of Na⁺-K⁺-ATPase from the plasma membrane was associated with a reduced pump function in the mature, but surprisingly not in the immature cells. This may suggest that the activity of the Na⁺-K⁺-ATPase that was still attached to the plasma membrane was increased in the immature cells in an attempt to compensate or that the method was not sensitive enough to detect a small decrease in its activity. Expression of Na⁺-K⁺-ATPase- α subunit was markedly reduced in mature RPT cells after 30 min of hypoxia whereas no such down-regulation was found in the immature RPT cells.

Renal hypoxia results in an up- or down-regulation of more than 1000 genes. Some of these gene products have been shown to mediate hypoxia-induced injury whereas others have been shown to protect from such injury. Several of these factors may contribute to the tolerance of the immature tubules to hypoxia.

One enzyme suggested to have a key role in proximal tubular cell injury is calpain. Calpain is a Ca²⁺-dependent cytosolic cysteine protease with two isoforms, μ - and m-calpain. Only the μ -isoform is expressed in rat renal proximal tubules (17). Renal ischemia is known to induce disruption of the actin cytoskeleton. Calpain cleaves actin-binding proteins such as spectrin, talin, filamin, α -actinin, and ankyrin (18). Calpain inhibition promotes the recovery of mitochondrial respiration and active Na⁺ transport in renal proximal tubules subjected to hypoxia/reoxygenation (19). Mature renal cortex exposed to ischemia/reperfusion responded with an up-regulation of calpain whereas immature renal cortex responded with a down-regulation. Whether calpain promotes dissociation and inhibition of Na⁺-K⁺-ATPase in mature renal tubular cells is a topic for further studies.

Heat shock factor-1 (HSF-1) is a stress responsive transcriptional factor that is activated by hypoxia and ATP depletion (20). Evidence supports a major function of heat shock proteins (HSP), the target genes of HSF, in the regulation of actin dynamics. However, in contrast to calpain, HSP is suggested to have a cytoprotective effect (21, 22). The hypoxic immature tubule has been demonstrated to exhibit more exuberant activation of HSF-1 and HSP-72 compared with the mature tubule, indicating a role of these factors in the tolerance of the immature tubules to hypoxia (23).

Another stress-responsive transcription factor activated by hypoxia is hypoxia-inducible factor 1 (HIF-1) (20). A target gene for HIF-1, heme oxygenase-1 (HO-1), is an enzyme shown to ameliorate renal ischemia and reperfusion injury (24). Renal tubular cells deficient of HO-1 are more susceptible to oxidative stress (25). HO-1 is an antioxidant enzyme and, in general, biochemical studies have demonstrated a developmental up-regulation of antioxidant enzymes. However, renal proximal tubules are shown to have a developmental down-regulation of antioxidants such as manganese superoxide dismutase, copper, zinc superoxidase and glutathione S trans-

ferase (26). We found that the expression of HO-1 was 2-fold increased in immature compared with the mature renal cortex.

CONCLUSION

In summary, these studies add further data toward the understanding of increased tolerance to stress in renal immature tubules. Renal ischemia is a common cause of acute renal failure (ARF) in adults but not in infants. One characteristic feature of ARF is a redistribution of Na⁺-K⁺-ATPase from the basolateral plasma membrane to the cytosol and the apical plasma membrane. Here we demonstrate that hypoxia-induced internalization of Na⁺-K⁺-ATPase is less pronounced in immature compared with mature renal proximal tubules. In addition, the immature kidney appears to have a higher capacity to tolerate ischemia/oxidative stress with a lower expression of cytotoxic proteins and a higher expression of cytoprotective factors.

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