Lipopolysaccharide-Induced Acute Renal Failure in Conscious Rats: Effects of Specific Phosphodiesterase Type 3 and 4 Inhibition

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ABSTRACT

In conscious, chronically instrumented rats we examined 1) renal tubular functional changes involved in lipopolysaccharide (LPS)-induced acute renal failure; 2) the effects of LPS on the expression of selected renal tubular water and sodium transporters; and 3) effects of milrinone, a phosphodiesterase type 3 (PDE3) inhibitor, and Ro-20-1724, a PDE4 inhibitor, on LPS-induced changes in renal function. Intravenous infusion of LPS (4 mg/kg b.wt. over 1 h) caused an immediate decrease in glomerular filtration rate (GFR) and proximal tubular outflow without changes in mean arterial pressure (MAP). LPS-induced fall in GFR and proximal tubular outflow were sustained on day 2. Furthermore, LPS-treated rats showed a marked increase in fractional distal water excretion, despite significantly elevated levels of plasma vasopressin (AVP). Semiquantitative immuno-

blotting showed that LPS increased the expression of the Na⁺,K⁺,2Cl⁻ co-transporter (BSC1) in the thick ascending limb, whereas the expression of the AVP-regulated water channel aquaporin-2 in the collecting duct (CD) was unchanged. Pretreatment with milrinone or Ro-20-1724 enhanced LPS-induced increases in plasma tumor necrosis factor-α and lactate, inhibited the LPS-induced tachycardia, and exacerbated the acute LPS-induced fall in GFR. Furthermore, Ro-20-1724-treated rats were unable to maintain MAP. We conclude 1) PDE3 or PDE4 inhibition exacerbates LPS-induced renal failure in conscious rats; and 2) LPS treated rats develop an escape from AVP in the CDs, which could be aimed to protect against water intoxication in septic conditions associated with decreased GFR and high levels of AVP.

Acute renal failure (ARF) is a frequent complication to the systemic inflammatory response syndrome (SIRS). SIRS is associated with an inflammatory host response to endotoxins released from infectious agents characterized by massive production of cell-derived mediators such as tumor necrosis factor (TNF)-α, interleukins (IL-1β and IL-8), nitric oxide, and free oxygen radicals (Camussi et al., 1998). This will eventually induce widespread endothelial damage with loss of arteriolar tonus in systemic vessels, increased capillary permeability, and sustained hypotension. Furthermore, sepsis-induced ARF with deterioration of glomerular filtration rate (GFR) is associated with renal vasoconstriction in the presence of a decrease in the systemic vascular resistance (Schwartz et al., 1997). Sepsis induced ARF is therefore most probably caused by a combination of ischemia due to hypoperfusion and direct cytotoxic renal effects.

Little is known about tubular function and the regulation of renal sodium and water transporters in SIRS-induced ARF. It is well described that polyuria and failure to concentrate urine maximally are frequent consequences of mild-to-moderate ischemic ARF. Recently, it has been shown that rats with ARF induced by ischemia have an almost ubiquitous down-regulation of all major renal tubular sodium and water transporters (Fernandez-Llama et al., 1999; Kwon et al., 1999, 2000). However, whether these tubular changes are present in SIRS-induced ARF is unknown.

Degradation of intracellular cAMP and cGMP is catalyzed by phosphodiesterases (PDEs), which have been classified...
into nine isozyme families (Dousa, 1999). PDE3 and PDE4 isoforms have been shown to be present in both renal vasculature and tubules (Dousa, 1999) and have been shown to play important roles in regulation of renal circulation and tubular function (Jackson et al., 1997; Kurtz et al., 1998; Dousa, 1999; Sandner et al., 1999). It has been shown that renal excretion of cAMP (Begany et al., 1996), possibly through TNF-α-induced desensitization of adrenergic β2-receptors, inactivation of adenylate cyclase (Bernardin et al., 1998), and/or increased PDE4 activity (Koga et al., 1995) are present in experimental models of SIRS induced by lipopolysaccharide (LPS) administration. PDE inhibitors may reduce LPS-induced synthesis and release of cytokines (Dousa, 1999), and a number of studies have examined the effect of PDE inhibitors on LPS-induced ARF. Specific PDE4 inhibitors (Rolipram and Ro-20-1724) have been shown to counteract LPS-induced up-regulation of PDE activity (Koga et al., 1995), and it has been reported that pretreatment with Ro-20-1724 significantly attenuated LPS-induced fall in renal blood flow, renal vascular resistance, and GFR in anesthetized rats (Begany et al., 1996; Carcillo et al., 1996). However, the described beneficial effects of PDE inhibitors were found in anesthetized rats. Anesthetics are known to have profound effects on renal hemodynamics and tubular function (Schaller et al., 1985; Petersen et al., 1991; Beno and Kimura, 1999), and Schwartz et al. (1997) have shown that blood pressure was preserved in conscious rats treated with LPS, whereas similar treatment to anesthetized rats induced an acute and severe fall in blood pressure.

