Cytokine-mediated regulation of urea transporters during experimental endotoxemia

Christoph Schmidt,1 Klaus Höcherl,2 and Michael Bucher1
Departments of 1Anesthesiology and 2Physiology, Regensburg University, Regensburg, Germany
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Schmidt C, Höcherl K, Bucher M. Cytokine-mediated regulation of urea transporters during experimental endotoxemia. Am J Physiol Renal Physiol 292: F1479–F1489, 2007. First published January 16, 2007; doi:10.1152/ajprenal.00460.2006.—Acute renal failure (ARF) is a frequent complication of sepsis and has a high mortality. Sepsis-induced ARF is known to be associated with significant impairment of tubular capacity. However, the pathogenesis of endotoxemic tubular dysfunction with failure of urine concentration is poorly understood. Urea plays an important role in the urinary concentrating mechanism and expression of the urea transporters UT-A1, UT-A2, UT-A3, UT-A4, and UT-B is essential for tubular urea resorption. The present study attempts to assess the regulation of renal urea transporters during severe inflammation in vivo. Lipopolysaccharide (LPS)-injected mice presented with reduced glomerular filtration rate, fractional urea excretion, and inner medulla osmolality associated with a marked decrease in expression of all renal urea transporters. Similar alterations were observed after application of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interferon (IFN)-γ, or IL-6. LPS-induced ARF is known to be affected by myocardial infarction in the United States (54), and exceeds the number of deaths per year due to myocardial infarction in the United States (54). ARF is defined as the abrupt decline in glomerular filtration rate (GFR) and tubular function. Although the reduced GFR in sepsis could be secondary to altered glomerular hemodynamics (15, 39), the pathophysiology of endotoxemic tubular dysfunction with failure of urine concentration and decreased urine osmolality is poorly understood.

It has long been known that urea plays an important role in the urinary concentrating mechanism and, as the primary nitrogenous end product of amino acid catabolism, is essential for the excretion of nitrogenous waste (64). The kidney’s permeability to the highly polar molecule urea is greatly increased by the insertion of five urea transporters (UTs) that allow facilitated urea transport (52, 53, 64). UT-B has been found in the endothelium of the descending vasa recta (DVR) (see Refs. 49, 58, and 63). The vasopressin-regulated UT-A1 as well as UT-A3 and UT-A4 are expressed in the inner medulla (IM) of the collecting duct (CD, IMCD; see Ref. 43). UT-A2 is located in the thick descending limbs of the Henle’s loops (TDL), the primary site of urea secretion (43, 62). Urea-dependent urine concentration is based on the following three associated processes: 1) urea becomes progressively concentrated along the CD because of-osmoprotective tubular liquid; 2) a vasopressin-dependent increase in the urea permeability of the terminal IMCD because of UT-A1, UT-A3, and UT-A4 enables transport of the concentrated urea in the interstitial tissue of the deep IM; and 3) medullary urea, which escapes the IM via the ascending venous vasa recta, is continuously returned to the IM by a complex intrarenal urea recycling process involving reentry of urea along the DVR through UT-B and the TDL through UT-A2. This recycling results in a hyperosmolar medullary interstitium that is crucial for the potent urinary concentrating (5, 64).

The finding that endotoxemia diminishes the expression of several renal tubular transporters directed our interest toward the regulation of renal urea transporters during experimental sepsis (22). We hypothesized that endotoxemia downregulates renal urea transporters. Based on our previous findings that cytokines downregulate several vasoconstrictive receptors in renal tissue, we also hypothesized that proinflammatory cytokines affect the expression of renal urea transporters (11–14). To test our hypotheses, we performed experiments with 1) lipopolysaccharide (LPS)-injected mice as a model for severe experimental inflammation; 2) mice injected with the cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interferon (IFN)-γ, or IL-6; 3) LPS-injected knockout mice with a deficiency for TNF-α, IL-1-receptor-1, IFN-γ, or IL-6; 4) LPS-injected mice with or without glucocorticoid pretreatment, which reduces LPS-induced cytokine production (8, 27, 28);

Address for reprint requests and other correspondence: M. Bucher, Dept. of Anesthesiology, Regensburg Univ., 93042 Regensburg, Germany (e-mail: michael.bucher@klinik.uni-regensburg.de).

