Role of AQP1 in endotoxemia-induced acute kidney injury

Weidong Wang,1 Chunling Li,1 Sandra N. Summer,1 Sander Falk,1 Wei Wang,1 Danica Ljubanovic,2 and Robert W. Schrier1

1Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado; and 2Department of Pathology, Dubrava University Hospital, Zagreb, Croatia

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Wang W, Li C, Summer SN, Falk S, Wang W, Ljubanovic D, Schrier RW. Role of AQP1 in endotoxemia-induced acute kidney injury. Am J Physiol Renal Physiol 294: F1473–F1480, 2008. First published April 23, 2008; doi:10.1152/ajprenal.00036.2008.—The effect of endotoxemia (lipopolysaccharide, 2.5 mg/kg ip) was investigated in aquaporin (AQP) 1 knockout (KO) compared with wild-type (WT) mice. At baseline, KO mice exhibited higher water intake (WI) and urine output (UO). After endotoxemia, WI and UO remained higher in the KO than WT mice, and urine osmolality was lower. The higher serum osmolality in AQP1-KO mice during endotoxemia was associated with higher AQP2 (133 ± 8 vs. 100 ± 3%, P < 0.01), AQP3 (140 ± 8 vs. 100 ± 4%, P < 0.001) and Na+/K+2Cl− cotransporter type 2 (NKCC2; 152 ± 14 vs. 100 ± 15%, P < 0.05) expression than that in WT mice. These responses during endotoxemia in the AQP1-KO mice compared with WT were associated with lower glomerular filtration rate (GFR) (69 ± 8 vs. 96 ± 8 ml/min, P < 0.05) and renal blood flow (0.77 ± 0.1 vs. 1.01 ± 0.1 ml/min, P < 0.01). Urinary sodium excretion and fractional sodium excretion were higher in KO compared with WT mice in endotoxemia and were accompanied by more severe tubular injury. With water repletion and comparable serum osmolalities, GFR was still lower in KO (57 ± 13 vs. 120 ± 6 ml/min, P < 0.01) compared with WT during endotoxemia. The abundance of AQP2 and AQP3 protein in KO mice was not different from WT mice; however, NKCC2, Na+/H+ exchanger type 3, and fractional sodium excretion remained higher in KO compared with WT. Thus the polyuria in AQP1-KO mice does not protect against endotoxin-induced acute kidney injury but rather absence of AQP1 predisposed to enhanced endotoxic renal injury. The AQUABORIN-1 (AQP1) water channel is expressed at the apical and basolateral plasma membranes in epithelial cells of the proximal tubule and the thin descending limb of Henle and in the plasma membrane of endothelial cells in the outer medullary descending vasa recta (11–13). Water moves across the cell membranes through these water channels. Targeted AQP1 gene disruption in mice impairs urinary concentrating capacity (10). These AQP1 knockout (KO) mice exhibit reduced basal urine osmolality and polyuria. In response to water deprivation or vasopressin administration, maximal urinary osmolality is reduced (10). A selective defect in the cell migration has been shown in AQP1-deficient mice. This AQP1-facilitated cell migration may be important in the structural and functional regeneration of tubules after acute kidney injury (AKI) (5). Alternatively, the increase in urine flow in AQP1 KO mice might attenuate AKI. These observations suggested that AQP1 might be important in endotoxin-induced AKI.

Sepsis-related endotoxemia is associated frequently with AKI, characterized by deterioration of glomerular filtration rate (GFR) and tubular dysfunction (14). Adequate tubular function for urinary concentration and sodium reabsorption depends on the functional expression of water channels and several ion transporters. The present study was therefore undertaken to examine the effect of endotoxemia on renal hemodynamics and tubular function in AQP1 null mice compared with wild-type (WT) mice.

MATERIALS AND METHODS

AQP1 null mice. AQP1 null mice were generated by targeted gene disruption as described (10). The experimental protocol was approved by the Animal Ethics Review Committee at the University of Colorado Health Sciences Center. Male mice aged 5–7 wk were used throughout the study. Mice were maintained on a standard rodent chow and had free access to water.

Materials. Chemicals were purchased from Sigma (St. Louis, MO) unless otherwise specified.