The purpose of the present study was therefore to examine 1) renal tubular functional changes involved in sepsis-induced ARF in a model of LPS-induced sepsis in conscious Wistar rats; 2) effects of LPS on protein levels (by Western blotting) of the water channel aquaporin 1 (AQP1) present in the proximal tubules and the thin descending limb of Henle’s; the arginine-vasopressin (AVP)-regulated AQP2 water channel present in the collecting ducts; the widely distributed basolateral Na⁺-K⁺-ATPase; and the bumetanide-sensitive type-1 Na⁺-K⁺-2Cl⁻ cotransporter (BSC1) exclusively expressed in the thick ascending limb of Henle’s (TAL); and finally 3) the effect of the PDE3 inhibitor milrinone in a low dose without bradycardic/hypotensive effects in normal rats or the PDE4 inhibitor Ro-20-1724 in the same doses previously used in anesthetized rats (Begany et al., 1996; Carcillo et al., 1996) on LPS-induced changes in renal hemodynamics and tubular function in conscious, chronically instrumented rats.

### Materials and Methods

#### Experimental Animals

Female Wistar rats (220–240 g) were obtained from Charles River (Sulzfeld, Germany) and housed in a temperature- (22–24°C) and moisture-controlled room (40–70%) with a 12-h light/dark cycle (light on from 6:00 AM to 6:00 PM). The rats were maintained on a standard rodent diet with 140 mmol/kg sodium, 275 mmol/kg potassium, and 25% protein (Altromin International, Lage, Germany) and had free access to water.

#### Animal Preparation

In halothane-nitrous oxide anesthesia, the animals were implanted with permanent medical grade Tygon catheters into the abdominal aorta and the inferior caval vein, respectively, via a femoral artery and vein. Catheters were produced, fixed, and sealed as described previously (Petersen et al., 1991). A permanent suprapubic catheter was implanted into the urinary bladder, which was sealed with a silicone-coated stainless steel pin after flushing the bladder with ampicillin (0.6 mg/ml). After instrumentation, the animals were housed individually for 7 to 10 days until the day of the experiment.

#### Experimental Protocol

Six different groups of conscious, instrumented animals were studied (n = 8–13 in all groups):

1. Vehicle: vehicle (150 mM glucose)-treated control rats.
2. Vehicle-Mil: rats were treated with i.v infusion of milrinone (bolus, 50 μg/kg; infusion rate, 1 μg/kg/min) for 7 h.
3. Vehicle-Ro: rats were treated with i.v infusion of Ro-20-1724 (10 μg/kg/min) for 7 h.
4. LPS: rats received LPS (Escherichia coli serotype 0127 B8, L 3129, Sigma-Aldrich, St. Louis, MO) at a dose of 4 mg/kg delivered as an i.v infusion over 1 h, starting the 2nd h of the 7-h study.
5. LPS-Mil: rats were treated with i.v infusion of milrinone for 7 h and LPS, as described for group 4.
6. LPS-Ro: rats were treated with i.v infusion of Ro-20-1724 for 7 h and LPS, as described for group 4.

LPS and PDE inhibitors were administered via the femoral vein catheter. Milrinone and LPS were dissolved in 150 mM glucose. Ro-20-1724 was dissolved in ethanol/150 mM glucose (1:9).