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MATERIALS AND METHODS

Animal Preparation. Mice with deficiencies for TNF-α (B6.129S-Tnf<sup>fltm1Lka</sup>), IL-1-receptor-1 (B6.129S7-Il1r1<sup>tm1lms</sup>), IFN-γ (B6.129S-Ifng<sup>tm1ts</sup>), and the wild-type strains B6129S2F2/J and C57BL/6J were purchased from The Jackson Laboratory. To determine the appropriate LPS dose (Escherichia coli, serotype 0111:B4; Sigma) for the induction of severe inflammation, wild-type mice received several LPS application rates (1, 5, and 10 mg/kg ip). Hemodynamic and renal parameters of LPS-treated mice were measured 12 h after injection and compared with NaCl-treated mice (control). Mice were killed immediately following identification of these parameters.

Table 1. Primer set for urea transporter analysis by real-time PCR

<table>
<thead>
<tr>
<th>mRNAm</th>
<th>Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>UT-A1</td>
<td>s GAC AGT GAG GAG GAG TGA AG</td>
</tr>
<tr>
<td></td>
<td>r ACG GTC TCA GAG GTC TCT TC</td>
</tr>
<tr>
<td>UT-A2</td>
<td>s TTT CTC CAG TGC TAT CTA GAG</td>
</tr>
<tr>
<td></td>
<td>r ACG GTC TCA GAG GTC TCT TC</td>
</tr>
<tr>
<td>UT-A3</td>
<td>s AGG GCA ACC GGA TCT ACT TC</td>
</tr>
<tr>
<td></td>
<td>r AGA GTG GAC GCC ACA CAG AT</td>
</tr>
<tr>
<td>UT-A4</td>
<td>s GCC CTT CAA CAT TGC CTT AAC A</td>
</tr>
<tr>
<td></td>
<td>r ATG TTG GGT GGA GTG GTG GT</td>
</tr>
<tr>
<td>UT-B</td>
<td>s TCT TCT CAA ACA AGG GCC AC</td>
</tr>
<tr>
<td></td>
<td>r TCG CTC ACG AGG GAG CTC AA</td>
</tr>
<tr>
<td>β-Actin</td>
<td>s AGA CCT TCA AGA CCC CAG GC</td>
</tr>
<tr>
<td></td>
<td>r ACA GCA CTC TGT TGG CAT AG</td>
</tr>
</tbody>
</table>

UT, urea transporter; s, sense; r, reverse.

Hemodynamic and renal parameters of mice (n = 6/group) were injected with 10 mg/kg LPS or NaCl (control) and hemodynamic as well as renal parameters were determined after 6, 12, and 24 h. Because the strongest effect of LPS was observed at the 12-h time point, we focused further investigations at this time point. Wild-type and cytokine knockout mice received NaCl (control) or 10 mg/kg ip LPS (n = 6/group) and were killed 12 h after injection. Wild-type mice were additionally treated with NaCl (control) or the cytokines TNF-α, IL-1β, IFN-γ or IL-6 (1 μg/g; Peprotech) intraperitoneally and were killed 12 h after injection (n = 6/group). Furthermore, we investigated the effect of both a single dose of dexamethasone alone (10 mg/kg ip) and dexamethasone 2 h before LPS injection in wild-type mice (n = 6/group). The dose of LPS, cytokines, and dexamethasone was chosen from data taken from the literature (11–13, 44, 61).

Measurement of hemodynamic, renal, and blood parameters. Animals were anesthetized with Sevoflurane, using a Trajan 808 (Draeger) and the left renal artery was only touched with forceps.

Arteries of mice (n = 12/group) were clipped with 0.11 mm ID silver rings and finally 5) animals with renal ischemia caused by renal artery clipping.

and was used to determine tissue cytokine concentration. The resultant pellet was reconstituted in blotting buffer. After centrifugation, the resultant pellet (membrane fraction) was reconstituted in blotting buffer and used for the determination of urea transporter protein by Western blot. Protein concentration was measured by the method of Lowry.

