Uremia induces proximal tubular cytoresistance and heme oxygenase-1 expression in the absence of acute kidney injury

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Submitted 28 October 2008; accepted in final form 19 November 2008

Zager RA. Uremia induces proximal tubular cytoresistance and heme oxygenase-1 expression in the absence of acute kidney injury. Am J Physiol Renal Physiol 296: F362–F368, 2009. First published November 26, 2008; doi:10.1152/ajprenal.90645.2008.—Acute kidney injury (AKI) induces adaptive responses within proximal tubular (PT) cells that serve to protect them from further ischemic or toxic injury. However, it is not known whether uremia, a potential consequence of AKI, independently alters susceptibility to tubular injury. To address this issue, we subjected CD-1 mice to bilateral ureteral transection (BUTx), which produces uremia (blood urea nitrogen ~150 mg/dl) in the absence of direct renal damage. PT segments were then isolated from BUTx and control mice and subjected to in vitro hypoxic injury. Additionally, “in vitro uremia” was modeled in isolated tubules or in cultured PT (HK-2) cells by addition of 1) peritoneal dialysate (obtained from mice with bilateral ureteral obstruction), 2) peritoneal fluid (from BUTx mice), or 3) normal human urine (pH 7.4, with and without boiling). Effects on injury severity (lactate dehydrogenase release) were assessed. Finally, because uremia is a prooxidant state, it was hypothesized that BUTx would increase renal lipid peroxidation (malondialdehyde) and induce heme oxygenase-1 (HO-1), a redox-sensitive cytoprotective protein. BUTx conferred striking protection against hypoxic damage. This could be partially modeled in tubules and HK-2 cells by induction of in vitro uremia. Urine’s protective action was heat labile (largely destroyed by boiling). BUTx caused a tripling of renal malondialdehyde and HO-1 protein levels. Increased HO-1 transcription was likely involved, as indicated by a tripling of HO-1 mRNA and RNA polymerase II binding along the HO-1 gene (chromatin immunoprecipitation assay). “Gene-activating” histone modifications [H3K4 trimethylation (H3K4m3)] and histone 2 variant (H2A.Z)] at HO-1 gene loci were also observed. Uremia, per se, can contribute to the AKI-induced cytoresistance. Low-molecular-weight, heat-labile, cytoprotective factor(s) and uremia-induced renal stress responses (e.g., HO-1 gene activation) are likely involved. Finally, renal HO-1 induction following AKI may reflect direct cell injury effects and adaptations to uremia.

METHODS

Uremia Model

Male CD-1 mice (25–30 g body wt; Charles River Laboratories, Wilmington, MA) were maintained under normal vivarium conditions with free access to food and water. After induction of deep anesthesia with pentobarbital sodium (40–50 mg/kg), the mice were subjected to a midline abdominal incision, and both ureters were exposed. Half of the mice underwent BUTx, which was performed approximately half the distance from the ureteral origin at the renal pelvis. The other half of the mice (controls) underwent sham surgery. The abdominal walls were then sutured in two layers, and the mice were allowed to recover from anesthesia. After ~18 h, the mice were reanesthetized with pentobarbital sodium, a blood sample was obtained from the inferior vena cava for blood urea nitrogen (BUN) determination, and both the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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kidneys were resected and iced. The animal surgery protocols were approved by the Institutional Animal Care and Use Committee at the Fred Hutchinson Cancer Research Center.

Preparation of Isolated PT Segments

PT segments were prepared from mice that were subjected to BUTx or sham surgery (controls) as previously described (31). Briefly, after bilateral renal resection, the kidneys were iced and both cortices were resected. The tissues were minced with a razor blade, digested with collagenase, passed through a stainless steel sieve, and pelleted by centrifugation (4°C). PTs were recovered by centrifugation through 32% Percoll (Pharmacia, Piscataway, NJ). After they were washed multiple times in iced buffer, the PTs were suspended (~2–3 mg protein/ml) in experimentation buffer (100 mM NaCl, 2.1 mM KCl, 25 mM NaHCO3, 2.4 mM KH2PO4, 1.2 mM MgSO4, 1.2 mM CaCl2, 5 mM glucose, 1 mM alanine, 4 mM sodium lactate, 10 mM sodium butyrate, and 0.6% 36-kDa dextran, gassed with 95% O2-5% CO2, final pH 7.4). They were warmed from 4°C to 37°C in a heated shaking water bath for 15 min and then divided into four to six equal aliquots for experimentation (see below). Each preparation consisted of tubules isolated from a single mouse.