#### Renal Clearance Studies

Three days before the renal clearance experiments, the diet was changed to a standard diet (catalog 1014; Altromin International) containing lithium citrate (12 mmol of Li⁺/kg of dry diet). This mode of lithium administration results in steady-state plasma concentrations of lithium in the range from 0.1 to 0.2 mol/l that do not influence renal function (Leysac et al., 1994). The renal clearance of lithium was used as an index of proximal tubular outflow (Leysac et al., 1994; Thomsen and Shirlet, 1997). Before the renal clearance experiments all rats were adapted to the restraining cage used for these experiments by training them for two periods of 2 h each.

#### Clearance Experiment 1

On the first day of the experiment, the animals were transferred to a restraining cage, and an intravenous infusion (150 mM glucose, 13 mM NaCl, and 3 mM LiCl) with [³H]inulin was started. The infusion rate was 2 ml/h in the first 15 min and was thereafter reduced to 0.5 ml/h. The infusion rate of [³H]inulin was 3.5 μCi/h. After a 90-min equilibration period, the i.v infusion of milrinone, Ro-20-1724, or vehicle was started and continued for seven consecutive 1-h clearance periods with urine collections. LPS or 150 mM glucose was given during the second 1-h period. Arterial blood samples (300 μl) were collected in the middle of each urine collection period and replaced immediately with heparinized blood from a normal donor rat. After the renal clearance experiment, all catheters were sealed, the bladder was flushed with ampicillin (0.6 mg/ml), and the animals were returned to their home cages.

#### Clearance Experiment 2

On the following day, rats were transferred to restraining cages, and a 4-h clearance study as described above was performed without administration of LPS and PDE inhibitors. On days 1 and 2, GFR, renal clearance of [³H]inulin, lithium clearance (C_Li), and sodium clearance (C_Na) were calculated for each 1-h clearance period. The urine collection periods were urine collections. LPS or 150 mM glucose was given during the second 1-h period. Arterial blood samples (300 μl) were collected in the middle of each urine collection period and replaced immediately with heparinized blood from a normal donor rat. After the renal clearance experiment, all catheters were sealed, the bladder was flushed with ampicillin (0.6 mg/ml), and the animals were returned to their home cages.

#### Mean Arterial Blood Pressure (MAP), Heart Rate (HR), Arterial Blood Gases, and p-Glucose

MAP and HR were measured continuously by the use of pressure transducers (Bentley Laboratories, Uden, Holland) and sampled on-
Hematocrit was measured in each period. Arterial tensions of oxygen and CO₂, arterial pH, and arterial concentrations of glucose were measured in period 6 on day 1. TNF-α, AVP, and Lactate

Arterial blood samples for the measurement of TNF-α were drawn in the middle of period 3, about 90 min after the start of the LPS infusion.1 Samples of 300 μl were collected into heparinized test tubes. Plasma concentration of AVP was measured in period 7 on day 1 and in period 4 on day 2. A 1.0-ml blood sample was collected in a prechilled test tube with 20 μl of 0.5 mM EDTA, pH 7.4, and 10 μl of 20 × 10⁶ IU/ml aprotinin. After centrifugation at 4°C, plasma samples were transferred to prechilled test tubes and stored at −20°C for later measurements of TNF-α and AVP. Plasma lactate concentration was measured in period 6 on day 1. All blood samples were replaced immediately with heparinized blood from a normal donor rat.

Analytical Methods

Urine volume was determined gravimetrically. Concentrations of lithium and sodium in plasma and urine were measured by atomic absorption spectrophotometry (model 2380; PerkinElmer, Alerød, Denmark). [3H]Inulin was determined by liquid scintillation counting on a Tri-Carb liquid scintillation analyzer (model 2250CA; Packard Instruments, Greve, Denmark). Arterial blood gases, pH, and plasma concentrations of glucose and lactate were measured by an ABL 600 blood gas analyzer (Radiometer, Copenhagen, Denmark). TNF-α in plasma was determined by an enzyme-linked immunosorbent assay (Biotrak; Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, UK). AVP was extracted from plasma on C18 Sep-Pak cartridges and measured by radioimmunoassay, as described previously (Kjaer et al., 1994).