Plasma/urine/tissue concentrations of cytokines/osmolality/urea. Tissue concentrations of TNF-α, IL-1β, IFN-γ, and IL-6 were determined using ELISA kits (R&D Systems) and set into proportion to total protein. Serum, urine, and IM osmolality were measured by freezing-point depression (Knauer Osmometer). Plasma, urine, and IM urea concentration was assayed using the Quantichrom Urea Assay Kit (BioAssay Systems).

Western blot. Protein samples were electrophoretically separated on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane that was blocked overnight either in 5% nonfat dry milk or in BSA diluted in Tris-buffered saline with 0.1% Tween 20. The membrane was then incubated for 1 h with rabbit polyclonal antibodies against UT-A1/UT-A2 (1:200; Alpha Diagnostics), UT-A3 [446/447] (Sigma), and β-actin (1:5,000; Sigma). After being washed, the membrane was incubated for 1 h with a horseradish peroxidase-conjugated secondary antibody (1:2,000; β-actin 1:5,000; Santa Cruz) and subjected to a chemiluminescence detection system. Semi-quantitative assessment of bands was performed densitometrically.

Statistical analysis. Data were analyzed by ANOVA with multiple comparisons followed by the t-test with Bonferroni’s adjustment. P < 0.05 was considered significant.

RESULTS

Appropriate LPS dose for induction of severe inflammation. To determine the appropriate LPS dose for the induction of severe inflammation, mice were treated with 1, 5, and 10 mg/kg. LPS (1 and 5 mg/kg) caused a significant regulation urea transporters and aggravation of renal parameters, whereas hemodynamic parameters remained stable. LPS (10 mg/kg) reduced hemodynamic, renal parameters and expression of renal urea transporters, indicating severe septic conditions at that LPS dosage. Therefore, we performed further experiments with 10 mg/kg LPS (Table 2 and Fig. 1).

Hemodynamic and blood parameters. Animals became lethargic, developed diarrhea, and showed piloerection starting 2 h after LPS injection (10 mg/kg). MAP decreased 12 and 24 h after LPS injection to 65 and 58% of control values, and heart rate of septic animals was significantly higher than in the
control group. Hemoglobin and hematocrit values showed no differences between control and septic groups, whereas plasma osmolality and plasma urea increased consistently after injection of LPS (Table 3).

IL-1β also led to tachycardia and arterial hypotension with hyperosmolality and elevated plasma urea levels. Injection only of dexamethasone did not influence hemodynamic parameters in wild-type mice compared with control levels, and plasma osmolality as well as plasma urea did not distinguish significantly from control values.

Supplementary treatment of LPS-injected mice with dexamethasone attenuated LPS-induced arterial hypotension after 12 and 24 h. Furthermore, supplementary glucocorticoid treatment partially averted the LPS-induced rise of plasma osmolality and plasma urea 12 and 24 h after LPS administration.

Renal parameters. Both urine flow and GFR were lower in LPS-injected mice, whereas GFR decreased to ~25% of control levels after LPS injection (Fig. 2, A and B, and Table 3).

Urine osmolality decreased approximately threefold in mice treated with LPS compared with control animals. Therefore, the ratio of urine to plasma osmolality declined more than threefold. LPS-injected mice showed significantly reduced osmolar excretion, and IM osmolality was 3.2 times lower than in control animals 24 h after induction of sepsis (Fig. 2D). Urea excretion declined to ~20% of control levels 24 h after LPS injection, and given as %vehicle control. Shown are means ± SE; n = 6 mice. LPS, lipopolysaccharide; MAP, mean arterial pressure; GFR, glomerular filtration rate. *P < 0.05 vs. control.

![Fig. 1. Effect of lipopolysaccharide (LPS: 1, 5, and 10 mg/kg) on urea transporter (UT)-A1, UT-A2, UT-A3, UT-A4, and UT-B mRNA in the kidney 12 h after ip injection. Values are related to signals obtained for β-actin mRNA and given as %vehicle control. Shown are means ± SE of 6 animals/group.](Image)