Animal protocols. AKI study (protocol 1). Mice were injected intraperitonally with a 2.5 mg/kg dose of lipopolysaccharide (LPS) (Escherichia coli O111:B4 from LIST Biological Laboratories, Campbell, CA) in both WT (WT-LPS) and KO (KO-LPS) groups. Functional studies and kidney removal were performed at 12 h after LPS injection. This endotoxemic model mimics that observed in patients with sepsis, whereby tubular dysfunction is more characteristic than morphological evidence of apoptosis and necrosis (6).

AKI with water loading study (protocol 2). Mice were injected intraperitonally with a 2.5 mg/kg dose of LPS (E. coli O111:B4 from LIST Biological Laboratories) in both groups. During the 12 h after LPS injection, mice were given water gavage every 3 h for three times in WT (WT-LPS-WL) and KO (KO-LPS-WL) groups. The water volume given was determined from our preliminary experiments. Functional studies and kidney removal were performed at 12 h after LPS injection.

Measurement of renal blood flow, GFR, and mean arterial pressure. The animals were anesthetized with pentobarbital sodium (60 mg/kg) and placed on a thermostatically controlled surgical table. A tracheotomy was performed in all mice. Catheters (custom pulled from PE-250) were placed in the jugular vein for maintenance infusion and in the carotid artery for blood pressure measurement. The kidney was exposed by a left subcostal incision and was dissected free from perirenal tissue. The renal arteries were isolated for the determination of renal blood flow (RBF) using a blood flowmeter and probe (Transonic Systems, Ithaca, NY) (18). Mean arterial pressure (MAP) was measured via a carotid artery catheter connected to a Transpac IV transducer and monitored continuously using Windaq Waveform recording software (Dataq Instruments). An intravenous wave form recording was used to record the MAP in both WT and KO mice. The RBF was measured using transonic flow prober (Transonic Systems, Inc.).

RESULTS

Effects of endotoxemia on renal function in WT and KO mice. The abundance of AQP2 and AQP3 protein in KO mice was not different from WT mice; however, NKCC2, Na+/H+ exchanger type 3, and fractional sodium excretion remained higher in KO compared with WT. Thus the polyuria in AQP1-KO mice does not protect against endotoxin-induced acute kidney injury but rather absence of AQP1 predisposed to enhanced endotoxic renal injury.

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maintenance infusion of 2.25% BSA in normal saline at a rate of 0.25 μL·body wt⁻¹·min⁻¹ was started 1 h before experimentation; 0.75% fluorescein isothiocyanate (FITC)-inulin was added to the infusion solution for the determination of GFR (18). A bladder catheter (PE-10) was used to collect urine. Two 30-min collections of urine were obtained under oil and weighed for volume determination. Blood protein concentration was determined for each sample by the Bradford method with 0.1 M phenylmethylsulfonyl fluoride. Tissue samples were immediately homogenized in a glass homogenizer at 4°C. After homogenization, protein concentration was determined for each sample by the Bradford method.

Antibodies. Antibodies to AQP2 (17), AQP2 phosphorylated at the protein kinase A phosphorylation consensus site (Ser 256; pAQP2, kindly provided by Dr. Soren Nielsen, University of Aarhus, Aarhus, Denmark) (1), AQP3 (17), Na⁺/H⁺ exchanger type 3 (NHE3) (7), Na⁺/H⁺-2Cl⁻ cotransporter type 2 (NKCC2, kindly provided by Dr. Mark A. Kenpper, National Institutes of Health) (8), and α-, β-, and γ-subunits of the epithelial sodium channel (3) (ENaC, kindly provided by Dr. Carolyn A. Ecelbarger, Department of Medicine, Georgetown University, Washington, DC) have been previously characterized.

Protein isolation. Kidneys were placed in ice-cold isolation solution containing 250 mM sucrose, 25 mM imidazole, and 1 mM EDTA, pH 7.2, with 0.1 vol/100 vol protease inhibitors (0.7 μg/ml pepstatin, 0.5 μg/ml leupeptin, and 1 μg/ml aprotinin) and 200 μM phenylmethylsulfonyl fluoride. Tissue samples were immediately homogenized in a glass homogenizer at 4°C. After homogenization, protein concentration was determined for each sample by the Bradford method.