Effect of BUTx on Response to Hypoxic Injury

PTs were prepared from mice that were subjected to BUTx or sham surgery (n = 5 from each group). One set of tubules from each of the BUTx and control groups was studied simultaneously on any given day. The following groups were prepared: 1) control tubules incubated for 45 min under oxygenated conditions (95% O2-5% CO2), 2) BUTx tubules incubated for 45 min under oxygenated conditions, 3) control tubules subjected to 15 min of hypoxia (gassed with 95% N2-5% CO2), and 4) BUTx tubules subjected to 15 min of hypoxia. After the incubations were completed, lethal cell injury was assessed by calculation of percent lactate dehydrogenase (LDH) release (LDH concentration in tubule incubation buffer after centrifugation / LDH concentration in total tubule suspension).

Effect of Uremic Environment on PT Susceptibility to Injury

The ~0.5 ml of intraperitoneal fluid (IPF) that accumulated in BUTx mice was saved. Its urea concentration closely matched that observed in terminal blood samples (~150 mg/dl; see RESULTS), implying approximate dialytic equilibrium. The following experiment was undertaken to ascertain whether this “uremic” IPF was able to confer a direct cytoprotective effect. Five sets of tubules were prepared from normal mice, and each was divided into aliquots as follows: 1) control oxygenated incubation (with addition of 0.25 ml of saline, 1 ml final incubation volume), 2) oxygenated incubation in the presence of 0.25 ml of IPF (after passage through a 50,000-Da-cutoff filter to remove high-molecular-weight molecules), 3) saline addition followed by 15 min of hypoxic challenge, and 4) filtered IPF addition followed by 15 min of hypoxic challenge. After the incubations were completed, percent LDH release was assessed. [The lack of effect of urea on tubule response to injury was assessed in subsequent experiments (see below).]

Effect of an Uremic Environment on HK-2 Cell Susceptibility to Injury

The HK-2 immortalized human PT cell line (25) was employed to test whether the results observed in PT segments could be recapitulated in a cell culture system. Briefly, the cells were maintained in T75 Costar flasks with keratinocyte serum-free medium (K-SFM) supplemented with glutamine and pituitary extract, as previously described (25). On the day before experimentation, the cells were trypsinized and then seeded into 24-well Costar plates with K-SFM. On the next day, the following experimental groups were prepared: 1) control incubation, 2) incubation with IPF obtained from mice subjected to BUTx (40% IPF-60% K-SFM), 3) ATP depletion injury induced by inhibition of mitochondrial respiration and glycolysis [with 7.5 μM antimycin + 5 μM Ca ionophore A-23187 + 20 mM 2-deoxyglucose (8)], 4) ATP depletion in the presence of IPF, 5) oxidant injury induced by 10 μM ferrous ammonium sulfate complexed to equimolar hydroxyquinolone (FeHQ) to permit Fe to gain intracellular access, and 6) FeHQ-mediated oxidant injury in the presence of IPF (n = 6 replicates, 3 different occasions). After ~18 h, lethal cell injury was assessed as percent LDH release.

Effect of Peritoneal Dialysis Fluid on HK-2 Cell Injury

The following experiment was undertaken to ascertain whether a dialyzable uremic solute can confer a cytoprotective effect. Acute uremia was induced in normal mice by bilateral ureteral ligation (performed through a midline abdominal incision with ligation of the ureters at the approximate midpoint with silk ligatures). After ~18 h, when severe uremia was present (BUN ~150 mg/dl), the mice were reanesthetized with pentobarbital sodium and injected intra-abdominally with 2 ml of 0.9% saline at 37°C. After 30 min, the abdominal cavity was opened, ~0.5 ml of peritoneal fluid was recovered, and the urea nitrogen concentrations were determined. This fluid was utilized in the HK-2 cell injury protocol as described above (with substitution of 40% dialysate fluid for IPF). The control cells were treated in the same fashion, with substitution of 0.9% saline for dialysate fluid. (The obstruction, rather than the BUTx, model of ARF was used, because peritoneal dialysate in the latter model would be contaminated with spontaneously occurring IPF.)

Effect of Urea Addition on HK-2 Cell and Isolated PT Injury

The following two experiments tested whether urea loading, a result of IPF or peritoneal dialysate addition, accounted for the protection that was observed.

