Renal cortical hexokinase and pentose phosphate pathway activation through the EGFR/Akt signaling pathway in endotoxin-induced acute kidney injury

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The pathophysiology of sepsis-induced AKI is widely recognized as multifactorial, involving microvascular, immunological, and tubular components that contribute to renal dysfunction. The microvascular component is characterized by reduced capillary flow, leading to local areas of hypoperfusion (29, 30, 66). Infiltration of both macrophages and neutrophils also exposes the septic kidney to a diverse array of proinflammatory factors (56, 72). Finally, tubular damage characterized by tubular cell vacuolization, mild tubular dilatation, and mitochondrial swelling has been noted in septic AKI (57, 62). Although histopathology in sepsis-induced AKI appears modest, preservation of renal function depends heavily on the proximal tubule for reabsorption and secretion of solutes, including sodium, glucose, and amino acids, from the glomerular filtrate via active transport processes. Therefore, any pathological condition resulting in reduced ATP levels may contribute to tubular deenergization and subsequent loss of renal function characteristic of AKI (51, 55).

Our laboratory demonstrated that I/R- and glycerol-induced AKI leads to rapid and sustained mitochondrial dysfunction in the renal cortex, characterized by suppressed expression of mitochondrial biogenesis markers and electron transport chain components at the mRNA and protein levels (16). Disruption of normal mitochondrial homeostasis in these models was closely associated with proximal tubule cell injury and loss of renal function (16). Tran et al. (62) recently reported similar findings following systemic exposure to LPS (a component of gram-negative bacterial cell walls), a well-established model of sepsis-induced AKI. In particular, LPS administration resulted in a marked reduction in cytochrome c oxidase activity and protein levels as well as downregulation of a number of mitochondrial genes in the renal cortex. Suppression of mitochondrial mRNAs correlated with renal function as measured by blood urea nitrogen (BUN) (62). LPS-induced mitochondrial dysfunction results in a decline (~50%) in renal ATP content within 5 h of exposure in rodents, likely contributing to changes in renal function in this model of septic AKI (32). Disruption of renal mitochondrial function and ATP production has also been reported in the cecal ligation and puncture model (CLP) of sepsis (40). What remains understudied are metabolic alterations which may facilitate both proximal tubule cell energy production and transport function in the presence of mitochondrial suppression.

Under normal conditions in vivo, renal proximal tubule cells (RPTC) primarily utilize oxidative phosphorylation via mitochondria to generate ATP and have very low glycolytic capacity. Early work characterizing renal metabolism demonstrated the proximal tubule possesses the lowest activities of glycolytic enzymes, including hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK) (the 3 major regulatory enzymes, including hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK) (the 3 major regulatory
steps), along the entire nephron segment (19, 70). These findings have led to considerable debate concerning the ability of the proximal tubule to induce glycolytic metabolism as an alternate means of ATP generation. However, both in vitro and in vivo work indicate that a variety of stressors including ischemia-hypoxia, nephrotoxins, elevated intracellular calcium, and inhibition of mitochondrial respiration may increase glycolytic flux in the proximal tubule (4, 14, 20). Glycolytic induction appears to play an important role in maintaining tubular cell viability, producing ATP, and preserving transport processes (14, 47, 72). In addition, recent studies have demonstrated that glycolytic metabolism is rapidly increased in the cortex in response to renal ischemia in vivo (75). These findings indicate that glycolysis may provide an alternate means of energy generation in response to tubular insult.

We hypothesized that glycolysis would be rapidly induced in the renal cortex in response to LPS-induced renal injury. To test this hypothesis, an extensive characterization of renal cortical glycolytic enzyme activity and expression after LPS challenge was performed. We report herein that LPS exposure results in a rapid (within 3 h) and specific induction of HK activity mediated by the EGF receptor (EGFR)/phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway. Surprisingly, HK activation by LPS was not sufficient to enhance glycolysis in the renal cortex but was associated with upregulation of the pentose phosphate pathway.