Table 1. Functional data on AQP1-KO and AQP1-WT mice with LPS injection and water repletion

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>KO</th>
<th>WT-LPS</th>
<th>KO-LPS</th>
<th>WT-LPS-WL</th>
<th>KO-LPS-WL</th>
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<tr>
<td>n</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
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<tr>
<td>Water intake, ml·100 g⁻¹·12 h⁻¹</td>
<td>5.2 ± 0.7</td>
<td>19.1 ± 1.9</td>
<td>2.9 ± 0.6</td>
<td>5.4 ± 0.5</td>
<td>5.2</td>
<td>9.0</td>
</tr>
<tr>
<td>Urine output, ml·100 g⁻¹·12 h⁻¹</td>
<td>2.8 ± 0.7</td>
<td>21.3 ± 1.8</td>
<td>1.0 ± 0.1</td>
<td>5.0 ± 0.5</td>
<td>3.5 ± 0.5</td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td>U_{Osm}, mosmol/kg H₂O</td>
<td>3,398 ± 192</td>
<td>487 ± 28</td>
<td>1,435 ± 94</td>
<td>409 ± 35b</td>
<td>527 ± 55</td>
<td>325 ± 21b</td>
</tr>
<tr>
<td>S_{Osm}, mosmol/kg H₂O</td>
<td>312 ± 3</td>
<td>313 ± 7</td>
<td>324 ± 3</td>
<td>342 ± 61b</td>
<td>316 ± 4</td>
<td>314 ± 5</td>
</tr>
<tr>
<td>S_{Na}, μmol·kg⁻¹·min⁻¹</td>
<td>144 ± 1</td>
<td>143 ± 0.5</td>
<td>146 ± 0.6</td>
<td>153 ± 0.7</td>
<td>139 ± 0.3</td>
<td>140 ± 0.6</td>
</tr>
<tr>
<td>U_{NaV}, μmol·kg⁻¹·min⁻¹</td>
<td>5.3 ± 0.1</td>
<td>9.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>2.6 ± 0.6</td>
<td>0.4 ± 0.1</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>F_{ENa}, %</td>
<td>0.8 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>1.1 ± 0.3</td>
<td>0.2 ± 0.05</td>
<td>1.1 ± 0.2</td>
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</table>

Values are means ± SE; n, no. of mice. WT, wild type; KO, knockout; WT-LPS, WT with lipopolysaccharide (LPS) injection; KO-LPS, KO with LPS injection; WT-LPS-WL, WT with LPS injection given water repletion; KO-LPS-WL, KO with LPS injection given water repletion; U_{Osm}, urine osmolality; S_{Osm}, serum osmolality; S_{Na}, serum sodium concentration; U_{NaV}, urinary sodium excretion; F_{ENa}, fractional excretion of sodium. KO compared with WT (*), KO-LPS compared with WT-LPS (†), and KO-LPS-WL compared with WT-LPS-WL (‡): *P < 0.05, †P < 0.01, and ‡P < 0.001.

Fig. 1. Mean arterial pressure (MAP), renal blood flow (RBF), and glomerular filtration rate (GFR) during en-dotoxemia in wild-type (WT; A, C, and E) and aquaporin (AQP) 1 knockout (KO; B, D, and F) mice. MAP, GFR, and RBF were measured at 12 h after ip injection of LPS (2.5 mg/kg). MAP was measured through carotid artery, GFR was measured by inulin clearance, and RBF was measured by blood flowmeter. WT-LPS, WT with lipopolysaccharide (LPS) injection; KO-LPS, knock-out with LPS injection; WT-LPS-WL, WT with LPS injection given water repletion; KO-LPS-WL, knock-out with LPS injection given water repletion.
stained with periodic acid-Schiff (PAS) by standard methods for dehydrated, embedded in paraffin, and cut into 2-

histological examination. All histological examinations were performed by the experienced renal pathologist. Histological changes due to acute tubular necrosis (ATN score) were evaluated in the outer stripe of the outer medulla and the cortex on PAS-stained tissue and were quantified by counting the percentage of tubules that displayed cell necrosis, loss of brush border, and cytoplasm vacuolation as follows: 0 = none, 1 = <10%, 2 = 11–25%, 3 = 26–45%, 4 = 46–75%, and 5 = >76%. At least 10 fields (×400) were reviewed for each slide. Morphological criteria were used to count apoptotic cells on PAS-stained tissue by a pathologist experienced in the evaluation of renal apoptosis. Morphological characteristics included cellular rounding and shrinkage, nuclear chromatin compaction, and formation of apoptotic bodies (16). Apoptotic tubular cells were quantitatively assessed in the cortex and outer stripe of the outer medulla. At least 10 fields (×400) were counted for each slide.