HK-2 cells. HK-2 cells on 24-well plates were divided into treatment groups: 1) control incubation, 2) the ATP depletion protocol described above, 3) incubation with 10 μM FeHQ, 4) addition of urea (40% of a 300 mg/dl stock urea solution, with final concentration of 120 mg/dl, thereby approximating urea concentrations produced in the above-described HK-2 IPF or dialysate addition experiments), 5) the ATP depletion protocol in the presence of urea, and 6) the FeHQ protocol in the presence of urea. After 18 h, the extent of lethal cell injury (percent LDH release) was assessed (n = 5 determinations of each treatment).

Isolated PTs. PTs were prepared from five normal mice, and each PT was divided into four aliquots: 1) control incubation, 2) urea addition (final concentration 120 mg/dl), 3) 15 min of hypoxia, and 4) hypoxia in the presence of urea. After 15 min of incubation, percent LDH release was determined.

Initial Characterization of “In Vitro Uremic Cytoprotective Effect”

Addition of human urine aliquots to isolated tubules. The following experiment assessed whether exposure of isolated tubules to an “experimental uremic milieu” via the addition of human urine samples might transfer the above-observed cytoprotective effects. If so, this system might facilitate initial characterization of this uremic property. Nine sets of tubules were prepared from normal mouse kidneys and divided into four aliquots: 1) control oxygenated incubation for 15 min, 2) incubation with urine samples obtained from three healthy individuals (20% urine-80% incubation buffer), 3) 15 min of hypoxia, and 4) 15 min of hypoxia in the presence of 20% human urine. Lethal cell injury was then assessed by determination of percent LDH release. Because acidosis can confer an independent cytoprotective effect (44), pH of the urine samples was adjusted to 7.4 to match that of the tubule incubation medium. Non-urine-treated samples were exposed to equal-volume 0.9% saline additions to maintain a constant volume.
Size exclusion filtration. The above-described 20% urine addition experiment was repeated with urine samples that had first been passed through a 3,000-Da-cutoff filter to remove macromolecular species (n = 5).

Heat stability. Urine samples obtained after 3,000-mol-wt filtration were boiled for 30 min or subjected to three freeze-thaw cycles. The relative ability of these boiled or freeze-thawed samples to mitigate hypoxic injury was then compared with that of the same urine samples not subjected to these treatments (n = 5 each).

**Effects of Uremia on Renal Oxidative Stress and HO-1 Expression**

Uremia is recognized as a prooxidant state (22, 23). Furthermore, preexistent oxidative tissue damage, as manifested by malondialdehyde (MDA) elevations, is known to usher in a cytoresistant state (43). The following experiments were undertaken to assess whether 1) BUTx evokes oxidative stress in renal cortex and 2) there is an associated change in expression of HO-1 [a dominant cytoprotective enzyme expressed in renal tissues (16)]. Mice were subjected to BUTx or sham surgery (n = 8 each). After 18 h, bilateral nephrectomies were performed. The renal cortices were dissected at 4°C, and 1) MDA concentrations were assessed as an index of lipid peroxidation (43), 2) protein was extracted for subsequent HO-1 Western blotting (40), 3) total mRNA was extracted for subsequent assessment of HO-1 mRNA levels by competitive PCR (with values expressed as a ratio to simultaneously determined GAPDH mRNA (40)), and 4) chromatin was isolated for subsequent chromatin immunoprecipitation (ChIP) assay. ChIP assays were performed as previously described (13, 14, 41) to assess 1) RNA polymerase II (Pol II) binding to HO-1 gene loci [start and end exons; a correlative index of gene transcription (12)] and 2) histone modifications at the start (exon 1) and end (exon 5) of the HO-1 gene [H3K4 trimethylation (H3K4m3) and histone 2 variant (H2A.Z)].

**Renal Cortical Cholesterol Content**

Renal cortical cholesterol synthesis is a component of the renal stress response, and the resulting increases can help mediate the cytoresistant state (33, 37). This experiment assessed whether uremia can evoke cholesterol accumulation in otherwise normal kidney tissue. Renal cortical tissues were obtained from eight BUTx and eight sham-operated mice and subjected to lipid extraction (42). Cholesterol (free cholesterol and cholesterol esters) was analyzed by gas chromatography as previously described and expressed as nanomoles per micromole of phospholipid phosphate (42).

**Calculations and Statistics**

Values are means ± SE. Statistical comparisons were performed by unpaired Student’s t-test. Significance was judged by P < 0.05.