METHODS

Animal model. Male C57BL/6 mice (6–8 wk of age, 20–25 g body wt) were obtained from the National Institutes of Health National Cancer Institute (Bethesda, MD). Mice received an intraperitoneal injection of 10 mg/kg LPS from Escherichia coli O111:B4 (catalog no. L4130, lot no. 052M4016V, Sigma-Aldrich) as previously described (62). Control mice were injected intraperitoneally (ip) with an equal volume of 0.9% saline vehicle. Mice were euthanized at 1, 3, 6, and 18 h after LPS injection, and serum and kidneys were collected for biochemical analysis. All experiments were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animal use was approved by the Institutional Animal Care and Use Committee at the Medical University of South Carolina.

Administration of gefitinib and MK-2206. For studies examining the role of EGFR and PI3K/Akt signaling in LPS-induced renal injury, the EGFR inhibitor gefitinib and the Akt inhibitor MK-2206 were obtained from Selleckchem Chemicals (Houston, TX) (12, 23). Mice were randomly assigned to four groups: 1) saline+vehicle control, 2) LPS+vehicle control, 3) LPS+gefitinib (100 mg/kg), or 4) LPS+MK-2206 (100 mg/kg), and inhibitors were administered ip 1 h before LPS administration. Dosing of gefitinib and MK-2206 was based on previous studies demonstrating inhibition of EGFR and Akt in mouse models (21, 33).

Routine biochemical assays. Blood urea nitrogen (BUN) was measured using a QuantiChrom Urea Assay Kit (BioAssay Systems, Hayward, CA) according to the manufacturer’s protocol. Values are expressed as BUN concentration in milligrams per deciliter.

Renal cortical HK, PFK, PK, and glucose-6-phosphate dehydrogenase (G6PDH) activities and glucose, pyruvate, lactate, and glycogen contents were determined using kits from BioVision (Milpitas, CA) according to the manufacturer’s protocol. Briefly, renal cortical tissue was homogenized and centrifuged at 14,000 g for 10 min to clear debris. Values were normalized to protein content (determined by BCA assay) or wet tissue weight as described in each figure.

Analysis of mRNA expression. Total RNA was isolated from renal cortex samples using the TRIzol reagent (Life Technologies, Grand Island, NY). A cDNA library was generated from 2 μg RNA by reverse transcription reaction using an iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the manufacturer’s protocol (Bio-Rad, Hercules, CA). Quantitative real-time PCR was then performed with cDNA using the SsoAdvanced SYBR Green Supermix (Bio-Rad). mRNA expression of genes of interest was calculated by the ΔΔCt method and normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) as previously described (67). Primer sequences used for mRNA analysis are shown in Table 1.

Immunoblot analysis. Tissue samples from the renal cortex were lysed in RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton X-100, pH 7.4) containing protease inhibitor cocktail (1:100), 1 mM sodium orthovanadate, and 10 mM sodium fluoride (Sigma-Aldrich). Total protein content was determined by BCA protein assay. Equal amounts of protein (50–100 μg) were resolved on 4–15% SDS-PAGE gels (Bio-Rad). Proteins were then transferred to nitrocellulose membranes and blocked in 2.5% BSA for 1 h. Membranes were incubated with primary antibody overnight at 4°C. Primary antibodies included HK1, HK2, PFKP, PKM1/2, PDK, and phospho-Akt (Ser 473), total Akt (1:1,000, all from Cell Signaling Technology, Danvers, MA), VDAC/porin (1:1,000, Abcam, Cambridge, MA), and β-actin (1:10,000, Santa Cruz Biotechnology, Dallas, TX). Membranes were then incubated in horseradish peroxidase (HRP)-conjugated secondary for 1 h at room temperature. Proteins were visualized using enhanced chemiluminescence reagents (Thermo Scientific, Waltham, MA) and the GE ImageQuant LAS4000 digital imaging system. National Institutes of Health ImageJ software (v. 1.46) was used to measure optical density.

Mitochondrial fractionation. Mitochondria were isolated from whole kidneys by differential centrifugation as previously described (3). The resulting mitochondrial pellet was resuspended in RIPA buffer, and mitochondrial localization of HK1 and HK2 was determined by Western blot analysis using antibodies against HK1, HK2, VDAC/porin, and PDK.