For immunohistochemistry, staining was carried out using indirect immunoperoxidase (17). Briefly, the sections were dewaxed and rehydrated. Endogenous peroxidase was blocked by 0.5% H2O2 in absolute methanol for 10 min at room temperature. The sections were incubated overnight at 4°C with primary antibodies diluted in PBS supplemented with 0.1% BSA and 0.3% Triton X-100. The sections were then washed with PBS and incubated in horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (Dako) diluted in PBS supplemented with 0.1% BSA and 0.3% Triton X-100. The sections were examined with a Leica DMRE light microscope.

Biochemical measurements. Serum and urinary osmolality was measured by freezing-point depression (Advanced Instruments, Nor-

Western blot analysis. SDS-PAGE was performed on gradient acrylamide gels for NKCC2 and ENaCs and on 12% acrylamide gels for AQPs and NHE3. After transfer by electroelution to polyvinyliden difluoride membranes (Millipore, Bedford, MA), the blots were blocked with 5% nonfat dry milk in PBS and then probed with the respective antibodies at 4°C overnight. The membranes were washed with buffer containing PBS with 0.1% Tween 20 (J. T. Baker, Phillipsburg, NJ) and then exposed to secondary antibody for 1 h at room temperature. Subsequent detection of the specific proteins was carried out by enhanced chemiluminescence (Amersham, Arlington Heights, IL) according to the manufacturer’s instructions. Densitometric results were reported as integrated values (area × density of band) and expressed as a percentage compared with the mean value in controls (100%). Blots are representative of results obtained from all samples. Densitometry reflects means ± SE of all samples.

Histological examination and immunohistochemical studies. The kidneys from animals in protocol 1 were fixed with 3% paraormaldehyde in 0.1 M cacodylate buffer (pH 7.4). Kidney blocks were dehydrated, embedded in paraffin, and cut into 2-μm-thick slices and stained with periodic acid-Schiff (PAS) by standard methods for

Fig. 2. Semiquantitative immunoblots of AQP2, AQP3, Na+/K+-2Cl– cotransporter type 2 (NKCC2), Na+/H+ exchanger type 3 (NHE3), α-epithelial sodium channel (ENaC), β-ENaC, and γ-ENaC using kidney proteins prepared from WT and KO mice at baseline. Western immunoblots shown are representative of blots performed with a total sample size of n = 7 in WT and n = 7 KO mice. Densitometric results are expressed as a percentage compared with the mean value in controls (100%).
was reduced significantly in both WT-LPS (94 ± 7 vs. 148 ± 9 μl/min, P < 0.01; Fig. 1E) and KO-LPS (69 ± 8 vs. 160 ± 17 μl/min, P < 0.001; Fig. 1F). After LPS injection, serum osmolality was markedly increased in both the WT-LPS (324 ± 3 vs. 312 ± 3 mosmol/kgH2O, P < 0.05) and the KO-LPS (342 ± 6 vs. 313 ± 7 mosmol/kgH2O, P < 0.01) (Table 1). Thus the negative water balance and hyperosmolality with AKI were greater in the KO-LPS mice. Urinary sodium excretion and fractional sodium excretion were also significantly higher in KO-LPS compared with WT-LPS (Table 1).

Renal histology. An ATN score was used to quantitate a feature of the kidney histology changes with LPS. Brush-border loss and cytoplasm vacuolization predominated over cell necrosis and cast formation in the ATN score. The ATN score was significantly increased in LPS-treated AQP1 KO mice compared with WT mice [3.8 ± 0.6 vs. 0.8 ± 0.3, P < 0.01, in cortex and 1.8 ± 0.6 vs. 0, P < 0.05 in outer stripe of outer medulla, (n = 5/group)]. The number of tubular apoptotic cells was counted in the cortex and outer stripe of the outer medulla. The apoptosis score (apoptotic tubular cells/high-

RESULTS

Baseline blood pressure and renal function in AQP1 KO vs. WT mice. There was no significant difference in baseline MAP [94 ± 4 vs. 88 ± 9 mmHg, P = not significant (NS)], RBF (1.05 ± 0.1 vs. 1.01 ± 0.1 ml/min, P = NS), and GFR (160 ± 17 vs. 148 ± 9 μl/min, P = NS) between WT and AQP1 KO mice. No significant differences were noted in serum osmolality, sodium concentration, urinary sodium excretion, and fractional sodium excretion (Table 1). Water intake and urinary output in KO mice were significantly increased, and urinary osmolality was significantly decreased compared with WT mice (Table 1).