Table 2. Effect of LPS on hemodynamic and renal parameters 12 h after injection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>1 mg/kg ip</th>
<th>5 mg/kg ip</th>
<th>10 mg/kg ip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>492 ± 10</td>
<td>490 ± 18</td>
<td>513 ± 16</td>
<td>581 ± 14*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>75 ± 2</td>
<td>72 ± 3</td>
<td>71 ± 3</td>
<td>51 ± 3*</td>
</tr>
<tr>
<td>Urine flow, µl/min</td>
<td>0.63 ± 0.04</td>
<td>0.43 ± 0.03*</td>
<td>0.38 ± 0.03*</td>
<td>0.32 ± 0.03*</td>
</tr>
<tr>
<td>GFR, µl·min⁻¹·g⁻¹ body wt</td>
<td>10.1 ± 0.3</td>
<td>3.6 ± 0.3*</td>
<td>3.1 ± 0.2*</td>
<td>2.7 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 mice. LPS, lipopolysaccharide; MAP, mean arterial pressure; GFR, glomerular filtration rate. *P < 0.05 vs. control.

Table 3. Effect of LPS without or with dexamethasone and IL-1β on hemodynamic, hematopoietic, and renal parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>1 mg/kg ip</th>
<th>5 mg/kg ip</th>
<th>10 mg/kg ip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>20.2 ± 0.5</td>
<td>20.1 ± 0.3</td>
<td>19.9 ± 0.3</td>
<td>19.9 ± 0.3</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>79 ± 2</td>
<td>62 ± 3</td>
<td>51 ± 3</td>
<td>51 ± 3*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>112 ± 10</td>
<td>103 ± 14</td>
<td>98 ± 14</td>
<td>98 ± 14</td>
</tr>
<tr>
<td>Urine flow, µl/min</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>GFR, µl·min⁻¹·g⁻¹ body wt</td>
<td>6.8 ± 0.3</td>
<td>8.5 ± 0.3</td>
<td>13.5 ± 0.7*</td>
<td>13.5 ± 0.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 mice. LPS, lipopolysaccharide; MAP, mean arterial pressure; GFR, glomerular filtration rate. *P < 0.05 vs. control.
Fig. 2. Effect of LPS (10 mg/kg), dexamethasone (10 mg/kg), and the combination of both on urine flow (μl/min; A), glomerular filtration rate (GFR, μl·min⁻¹·g body wt⁻¹; B), osmolar excretion (mosmol·min⁻¹·g body wt⁻¹; C) and osmolality of the renal inner medulla (mosmol/kgH₂O; D) in mice 6, 12, and 24 h after ip injection. Shown are means ± SE of 6 animals/group. P < 0.05 vs. control (*) and vs. LPS treatment (#).

Fig. 3. Effect of LPS (10 mg/kg), dexamethasone (10 mg/kg), and the combination of both on urea excretion (nmol·min⁻¹·g body wt⁻¹; A), fractional urea excretion (%; B), tubular reabsorption of urea (nmol·min⁻¹·g body wt⁻¹; C), and urea concentration of the renal inner medulla (mmol/l; D) in mice 6, 12, and 24 h after ip injection. Shown are means ± SE of 6 animals/group. P < 0.05 vs. control (*) and vs. LPS treatment (#).
and this drop was accompanied by a significantly reduced fractional urea excretion and a 2.5-fold lowered tubular urea reabsorption (Fig. 3, A–C). Renal tissue perfusion decreased both in the renal medulla and, more severely, in the cortex after sepsis induction (Fig. 4).

After injection of IL-1β (12 h), the observed renal function of these animals was restricted comparable to LPS-injected animals (GFR: 3.1 ± 0.4 μl·min⁻¹·g body wt⁻¹, urine flow: 0.34 ± 0.05 μl/min, IM osmolality: 592 ± 38 mosmol/kgH₂O, IM urea concentration: 95 ± 15 mmol/l).

Injection only of dexamethasone induced no relevant changes in functional renal parameters in wild-type mice after injection compared with controls (Figs. 2–4).

Supplementary glucocorticoid treatment of LPS-injected animals diminished renal injury as indicated by increased GFR, urine flow, tubular urea reabsorption, and fractional urea excretion compared with untreated LPS injection (Figs. 2, A and B, and Fig. 3, B and C). Additional dexamethasone treatment led to an attenuation of the LPS effect on urinary and medullary osmolality and urea concentration (Figs. 2D and 3D). Furthermore, the urine-to-plasma ratio of osmolality and of urea increased compared with LPS-treated animals, and osmolar and urea excretion rose significantly (Figs. 2C and 3A), indicating more effective renal urinary concentration than in LPS-injected mice. Indeed, renal tissue perfusion both in the medulla and cortex increased smoothly, but not significantly, after supplementary glucocorticoid administration compared with LPS treatment alone (Fig. 4).