Table 1. Primers used in mRNA analysis

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LPS-induced HK activation occurs independently of isoform expression. Because HK activity in the renal cortex increased following LPS exposure, we determined the effects of LPS on the expression of HK isoforms at both the mRNA and protein level via qPCR and immunoblot analysis at 3 and 18 h post-LPS. HK1 mRNA levels did not change in response to LPS exposure at any time point. HK2 gene expression increased ~33-fold at 3 h in LPS-treated mice but returned to baseline levels by 18 h post-LPS. LPS administration also resulted in increases in HK3 mRNA levels at both 3 (~2-fold) and 18 h (~5-fold) (Fig. 2, A and B). Although increased gene expression of HK2 and HK3 was observed in LPS-treated mice, immunoblot analysis revealed no changes in protein levels of HK1 or HK2 at 18 h after LPS administration (Fig. 2, C and D). These data demonstrate that rapid HK activation in the renal cortex of LPS-treated mice is independent of changes in HK isoform expression.

RESULTS

LPS increases renal cortical HK activity. Because suppression of both mitochondrial function and gene expression has been reported in multiple animal models of AKI, including sepsis-induced AKI, we hypothesized that glycolytic metabolism would increase in the renal cortex following LPS exposure (16, 62). We first measured activities of the three major rate-limiting enzymes in the glycolytic pathway, HK, PFK, and PK, in the renal cortex at 3, 6, and 18 h after LPS exposure. Renal cortical HK activity increased approximately twofold as early as 3 h post-LPS and remained elevated for at least 18 h (Fig. 1A). However, PFK and PK activities were unchanged through 18 h (Fig. 1, C and D). BUN progressively increased from ~32 mg/dl in vehicle-treated mice to 61 and 122 mg/dl at 3 and 18 h after LPS administration, respectively (Fig. 1B). Taken together, these findings indicate that HK is specifically and rapidly activated in the renal cortex of mice subjected to LPS-induced AKI.

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LPS exposure does not alter mitochondrial localization of HK1 or HK2. Both HK1 and HK2 isoforms possess mitochondrial binding domains which allow for their association with the outer mitochondrial membrane (45, 68). Because mitochondrial binding of HK has been demonstrated in the kidney and may result in increased HK activity, we determined the effects of LPS administration on HK1 and HK2 localization to mitochondria. Mitochondria were isolated by differential centrifugation from kidneys of vehicle- and LPS-treated mice at 18 h. Protein levels of HK1 and HK2 in the mitochondrial fraction were determined by immunoblot analysis using VDAC as a

Fig. 1. Hexokinase (HK) activation and renal dysfunction after LPS exposure. Male C57BL/6 mice were injected with LPS (10 mg/kg ip) or saline vehicle and euthanized at 3, 6, and 18 h. Renal cortical tissue was isolated and activities of HK (A), phosphofructokinase (PFK; C), and pyruvate kinase (PK; D) were measured and normalized to total protein content. Renal function at 3 and 18 h post-LPS was assessed by measuring blood urea nitrogen (BUN; B). The number of animals used for analysis in each group is denoted in the bar. Data are expressed as means ± SE for each group. Different superscripts above bars indicate statistically significant differences (P < 0.05) compared with time-point controls. Bars with multiple superscripts (a, b) are results not significantly different from other groups.
mitochondrial loading control. No differences were noted in the mitochondrial localization of either HK1 or HK2 following LPS administration (Fig. 3, A, C, and D). The purity of mitochondrial fractions was confirmed by immunoblot analysis for VDAC, α-tubulin, and lamin B1 (Fig. 3B). These findings demonstrate that LPS-induced increases in renal cortical HK activity cannot be attributed to mitochondrial localization.

LPS administration does not alter expression of other glycolysis-related enzymes in the renal cortex. We also examined expression of a number of other glycolysis-related enzymes in the renal cortex of mice exposed to LPS. In particular were genes and proteins involved in cellular glucose uptake (GLUT1/Slc2a1), glycolytic metabolism (PFKP, PFKL, PFKM, PGK1, PKM), pyruvate conversion into...
lactate (LDHA), and pyruvate entry into the TCA cycle (PDK1, PDH). No increases were noted in gene expression of glycolysis-related enzymes at 18 h post-LPS (Fig. 4A). Transcript levels of the glycolytic enzymes PFK, muscle type (PFKM), and phosphoglycerate kinase 1 (PGK1) were significantly decreased (~40%) following LPS exposure (Fig. 4A). In addition, no changes in protein expression of glycolysis-related enzymes were observed in mice treated with LPS (Fig. 4, B and C). Taken together, these data indicate that LPS exposure does not result in changes in gene or protein expression to facilitate glycolytic metabolism in the renal cortex.