Renal function during endotoxemia. After LPS was administered, there was no change in MAP in either the WT-LPS (86 ± 3 vs. 88 ± 9 mmHg, P = NS; Fig. 1A) or the KO-LPS (88 ± 6 vs. 94 ± 4 mmHg, P = NS; Fig. 1B) mice. RBF (0.77 ± 0.1 vs. 1.05 ± 0.1 ml/min, P < 0.05; Fig. 1D) was decreased significantly in the AQP1 KO mice but not in the WT (1.01 ± 0.1 vs. 1.01 ± 0.1 ml/min, P = NS, Fig. 1C). GFR...
power field) was also increased in LPS-treated AQP1 KO mice (3.5 ± 1.2 vs. 0, *P* < 0.05 in cortex and 3.8 ± 1 vs. 0, *P* < 0.05 in the outer stripe of the outer medulla, *n* = 5/group) compared with WT mice.

Renal function with endotoxemia and water repletion. To evaluate the effects of dehydration on renal function and vasopressin-regulated renal protein expression, mice with endotoxemia from both groups were subjected to water replacement. After water repletion, serum osmolality and serum sodium concentration returned to normal in both groups (Table 1), indicating that the water volume given was adequate. GFR in WT-LPS-WL improved (120 ± 6 vs. 96 ± 8 μl/min in WT-LPS, *P* < 0.05, Fig. 1E), although not to normal levels, whereas in KO-LPS-WL, GFR remained low (57 ± 13 vs. 69 ± 8 μl/min in KO-LPS, *P* > 0.05, Fig. 1F). Urinary sodium excretion and fractional sodium excretion were still higher in KO-LPS-WL than WT-LPS-WL (Table 1).

Baseline renal AQP and ion transporter abundance in KO mice compared with WT mice. KO mice demonstrated a significant increase in AQP2 (187 ± 24 vs. 100 ± 12%, *P* < 0.01), pAQP2 (172 ± 3 vs. 100 ± 5%, *P* < 0.05), and AQP3 abundance (155 ± 17 vs. 100 ± 10%, *P* < 0.05) compared with WT mice (Fig. 2). Compared with WT, KO mice showed a marked increase in NKCC2 protein expression (142 ± 10 vs. 100 ± 14%, *P* < 0.05) (Fig. 1E). In contrast, the abundance of NHE3 (75 ± 6 vs. 100 ± 7%, *P* < 0.05, Fig. 2) was decreased significantly in KO compared with WT mice (Fig. 2).

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Endotoxemia was associated with reduced renal AQP2 and AQP3 in WT mice but increased AQP2 and AQP3 expression in KO mice. After LPS treatment in WT-LPS mice, the abundance of AQP2 (75 ± 3 vs. 100 ± 6%, *P* < 0.01) and AQP3
Increased renal AQP and ion transporter abundance in KO mice with endotoxemia compared with WT. In protocol 1, KO-LPS mice demonstrated a significant increase in AQP2 (133 ± 8 vs. 100 ± 3%, P < 0.01), pAQP2 (180 ± 10 vs. 100 ± 20%, P < 0.01), and AQP3 abundance (140 ± 8 vs. 100 ± 4%, P < 0.001) (Fig. 5) compared with WT-LPS mice. Immunohistochemistry confirmed greater expression of apical AQP2 (Fig. 6, A–D) and basolateral AQP3 (Fig. 6, E–H) both in cortex and medulla in the kidneys of KO-LPS mice. Compared with WT-LPS, KO-LPS mice showed a substantial increase in NKCC2 protein expression (152 ± 14 vs. 100 ± 15%, P < 0.05) (Fig. 5). α-ENaC (149 ± 11 vs. 100 ± 7%, P < 0.01) was also increased in KO-LPS compared with WT-LPS mice (Fig. 5). In contrast, the abundance of NHE3 (120 ± 19 vs. 100 ± 10%, P = NS), β-ENaC (95 ± 4 vs. 100 ± 8%, P = NS, Fig. 5), and γ-ENaC (85 kDa) (92 ± 10 vs. 100 ± 6%, P = NS) was not different (Fig. 5). A weak band representing the 70-kDa γ-ENaC band was evident; no change in expression level was found in KO-LPS compared with WT-LPS when immunoblots were exposed for longer periods of time.