**Effect of LPS on renal urea transporter expression.** Treatment with LPS for 6, 12, or 24 h resulted in significantly decreased urea transporter gene expression in the kidney. UT-A1 and UT-A3 mRNA showed a concomitant fall to ~17% of control levels, and UT-A2 mRNA decreased to ~24% of control levels after LPS injection. UT-A4 mRNA was downregulated to ~40% of the control level after sepsis induction. This decrease was paralleled by a significant decline of UT-B mRNA to ~17% of control levels 6, 12, and 24 h after LPS injection (Fig. 5).

Protein expression of UT-A1, UT-A2, and UT-A3 was determined by Western blot analysis. The lack of available antibodies limited the analysis of UT-A4 and UT-B protein expression. UT-A1 protein fell to 49 and 38% of control, UT-A2 protein to 70 and 24% of control, and UT-A3 protein to 60 and 30% of control 12 and 24 h after LPS injection (Fig. 6A).

**Effect of cytokines on renal urea transporter gene expression.** For further characterization of the mechanisms by which renal urea transporters could be downregulated during endotoxemia, renal gene expression in mice injected with cytokines such as TNF-α, IL-1β, IFN-γ, and IL-6 was examined, since the potent induction of these cytokines is the hallmark of sepsis (47). mRNA expression of all renal urea transporters was strongly depressed after injection of IL-1β, TNF-α, or IFN-γ (apart from UT-A4). IL-6 only downregulated UT-A3 and UT-B (Fig. 7).

**Effect of LPS on urea transporter gene expression in cytokine knockout mice.** To investigate whether LPS-induced downregulation of renal urea transporter expression could be inhibited by the expression of individual cytokines, we measured mRNA levels of urea transporters in LPS-treated knockout mice with deficiencies of TNF-α, IL-1-receptor-1, IFN-γ, or IL-6 and compared the effect of LPS injection on urea transporter levels with that in the wild-type control strain. NaCl-injected cytokine knockout and wild-type mice revealed comparable urea transporter mRNA levels (data not shown). After LPS injection (12 h), mRNA levels of all renal urea transporters were depressed (UT-A1, UT-A2, UT-A3, and UT-B ~20% and UT-A4 ~42% of wild-type and of cytokine knockout control levels), and the downregulative effect of LPS on renal urea transporters was not diminished by the absence of single cytokines (Fig. 8).

**Effect of dexamethasone on urea transporter expression in LPS-treated mice.** Because absence of individual cytokines had no effect on LPS-induced downregulation of renal urea transporters, mice were additionally treated with dexamethasone (10 mg/kg ip). As shown in Table 4, treatment with dexamethasone markedly attenuated tissue cytokine concentration 6, 12, and 24 h after LPS injection. We used this model to investigate the impact of the diminished action of multiple cytokines on renal urea transporter gene expression.

Dexamethasone administration alone caused no significant differences in the expression of renal urea transporters compared with control levels (Fig. 5). Animals treated with LPS plus dexamethasone still revealed a significant downregulation of urea transporter gene expression; however, supplementary injection of dexamethasone substantially increased urea.
transporter gene expression compared with sole LPS treatment (Fig. 5).

This effect might also be evidenced on the level of protein expression. Supplementary glucocorticoid treatment led to a clear attenuation of the LPS effect on UT-A1, UT-A2, and UT-A3 membrane protein, whereas dexamethasone administration alone did not affect UT-A1, UT-A2, and UT-A3 membrane protein level (Fig. 6).

Effect of renal ischemia on urea transporter expression. Because renal ischemia is potentially present in our model of sepsis with cardiovascular depression and decreased renal perfusion, one could assume that the reduced expression of renal urea transporter is the result of ischemia. Therefore, we assessed the impact of ischemia on the gene expression of renal urea transporters by investigating animals with left renal artery clipping, which decreases renal tissue perfusion to values comparable to those found in septic animals in our and previous investigations (40). However, renal clipping did not bias gene expression of renal urea transporters compared with sham-clipped animals 6, 12, and 24 h following clipping (Fig. 9).