**LPS-induced HK activation does not enhance glycolytic flux in the renal cortex.** To determine whether LPS-induced activation of HK was sufficient to increase glycolytic metabolism, we next measured end products of glycolysis. Mice were treated with LPS or vehicle control and kidneys were harvested at 3 and 18 h after injection to determine pyruvate and lactate in renal cortical tissue. LPS exposure decreased pyruvate content (~50%) at both the 3 and 18-h time points (Fig. 5A). Cortical lactate levels were also reduced to ~50% of control levels at 3 h post-LPS. However, lactate content returned to baseline levels by the 18-h time point (Fig. 5B). The changes observed in both pyruvate and lactate levels did not result in an increase in the lactate-to-pyruvate ratio at 18 h post-LPS (Fig. 5C). These findings demonstrate that, although LPS exposure results in activation of HK in the renal cortex, increased HK activity is not adequate to increase flux of glucose through the glycolytic pathway.

*Increased HK activity after LPS exposure does not promote glycogen synthesis.* Because increased HK activity due to LPS exposure in the renal cortex did not result in enhanced glycolytic flux, we sought to determine whether HK activation in this setting has an alternate physiological role. Conversion of glucose to glucose-6-phosphate by HK has been described as a rate-limiting step in glycogen synthesis in a variety of model systems, including rodent skeletal muscle (10, 41, 50). Thus we measured glucose and glycogen levels following LPS exposure to determine whether HK activation in this model may result in glycogenesis. Glucose levels in the renal cortex were reduced 3 h after LPS administration and remained lower at 18 h (Fig. 6A). Measurement of glycogen content revealed no change in LPS-treated mice at 3 h and a ~60% reduction at 18 h (Fig. 6B). Our results demonstrate that LPS-induced HK activation does not stimulate glycogen synthesis.

**HK activation after LPS exposure increased pentose phosphate pathway activity.** We next questioned whether renal cortical HK activation following systemic LPS exposure might facilitate glucose flux through the pentose phosphate pathway (PPP). G6PDH activity, the rate-limiting step of the PPP, was measured (53). Renal cortical G6PDH activity increased ~3.5-fold at 3 h after LPS administration (Fig. 6C). Thus HK activation following LPS exposure is associated with an increase in G6PDH activity.

**LPS-induced HK activity in the renal cortex is mediated by EGFR and Akt.** To understand the mechanism by which LPS exposure increases HK activation, we investigated the role of EGFR. Earlier studies from our laboratory and others have shown that EGFR ligands (EGF, HB-EGF) stimulate HK activity in RPTC (9, 36, 46, 54). LPS is also known to induce EGFR transactivation in a variety of cell types, including epithelial cells and renal medullary collecting duct cells (15, 24, 28, 34, 61). One hour before LPS administration, mice were treated with the EGFR inhibitor gefitinib (100 mg/kg) or vehicle control and kidneys were harvested 3 h post-LPS. This dose has previously been reported to inhibit EGFR signaling following AKI in mice (21). LPS-induced HK activity was completely blocked by gefitinib, suggesting an essential role for EGFR in this process (Fig. 7A).
Because EGFR is known to activate PI3K/Akt signaling, we determined whether Akt is responsible for EGFR-mediated HK activation. To demonstrate Akt activation following systemic LPS exposure, mice were treated with LPS or vehicle control and kidneys were harvested 1 h later for immunoblot analysis of phosphorylated and total Akt. LPS exposure resulted in a 1.5-fold increase in expression of phospho-Akt without a change in total Akt (Fig. 7, C and D). Based on these findings, another group of mice was pretreated with the pan-Akt inhibitor MK-2206 (100 mg/kg) 1 h before LPS injection and kidneys were harvested 3 h post-LPS. MK-2206 is an allosteric inhibitor of Akt and exhibits high selectivity over other kinases because it requires the pleckstrin homology domain for its activity (74). LPS-induced HK activity was attenuated following Akt inhibition by MK-2206 (Fig. 7B). These data indicate that the EGFR/PI3K/Akt signaling axis is responsible for activation of renal cortical HK in LPS-induced AKI.