**RESULTS**

**BUTx Protects Against Hypoxic Injury**

After 45-min control (oxygenated) incubations, percent LDH release was identical in isolated tubules harvested from control and BUTx mice (Fig. 1A). However, susceptibility to hypoxic injury was markedly reduced in tubules from BUTx mice (Fig. 1A); whereas control tubule LDH release rose from a baseline of 9% to 34% with hypoxia (Δ = 25%), the increase in BUTx tubules was only 5%. BUN concentrations from the BUTx mice were markedly elevated compared with values from normal animals (152 ± 4 vs. 22 ± 1 mg/dl).

**IPF From BUTx Mice Protects Against Hypoxic Cell Injury**

Urea nitrogen concentration in IPF was essentially identical to that in serum (148 ± 4 mg/dl), implying dialytic equilibrium. Addition of IPF to normal tubules did not alter percent LDH release under control oxygenated incubation conditions (Fig. 1B). However, IPF did confer a modicum of protection against hypoxic cell death (Fig. 1B). This protection could not be ascribed to a direct urea effect, inasmuch as addition of pure urea, equal to that in IPF, tended to slightly increase, not decrease, hypoxia-mediated LDH release (Fig. 1C).

**HK-2 Cell Experiments**

**IPF addition.** Addition of IPF to HK-2 cells did not exert an independent effect on cell viability, as assessed by percent LDH release (Fig. 2A). However, IPF virtually eliminated ATP depletion-mediated cell death: 8 ± 1% in the control condition, 75 ± 1% with ATP depletion, and 15 ± 1% with ATP depletion in presence of IPF (Fig. 2A).

**Peritoneal dialysate addition.** Addition of dialysate did not independently alter HK-2 viability under control conditions (Fig. 2B). However, it did decrease the extent of ATP depletion-mediated and FeHQ-mediated oxidant cell death (Fig. 2B).

**Urea addition.** IPF- or dialysate-mediated protection could not be ascribed to a direct effect of urea, because addition of urea did not alter the extent of ATP depletion or Fe-mediated cell death: 73 ± 1% and 72 ± 1% LDH release without and with urea, respectively, for ATP depletion and 24 ± 1 and 29 ± 1% LDH release without and with urea, respectively, for Fe-mediated injury.

**Initial Characterization of Uremia-Induced Cytoprotective Effect**

As shown in Fig. 3, the addition of normal human urine samples to isolated PTs did not alter LDH release under control (oxygenated) incubation. However, each of the urine samples
markedly diminished hypoxic injury, as denoted by a reduction of LDH release from ~52% to ~20% (Fig. 3). The cytoprotective factor(s) were low molecular weight, given that filtering the urine samples through 3,000-kDa-cutoff filters did not diminish urine’s cytoprotective effect. Freeze-thawing also did not impact urine’s cytoprotective property. However, the latter was largely destroyed by boiling, indicating that the active property was neither an inorganic salt nor urea.

Evaluation of Uremia-Induced Renal Cortical Oxidative Stress

MDA and HO-1 expression in response to BUTx. BUTx induced an approximately threefold increase in renal cortical MDA (Fig. 4A). To assess whether this change was associated with a change in HO-1 protein levels, the latter was assessed by Western blotting, and a threefold increase was observed. A quantitatively comparable (~3-fold) increase in HO-1 mRNA was observed (Fig. 5A).

To further assess whether BUTx leads to activation of the HO-1 gene, we assessed Pol II recruitment to HO-1 gene loci. BUTx induced a threefold increase in Pol II binding at the start (exon 1) and end (exon 5) of the HO-1 gene (Fig. 5B).

Histone modifications at the HO-1 gene. To ascertain whether BUTx might also alter histone expression at HO-1 gene loci, we assessed a representative histone modification [trimethylation of H3K4 (H3K4m3)] at HO-1 exons 1 and 5. An approximate doubling was observed at both loci (Fig. 6A).

To assess whether a normally repressed histone variant (H2A.Z) might be upregulated in BUTx, we determined H2A.Z levels. An approximate doubling at HO-1 exons 1 and 5 was observed.