DISCUSSION

ARF secondary to sepsis is a highly prevalent diagnosis in the ICU setting and continues to be associated with a high mortality rate. Therefore, understanding the pathogenesis of sepsis-related ARF is of critical importance. Several studies have demonstrated that reduced GFR during sepsis is secondary to altered glomerular hemodynamics (15, 39). In contrast, the pathophysiology of sepsis-associated renal tubular dysfunction with impaired urinary concentration mechanism and decreased urine osmolality is only poorly understood.

It has long been known that renal “urea recycling” depends on the functional expression of renal urea transporters such as UT-A1, UT-A2, UT-A3, UT-A4, and UT-B and is essential for adequate renal tubular function, including the ability to potentially concentrate urine (5, 21, 25, 33, 34, 64). In the present study, we characterized the regulation of renal urea transporters during severe experimental inflammation.

To induce experimental sepsis, we used intraperitoneal injection of LPS, an established, easy-to-apply in vivo sepsis model (46). To ensure induction of severe inflammation in mice, we performed primary studies with several LPS dose rates (1, 5, and 10 mg/kg). In contrast to 1 and 5 mg/kg LPS, 10 mg/kg LPS caused a pronounced, time-dependent arterial hypotension and tachycardia and was associated with reduced GFR and urine flow, indicating severe septic conditions at that dosage of LPS. Because 10 mg/kg LPS was most suitable for inducing severe septic conditions and the literature also made use of this LPS dose (10 –13, 16, 24, 37, 51), we performed our experiments with this application rate.

Fractional urea excretion, tubular urea reabsorption, and the urine-to-plasma ratio for osmolality, all indicators of tubular function in this study, decreased after LPS injection, indicating renal tubular damage consistent with the findings of previous studies (4, 21, 25). Notably, it has been shown in our and other investigations that LPS administration led to a fourfold decrease in both urea concentration and osmolality in the renal IM, indicating that urea may be crucial for the production of a hyperosmolar medullary interstitium (21, 25). Hu et al. (25)
even observed that accumulation of urea in the medullary interstitium was impaired to much greater extent than that of other solutes such as NaCl. This selective functional impairment in urinary urea-concentrating capacity can be explained by the marked downregulation of all renal UT transporters in this study, suggesting a possible causal linkage between impairment of renal urea reabsorption and expression of these transporters. This hypothesis is strengthened by studies describing coexistence of renal tubular dysfunction and downregulation of several renal urea transporters, especially UT-A1, UT-A2, and UT-B, in uremic animals (25, 30, 36). Concerning the UT-A4 expression in mice, relatively little is known about the mouse UT-A4 isofrom compared with rats, and some studies did not detect UT-A4 in mouse kidney (31). However, the literature reports about findings detecting UT-A4 mRNA and using the same primer set we disposed in this investigation (59).

We also studied the mechanisms and pathways by which septic conditions lead to downregulation of renal urea transporters. Proinflammatory cytokines such as TNF-α, IL-1β, IFN-γ, or IL-6 are known to be abundantly generated mediators during sepsis (47), as was clearly demonstrated in our study. Therefore, we were interested in the effect of these mediators on functional renal and hemodynamic parameters and on the expression of renal urea transporters. Injection of IL-1β similar to LPS caused a significant depression of hemodynamic and renal function. Additionally, we found that IL-1, TNF-α, and IFN-γ extensively downregulated expression of all renal UT-transporters. IL-6 inhibited expression of UT-A3 and UT-B. We conclude that cytokines may mediate the LPS-induced downregulation of renal urea transporters.

To test this assumption, additional experiments with knockout mice with a deficiency for TNF-α, IL-1-receptor-1, IFN-γ, or IL-6 were conducted. However, we found that the absence of individual cytokines does not prevent LPS-induced downregulation of renal urea transporters, suggesting that LPS-induced inhibition of urea transporter gene expression cannot be attributed to single cytokines. This observation may be because of the overlapping interactions of several proinflammatory cytokines. These findings are in accordance with reports of ineffective anti-single cytokine strategies in septic patients in clinical trials (1, 20, 45).