DISCUSSION

Mitochondrial dysfunction is an important contributor to the pathophysiology of multiple forms of AKI (16, 52). Although histological changes in sepsis-induced AKI are limited, post mortem and experimental studies have demonstrated mitochondrial swelling in the relative absence of overt tubular cell death (57, 62, 63). On the molecular level, sepsis-induced AKI results in suppression of mitochondrial genes which is associated with functional decline (62). Patil et al. (40) recently demonstrated reduced renal electron transport chain complex I and complex II/III activity occurring as early as 6 h after cecal ligation and puncture in mice. Furthermore, a reduction of renal ATP levels has been observed in rodent models of sepsis-induced AKI which correlated well with renal dysfunction (32, 40, 49). Taken together, these data provide strong evidence that mitochondrial dysfunction plays a central role in the pathophysiology of sepsis-induced AKI. To this point, therapies that restore mitochondrial function and/or reduce oxidative stress have proven beneficial in experimental models (40, 66).

We hypothesized that RPTC might respond to mitochondrial dysfunction by increasing glycolytic flux to generate ATP. Although the proximal tubule has been viewed as having very low glycolytic capacity, studies have revealed that glycolysis may be induced in RPTC in response to a cellular insult (5, 19, 70). Dickman and Mandel (14) demonstrated that proximal tubules in vitro increase glycolytic metabolism in response to hypoxia, mitochondrial uncoupling, and inhibition of complex I of the electron transport chain. Inhibition of the Na+/K+-ATPase and glycolysis in proximal tubules following complex I inhibition indicated that glycolytic induction serves to promote cellular function and survival (14). In vivo data also reveal that anaerobic glycolysis is increased in the renal cortex during ischemia but rapidly returns to baseline levels after reperfusion (75). These data provide strong evidence that glycolytic metabolism can be activated for energy production in the proximal tubule.

We performed a comprehensive evaluation of the glycolytic pathway to examine activities of the major regulatory enzymes, mRNA and protein levels of key components of glucose metabolism, and glucose flux in mouse kidneys following LPS exposure. The data obtained support the hypothesis that sepsis-induced AKI is accompanied by enhanced HK activity in RPTC, which is associated with increased glucose-6-phosphate dehydrogenase (G6PDH) activity, glucose production, and glycogen synthesis, and is accompanied by reduced renal AMPK activity. These findings provide novel evidence of a direct role for HK activation in the pathogenesis of sepsis-induced AKI, which may be amenable to therapeutic intervention.
metabolism, and end products of glycolysis (pyruvate and lactate). We observed an increase in renal cortical HK activity as early as 3 h after LPS exposure in mice that was maintained for at least 18 h without an upregulation of any other glycolytic enzymes nor an increase in flux through the glycolytic pathway as measured by renal cortical pyruvate and lactate levels.

To our knowledge, this is the first demonstration of rapid, specific activation of HK in the renal cortex after systemic LPS administration. Three high-affinity HK isoforms (Km values in the micromolar range) are expressed in the mammalian kidney (HK1, HK2, and HK3) (18, 45). HK1 is constitutively expressed/active and accounts for ~70% of total renal HK activity under normal conditions. In contrast, HK2 appears to be regulated in response to a variety of stimuli (37, 43, 58). Little is known about the regulation of HK3 expression/activity. Together, HK2 and HK3 account for the remainder of renal HK activity (~30%) (9).

The experimental method used to measure HK activity in this study does not distinguish which HK isoform is activated following systemic LPS exposure. Therefore, we measured HK isoforms at both the mRNA and protein levels in the renal cortex. Although early changes were noted in mRNA levels of HK2, no changes were seen in HK1 or HK2 isoforms at the protein level following LPS exposure. HK3 mRNA expression increased at both 3- and 18-h time points. We were not able to measure HK3 protein due to the lack of a validated antibody with reactivity to mouse HK3. An anabolic role (PPP or glycogen synthesis) has been proposed for HK2 and HK3 since these isoforms are subjected to inhibition by glucose-6-phosphate and Pi, whereas HK1 is thought to mainly facilitate glycolytic metabolism (69).