Effects of endotoxemia on KO and WT mice during water repletion. After water repletion (protocol 2), the abundance of AQP2 (106 ± 3 vs. 100 ± 2%, P = NS), pAQP2 (138 ± 20 vs. 100 ± 10%, P = NS), and AQP3 (115 ± 5 vs. 100 ± 6%, P = NS) (Fig. 7) in KO mice with endotoxemia was not different compared with WT mice. Increased NKCC2 abundance in KO mice with endotoxemia was maintained (268 ± 21 vs. 100 ± 7%, P < 0.001, Fig. 7) in spite of water repletion. The protein expression of NHE3 was also markedly increased in KO compared with WT mice (208 ± 23 vs 100 ± 14%, P < 0.01, Fig. 7). During endotoxemia, the abundance of collecting duct α-ENaC (60 ± 13 vs. 100 ± 9%, P < 0.05, Fig. 7), however, was significantly reduced in KO compared with WT mice. In contrast, β-ENaC (89 ± 7 vs. 100 ± 5%, P = NS) and γ-ENaC (85 kDa, 121 ± 9 vs. 100 ± 12%, P = NS) were not different from KO and WT mice during endotoxemia. γ-ENaC (70 kDa) was not different in KO mice compared with WT.

**DISCUSSION**

The present study was undertaken to examine the role of AQP1 in response to AKI secondary to endotoxemia. AQP1 KO mice have been shown to exhibit polyuria and polydipsia secondary to a urinary concentrating defect (10). AQP1 is located in the proximal tubule, descending limb of Henle’s loop, and medullary descending vasa rectae (11–13). The countercurrent mechanisms involve these AQP1 water channels in the descending limb, and the countercurrent exchange mechanism involves the AQP1 water channels in the vasa rectae.

Whether AQP1 KO mice would respond differently from WT mice during endotoxemia is not known. Theoretically, the increased urine flow in these KO mice might attenuate AKI. Such a protective renal effect of polyuria against AKI has been shown in experimental studies (2). Alternatively, AQP1 KO mice have been found to have impaired cell migration in response to ischemia-reperfusion injury that might enhance AKI during endotoxemia (5).

During the 12 h after the endotoxin insult, there was a substantial decrease in urine volume in the WT mice (2.8 ± 0.7 to 1.0 ± 0.1 ml·100 g⁻¹·12 h⁻¹) as GFR fell significantly. Water intake also decreased from 5.2 ± 0.7 to 2.9 ± 0.6 ml·100 g⁻¹·12 h⁻¹. This led to an increase in serum osmolality from 312 ± 3 to 324 ± 3 mosmol/kgH₂O as an index of water depletion. The changes in fluid balance in the AQP1 KO mice with endotoxin were in the same direction but were more dramatic. Urine volume decreased from 21 ± 3 to 5.0 ± 0.5 ml·100 g⁻¹·12 h⁻¹ as water intake decreased from 19 ± 1 to 5.4 ± 0.5 ml·100 g⁻¹·12 h⁻¹. This led to a large increase in mean serum osmolality from 313 ± 7 to 342 ± 6 mosmol/kgH₂O. The percentage decrease in GFR was greater in the AQP1 KO compared with WT mice (57 vs. 37%). There was a significant increase in fractional sodium excretion and a decrease in RBF in the AQP1 KO, but not WT, mice with endotoxemia.