Therefore, we performed experiments with glucocorticoid-injected mice to simultaneously reduce LPS-induced upregu-
Fig. 7. Effect of LPS (10 mg/kg) and proinflammatory cytokines on UT-A1 (A), UT-A2 (B), UT-A3 (C), UT-A4 (D), and UT-B (E) mRNA in the kidney 12 h after ip injection. Values are related to signals obtained for β-actin mRNA and given as %vehicle control. Expression of β-actin showed no changes after LPS or cytokine treatment. Shown are means ± SE of 6 animals/group. *P < 0.05 vs. vehicle control.

Fig. 8. Effect of LPS (10 mg/kg) on renal UT-A1 (A), UT-A2 (B), UT-A3 (C), UT-A4 (D), and UT-B (E) mRNA in cytokine knock-out mice 12 h after ip injection. Values are related to signals obtained for β-actin mRNA and given as %vehicle control. Expression of β-actin showed no changes after LPS treatment. Shown are means ± SE of 6 animals/group. *P < 0.05 vs. vehicle control.
urea transporters are related to signals obtained for medullary tissue perfusion are given as %sham treatment. Values of renal urea mRNA 6, 12, and 24 h following clipping. Values of renocortical and sion and on the expression of UT-A1, UT-A2, UT-A3, UT-A4, and UT-B

Fig. 9. Effect of renal ischemia on renal cortical and medullary tissue perfusion and on the expression of UT-A1, UT-A2, UT-A3, UT-A4, and UT-B mRNA 6, 12, and 24 h following clipping. Values of renocortical and medullary tissue perfusion are given as %sham treatment. Renal ischemia did not affect expression of β-actin. Shown are means ± SE of 6 animals/group. *P < 0.05 vs. sham treatment.

Because changes in urine flow resulting from altered GFR or hemodynamics can affect urea clearance, one could assume that the effects observed in the present study may be because of renal ischemia, particularly given the decreased renal tissue perfusion after LPS treatment in our current investigation and previous trials (40). Our finding that glucocorticoid treatment ameliorated LPS-induced alterations of renal urea transporters and renal dysfunction without affecting renal ischemia suggests that renal ischemia does not influence the expression of renal urea transporters. To further approve this hypothesis, we induced experimental renal ischemia by clipping the left renal artery so that renal tissue perfusion decreased to values comparable to septic animals in this and previous studies (40). We found that expression of renal urea transporters was not influenced by renal ischemia, consistent with previous studies investigating other tubular transporters following renal artery clipping (6, 65).

Our results demonstrate that renal urea transporters are downregulated during severe inflammation and indicate that this downregulation is mediated by proinflammatory cytokines independent of ischemia. Because of overlapping actions of different proinflammatory cytokines, the downregulation of urea transporters cannot be attributed to single cytokines, providing an explanation for the failure of single anti-cytokine strategies to improve the outcome of septic patients (1, 20, 45). In addition, our findings contribute to explaining and understanding the beneficial effects of glucocorticoids on the outcome of septic patients (2, 3, 7, 29).

ACKNOWLEDGMENTS

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GRANTS

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Table 4. Effect of LPS (10 mg/kg ip) without or with dexamethasone (10 mg/kg ip) on renal tissue cytokine concentrations

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LPS</th>
<th>LPS + Dexamethasone</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td>TNF-α</td>
<td>ND</td>
<td>2.2±0.3</td>
<td>2.3±0.5</td>
</tr>
<tr>
<td>IL-1β</td>
<td>2.0±0.3</td>
<td>75.8±5.4*</td>
<td>59.6±4.9*</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.6±0.1</td>
<td>98.3±7.5*</td>
<td>88.3±7.3*</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>ND</td>
<td>197±14</td>
<td>115±10</td>
</tr>
</tbody>
</table>

Values are means ± SE; *n = 6 mice. Units are pg/mg protein. TNF, tumor necrosis factor; IL, interleukin; IFN, interferon. P < 0.05 vs. NaCl (⁎) and vs. respective LPS (†), ND, not detectable.