Given that HK isoform expression did not change after LPS exposure, we investigated whether HK activation might be attributed to increased mitochondrial localization. Recent evidence revealed that mitochondrial localization of HK isoforms may serve a number of physiological roles, including direct coupling of glucose phosphorylation to the intramitochondrial ATP pool, reducing feedback inhibition by glucose-6-phosphate (G-6-P), and preventing initiation of apoptosis by pro-apoptotic members of the Bcl-2 family (2, 8, 38, 39, 68). However, we did not observe any changes in mitochondrial localization of either HK1 or HK2 in response to systemic LPS exposure. In contrast to HK1 and HK2, HK3 does not possess a mitochondrial localization sequence and is thought to be predominantly perinuclear in location (42). Taken together, these data suggest that renal cortical HK activation in endotoxin-induced AKI is likely due to a posttranslational modification (i.e., phosphorylation) which regulates HK activity (35, 44).

Although HK activity rapidly increased and was sustained for up to 18 h after LPS exposure, we observed no changes in pyruvate and lactate content indicative of increased glycolysis. For the purposes of this study, glycolytic flux was defined as the conversion of glucose to pyruvate as well as downstream generation of lactate. Lactate-to-pyruvate ratios were compared under different experimental conditions as an indicator of anaerobic glycolysis. Under anaerobic conditions, LDH would be expected to convert pyruvate to lactate, resulting in increased lactate with a corresponding equimolar reduction in pyruvate. Pyruvate content was decreased approximately twofold at both 3 and 18 h, whereas lactate levels trended toward a decrease at 3 h but returned to baseline levels by 18 h post-LPS. These findings are in agreement with recent reports of renal cortical pyruvate depletion in both I/R and glycerol-induced AKI in mice (75). The same study also reported glycolytic induction in the renal cortex only occurs during the ischemic period and is reversed after reperfusion (75). However, it should be noted that some of the analyses performed here, including pyruvate and lactate measurements, are not sufficiently powered to detect small changes between groups. Thus it is possible that there are minimal changes in glycolytic flux in the renal cortex up to 18 h after LPS exposure that we were not able to distinguish.

Using a combination of microultrasound and blood oxygen level-dependent MRI, Tran et al. (62) demonstrated that although renal perfusion is markedly decreased in LPS-treated mice, there is minimal change in tissue oxygenation (62). Thus the lack of renal hypoxia may explain why we did not detect increases in lactate following systemic LPS administration. However, the importance of hypoperfusion/hypoxia in renal
pathophysiology remains unclear. Changes in renal blood flow following endotoxin exposure are local and dynamic at the dose used in this study. Wu et al. (71) reported the disruption in cortical peritubular capillary flow at 10 h after LPS administration in mice. Peritubular capillary dysfunction observed in this study was correlated with an increase in tubular NAD(P)H autofluorescence, suggesting that local hypoxia may contribute to cellular injury after LPS (71). In light of these findings, it is important to note that our analyses are limited to only two time points (3 and 18 h) after endotoxin exposure.

Our analysis of glycolytic enzyme activities was restricted to the major rate-limiting enzymes in the glycolysis pathway (HK, PFK, PK). In addition, we measured mRNA and protein expression of other enzymes involved in glucose metabolism in the renal cortex following LPS exposure (e.g., GLUT1, PKG, LDHA, PDH, PDK1). There was no evidence of increased mRNA or protein expression of any of these components, indicating that LPS-induced AKI does not result in early activation of a transcriptional program to facilitate glycolysis in the renal cortex. We cannot rule out the possibility that activities of one or more of these enzymes are increased after endotoxin exposure without an associated increase in expression. However, the data presented here demonstrating minimal changes in the lactate-to-pyruvate ratio, specific HK activation, and an absence of increases in expression of other glycolytic components provide considerable evidence indicating that glycolysis is minimally activated at early time points (3 and 18 h) following endotoxin-induced AKI. Further studies are necessary to determine whether glycolytic metabolism might be activated in the chronic phase.