Renal Cortical Cholesterol Content

Free cholesterol levels were significantly higher in renal cortex obtained from BUTx mice than sham-operated controls: 292 ± 6 vs. 268 ± 7 nmol/μmol phospholipid phosphate (P < 0.005). Renal cortical cholesteryl esters (a reflection of cellular cholesterol excess, i.e., cholesterol flux into the ester storage pools) were approximately twice as great in the BUTx as in the control tissue samples: 9.3 ± 0.4 vs. 4.0 ± 0.3 nmol/μmol phospholipid phosphate (P < 0.0001). Thus, similar to HO-1, a lipid cytoprotectant was also induced in renal cortex by the uremic environment.

DISCUSSION

It has been widely demonstrated that diverse forms of AKI can confer cytoprotection against subsequent renal damage (for

AJP-Renal Physiol • VOL 296 • FEBRUARY 2009 • www.ajprenal.org
uremia, by itself, can confer a direct PT cytoprotective effect. As shown in Fig. 1A, whereas BUTx did not alter tubule viability during control incubations, when tubules were subjected to hypoxia, a marked reduction in lethal cell injury (LDH release) was observed. At least part of the cytoprotection observed in BUTx tubules could be rapidly transferred to normal tubules by exposure to an experimental “uremic milieu.” For example, as shown in Fig. 1B, within minutes of addition of small amounts of IPF (which was in dialytic equilibrium with plasma), hypoxic tubular injury was reduced. However, the extent of this protection was only ~50% of that observed in tubules harvested from BUTx mice. This suggests that, in addition to tubule exposure to “uremic molecules,” secondary tubular adaptations may also contribute to the cytoprotected state (see below).

Because the extraction of PTs from renal cortex induces some baseline cell injury, the impact of a uremic milieu on tubule injury susceptibility was also assessed in a cell culture system, i.e., where no isolation injury exists. Cultured HK-2 cells were exposed to a uremic environment for 18 h (via IPF addition), during which time ATP depletion injury was imposed by mitochondrial + glycolytic blockade. As shown in Fig. 2A, IPF conferred almost complete protection against ATP depletion injury, reducing LDH release from 73% to 12%. To underscore that the uremic environment can, indeed, protect against injury, ARF was induced in mice by bilateral ureteral obstruction, and 18 h later, peritoneal dialysis was performed. When the resulting dialysate was added to HK-2 cells, protection against hypoxic injury was observed. This protection against ATP depletion injury and oxidant (Fe-mediated) injury indicates a potentially broad-based cytoprotective effect. Finally, it is notable that equimolar urea exposure had no effect on ATP depletion injury, whether imposed on HK-2 cells or isolated PTs. Hence, the protection conferred by a uremic milieu cannot simply be attributed to a urea-induced hyperosmolar state.

The ability of peritoneal dialysate to directly transfer cytoprotection to tubular cells indicates that the responsive molecule(s) are dialyzable; hence, these compounds are likely of low molecular weight and undergo urinary excretion. To test this concept, PTs were exposed to normal human urine samples (pH corrected to 7.4) and then their impact on hypoxic tubule damage was assessed. As shown in Fig. 3, a dramatic reduction in hypoxic injury was observed. To confirm that this protection was, indeed, imparted by low-molecular-weight compound(s), the urine samples were passed through 3,000-Da-cutoff filters and then retested. A complete preservation of cytoprotection was observed. The responsible urine factor(s) was highly heat labile, as indicated by a dramatic loss of protective activity by boiling. This observation clearly indicates that inorganic urinary salts (e.g., Na, K, Cl, phosphates, and sulfates, which are heat stable) are not responsible for urine’s protective property. The exact uremic/urinary component(s) that elicits the cytoprotection is obviously of great experimental and potential pharmacological interest. As just one example, it seems possible that the excretion of a naturally occurring cytoprotectant molecule could help tubular cells withstand the low oxygen tensions that normally exist in the renal medulla (i.e., which is said to exist “on the brink of anoxia”). Also, these factor(s) might protect against the rapid osmotic shifts that are natural consequences of urinary concentration and dilution. Unfortunately, despite considerable efforts undertaken by this laboratory, the exact nature of these compound(s) remains to be defined. Hence, it is premature to speculate as to this issue.

Fig. 5. BUTx increases HO-1 mRNA and RNA polymerase II (Pol II) binding to the HO-1 gene. A: BUTx induced a ~3-fold increase in HO-1 mRNA levels. B: This likely arose from increased HO-1 gene transcription, because 3-fold increases in Pol II binding to HO-1 start and end exons (exons 1 and 5, respectively) were observed.