These results suggested that the degree of negative fluid balance during endotoxemia could be a factor in AKI during endotoxemia. Studies were therefore undertaken in which water was replaced during the 12 h after endotoxin administration.
This approach was successful in avoiding the negative water balance and hyperosmolality in both WT (312 ± 3 vs. 316 ± 4 mosmol/kgH2O) and AQPI (313 ± 7 vs. 314 ± 5 mosmol/kg) KO mice. In the WT mice, there was a significant improvement in the endotoxin-related AKI, as assessed by GFR, with the water repletion. These results indicate that the severity of endotoxin-related AKI can be attenuated by maintenance of fluid balance in the WT mice. This, however, was not the case in the AQPI KO mice. The decrease in GFR with endotoxin in AQPI KO mice was not improved with fluid repletion even though their negative fluid balance and hyperosmolality in the initial studies were more severe during endotoxemia.

With endotoxin, but without water repletion, the AQPI KO mice demonstrated an increase in AQP2 and AQP3 water channels and a decrease in NHE3 protein expression, whereas the WT mice demonstrated a decrease in AQP2 and AQP3 water channels in association with diminished NKCC2 and NHE3 ion transporters. Thus AQP2, AQP3, NKCC2, and α-ENaC were significantly higher in the AQPI KO than WT mice during endotoxemia in the absence of water repletion.

Decreased total AQP2 in the collecting duct cells during endotoxemia, as was observed in the WT mice, has been suggested to result from reduced V$_2$ receptor mRNA and/or decreased arginine vasopressin binding (4). However, in the absence of water repletion, AQP2 was increased significantly in KO mice with endotoxemia, perhaps indicating an overriding effect of the water depletion and hyperosmolality. Results from our laboratory, however, have shown that hyperosmolality upregulates AQP2 in vitro (15) and in vivo (9) in the absence of vasopressin. The increased AQP2 and AQP3 in the collecting ducts of KO mice during endotoxemia would expect to attenuate water loss during endotoxemia. Moreover, the increased expression of NKCC2 in the thick ascending limbs would be expected compensate for urinary sodium loss in KO mice with endotoxemia.

With water repletion, however, the differences in AQP2 and AQP3 water channels between the WT and KO mice were no longer observed. The NKCC2 and NHE3 ion transporters, however, remained higher in the AQPI KO mice, and a significant increase in fractional sodium excretion in the AQPI KO, but not WT, mice still occurred. Thus the persistent higher NKCC2 and NHE3 transporters in AQPI KO mice during endotoxemia with fluid repletion failed to compensate for the tubular injury, which resulted in diminished tubular sodium reabsorption and increased fractional sodium excretion. Histological examination confirmed more severe injury in the AQPI KO mice with endotoxemia. The AQPI KO mice also exhibited a significant diminution in the α-ENaC transporter compared with WT mice during endotoxemia with fluid repletion. This would be expected to contribute to the increase in fractional excretion of sodium in the AQPI KO mice with endotoxemia in spite of water repletion. The increase in α-ENaC, but not the β- and γ-ENaC subunits, in the AQPI mice in the presence of water depletion is compatible with the effect of aldosterone stimulation. In contrast, with water repletion, α-ENaC actually decreased during endotoxemia in the AQPI KO mice. This finding is further supportive of the observed increased fractional excretion of sodium during endotoxemia in the KO mice. In addition to any effect of tubular sodium wasting in the LPS-treated KO mice, there are other potential contributing factors for the increased susceptibility of the AQPI KO mice to endotoxin-related AKI. Increased fluid delivery to the macula densa could increase tubuloglomerular feedback and thereby accentuate the decrease in GFR. Perhaps most important, the epithelial cell migration that occurs during tubular injury and leads to dedifferentiation, spreading, and ultimately differentiation, is important in AKI repair.

In summary, the integrity of AQPI is clearly important in avoiding profound negative fluid balance during endotoxemia. The resultant polyuria in AQPI KO mice does not attenuate the endotoxin-related AKI, and, in fact, the AKI is more severe in these mice. Although fluid repletion was shown to have a beneficial effect in WT mice during endotoxemia, this was not the case in AQPI KO mice. More severe tubular injury was documented histologically and functionally in AQPI KO mice compared with WT mice. The more severe fall in GFR during endotoxemia despite water repletion implicates factors other than water depletion. The tubular sodium wasting could increase tubuloglomerular feedback and contribute to the greater fall in GFR in the AQPI KO mice. Perhaps even more important, the known impaired tubular cell migration in AQPI KO mice may contribute to the enhanced tubular injury, including impaired healing.

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GRANTS

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