In addition to glycolysis, G-6-P has multiple fates that include flux through the PPP to produce NADPH and nucleotide/amino acid precursors, glycogen synthesis, and the HK biosynthetic pathway. Zager et al. (75) reported that pyruvate depletion in the renal cortex following ischemic and nephrotoxic forms of AKI was partially attributed to an increase in gluconeogenesis and glycogen synthesis. Interestingly, generation of G-6-P by HK is thought to be a rate-limiting step in glycogen synthesis in a number of tissues and has been associated with glycogen supercompensation in rat skeletal muscle (10, 25, 41, 50). In contrast to changes observed in other mouse models of AKI, we observed a decrease in both renal cortical glucose and glycogen content. Together, these data indicate that gluconeogenesis and glycogen content are not upregulated in the septic kidney. These findings are consistent with the reduction in renal glucose and gluconeogenic enzymes in the kidney after endotoxin administration in rats (1, 22).

G-6-P generated following HK activation in the renal cortex may also be utilized by the PPP. Through the PPP, G-6-P is further metabolized by the rate-limiting enzyme G6PDH to generate NADPH and biosynthetic precursors (45). Reduced NADPH levels in G6PDH-deficient mice were associated with increased renal oxidative stress, inflammation, and dysfunction, indicating an important role for NADPH in antioxidant defense in the kidney (73). Given that oxidative stress rapidly develops in the proximal tubule following systemic LPS exposure, these findings suggest an alternate hypothesis that HK activation may contribute to PPP activity (26). Interestingly, G6PDH activity was increased (~3.5 fold) in the renal cortex of mice exposed to LPS. These findings provide strong evidence that G-6-P formed as a result of HK activation is selectively metabolized via the PPP. The importance of the PPP in NADPH production and thus antioxidant defense following AKI has received little attention.

Our laboratory previously demonstrated that activation of EGFR signaling in primary rabbit proximal tubule cells lead to a rapid increase in both glycolysis and PPP activity (36). Further studies demonstrated that EGFR ligands are capable of increasing HK activity in multiple renal cell types, including proximal tubule cells and mesangial cells (45, 46). A link between LPS and EGFR signaling has also been established in studies demonstrating that TLR4 rapidly transactivates the EGFR via protease-mediated EGFR ligand shedding (28, 34). In addition, EGFR activation can contribute to the pathology and recovery of multiple forms of AKI (59). Results presented here demonstrate that EGFR signaling is required for HK activation in LPS-induced AKI.

We next focused on Akt as a downstream mediator of EGFR-induced HK activation in LPS-treated mice based on extensive evidence indicating that Akt modulates both HK activity and localization through phosphorylation (17, 35). Site-directed mutagenesis has further revealed that HK2 is phosphorylated by Akt on threonine 473 within an Akt consensus sequence in cardiomyocytes in vitro (44). We examined signaling changes in the renal cortex that might precede HK activation and noted increased activation of Akt as early as 1 h after LPS administration, which is consistent with other reports (11, 31). Inhibition of Akt by MK-2206 attenuated increases in HK activity following LPS exposure. Taken together, these findings provide strong evidence that the EGFR/Akt signaling pathway is responsible for LPS-mediated HK activation in the renal cortex.

In conclusion, the present study reports the novel finding of rapid activation of renal cortical HK activity in a mouse model of sepsis-induced AKI. HK was activated following LPS-induced AKI via an EGFR/Akt-dependent signaling mechanism. Surprisingly, the increase in HK activity observed in this model was associated with minimal changes in glycolysis and glycogen synthesis and was strongly linked with an increase in G6PDH, the rate-limiting enzyme in the PPP. The production of reducing equivalents (i.e., NADPH) may be key in preserving oxidant defense pathways.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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analyzed data: J.A.S. and R.G.S. interpreted results of experiments; J.A.S. prepared figures; J.A.S. drafted manuscript; J.A.S. and R.G.S. edited and revised manuscript; J.A.S., L.J.S., and R.G.S. approved final version of manuscript.

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