Fig. 6. BUTx modifies selected histone expression at HO-1 exons 1 and 5. To assess whether BUTx can alter histone expression at the HO-1 gene, a representative histone modification, trimethylation of H3K4 (H3K4m3), and the levels of a normally repressed histone 2 variant (H2A.Z) were assessed at HO-1 start and end exons. BUTx induced increases in both instances and at both exons.
PTs that are harvested from BUTx mice and incubated in normal experimentation buffer are no longer exposed to a uremic environment. Nevertheless, these tubules manifested cytoresistance to hypoxic damage (see above). This finding suggests that prior in vivo exposure to uremia evoked secondary tubular adaptations that contributed to the cytoprotected state. Stated differently, cytoresistance might arise from two phenomena: 1) direct exposure to cytoprotective uremic compounds (e.g., as present in urine, IPF, or dialysate) and 2) secondary cellular adaptations to in vivo uremia. It is well known that uremia represents a prooxidant state (22, 23). This suggests that uremia, per se, induces renal oxidative stress, which leads to the induction of stress-activated cytoprotective molecules, e.g., HO-1. To test this hypothesis, MDAC concentrations, a marker of lipid peroxidation, were measured in renal cortices obtained from BUTx and control animals. Threefold-higher levels were observed in BUTx than in control tissue samples. This observation directly supports the concept that uremia-induced renal oxidant stress occurred. To explore whether there was a secondary increase in HO-1, HO-1 protein and its mRNA were measured. In both instances, approximately threefold elevations were observed. To test whether these HO-1 mRNA and protein increases reflected increased HO-1 transcription, Pol II recruitment to the start and end exons of the HO-1 gene was assessed (by ChIP assay). As with HO-1 protein and HO-1 mRNA, approximately threefold increases in Pol II levels at HO-1 start and end exons strongly suggest that increased HO-1 transcription caused, or contributed to, the HO-1 protein increases. Pol II recruitment to, and activity at, specific gene loci can be strongly influenced by epigenetic modifications at these sites. Two potential gene-activating histone modifications are trimethylation of H3K4 (H3K4m3) and increases in the histone-2 variant H2A.Z (13, 14). Hence, these two histone marks were quantified in control and BUTx tissue samples, and approximately twofold increases in both at start and end HO-1 exons were observed. The molecular inducers of these histone marks and whether they mechanistically contribute to HO-1 transcription remain unknown at this time. However, one additional point does seem clear: when AKI leads to increased renal HO-1 expression, direct cell injury effects, as well as the secondary effects of associated uremia, may each play a role.

It is highly unlikely that increased HO-1 expression is the only component of the renal “stress response” that is induced by BUTx-induced uremia. For example, many other cytoprotective pathways might also respond. As just one example, increased renal cholesterol synthesis is part of the acute renal stress response (39), and the cholesterol increases can help mediate a cyoresistant state. To test this hypothesis, free cholesterol and cholesterol ester levels were measured in renal cortex obtained from BUTx and control renal tissues, and substantial increases in each were observed in the BUTx samples. These data simply serve to underscore that uremia-induced cyoresistance likely reflects the impact of multiple, and potentially interactive, components of a renal stress response, rather than any single cellular adaptive event.

In conclusion, the present study demonstrates, we believe for the first time, that uremia, in the absence of primary kidney injury, exerts a cytoprotective effect on renal PTs. Thus the so-called phenomenon of acquired resistance to acute renal failure, whereby one renal insult confers protection against another, is not necessarily a sole consequence of direct tubular damage. Rather, if AKI induces uremia, the latter may also evoke cytoprotective effects. Uremia-initiated cyoresistance may arise from two general pathways: 1) the accumulation of yet-to-be-defined low-molecular-weight heat-labile cytoprotectants that are normally excreted in urine and 2) a uremia-initiated stress response within the renal tubules. Uremia-induced renal oxidant stress, with corresponding increases in HO-1 gene expression, and renal tubular cholesterol accumulation are illustrative of this latter pathway. Finally, because the present study documented heterogeneous biological adaptations within normal tubules in response to uremia (e.g., epigenetic modifications at, or Pol II recruitment to, the HO-1 gene or cholesterol accumulation) it would appear that the BUTx mouse model has substantial utility for studying interactions between uremia and renal homeostasis.

ACKNOWLEDGMENTS

The authors thank A. C. M. Johnson, S. Lund, K. Nayeon, and M. Naito for expert technical assistance.

GRANTS

This project was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-R37-38432 and DK-68520.

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