Calculations

Renal clearances of inulin (GFR), lithium (C_Li) and sodium (C_Na) were calculated according to the standard formula as the ratio of urinary excretion rate to the plasma concentration. Segmental tubular reabsorption rates of sodium and water were calculated based on the assumption that C_Li provides an accurate measure of the delivery of tubular fluid into the thin descending limb of Henle (Leyssac et al., 1994; Thomsen and Shirley, 1997). Fractional lithium excretion (FELi) was calculated as C_Li/GFR and used as a marker for the fractional delivery of fluid out of the proximal tubules. Fractional distal sodium excretion was calculated as C_Na/C_Li and used as a marker for the fractional excretion of the sodium load delivered from the proximal tubules. Fractional distal water excretion was calculated as V/C_Li and used as a marker for the fractional excretion of water delivered from the proximal tubules. Fractional sodium excretion (FENa) was calculated as C_Na/GFR, and fractional water excretion (FEH₂O) was calculated as V/GFR.

Measurement of AQP1, AQP2, Na⁺-K⁺-ATPase, and BSC1 by Semiquantitative Immunoblotting

The rats given vehicle or LPS alone were anesthetized with halothane/nitrous oxide at the end of clearance experiment 2. Then the right kidney was removed and processed for membrane fraction-
ation. Briefly, the kidneys were homogenized and the homogenates were centrifuged at 4000g for 15 min. The supernatant was centrifuged at 200,000g for 1 h, and the pellet containing plasma membranes and intracellular vesicles was used for immunoblotting. Membrane fractions were run on 12% polyacrylamide minigels for measurement of AQP1 and AQP2 and on 6 to 16% gradient polyacrylamide minigels for measurement of Na\(^+\)/K\(^+\)-ATPase and BSC1. For each gel an identical gel was run in parallel and subjected to Coomassie staining to ensure identical loading of protein. Blots were blocked with 5% milk in phosphate-buffered saline-Tween 20 for 1 h, and incubated with the primary antibody. The labeling was visualized with horseradish peroxidase-conjugated secondary antibody (diluted 1:3000; P448; DAKO, Glostrup, Denmark) using an enhanced chemiluminescence system (Amersham Biosciences UK, Ltd.). Enhanced chemiluminescence films with bands within the linear range were then scanned. For AQP1 a 29-kDa band was scanned. For AQP2 the 29-kDa and the 35- to 50-kDa band corresponding to nonglycosylated and glycosylated AQP2 were scanned. For Na\(^+\)/K\(^+\)-ATPase (α1 subunit) a 96-kDa band and for BSC1 a broad 161-kDa band were scanned. Samples from the LPS-treated rats were expressed relative to the mean expression in the corresponding material from vehicle-treated rats run on the same gel. For further details, including characterization of the antibodies see Nielsen et al. (1997) and Kwon et al. (2000).

Statistical Analyses

Results are presented as means ± S.E.M. A two-way analysis of variance for repeated measures was used to test for differences between groups. For \( P < 0.05 \), the differences between corresponding periods were evaluated by unpaired \( t \) tests with Bonferroni's correction of the level of significance. For variance nonhomogeneity the data were subjected to logarithmic transformation before statistical evaluation.

Results

Renal Function Study, Day 1

MAP, HR, and GFR (Fig. 1). Within 2 h, LPS caused a significant and sustained decrease in GFR. However, blood pressure remained unchanged throughout the clearance study, whereas HR increased significantly in the LPS-treated rats (ΔHR\(_{LPS} \) 95 ± 11 min\(^{-1} \) versus ΔHR\(_{Vehicle} \) −21 ± 8 min\(^{-1}, P < 0.01 \)).

Pretreatment with milrinone or Ro-20-1724 significantly enhanced the LPS-induced decrease in GFR. In addition, Ro-20-1724 but not milrinone induced a significant and sustained fall in MAP in LPS rats. The LPS-induced tachycardia was absent in the rats pretreated with either milrinone or Ro-20-1724 (ΔHR\(_{LPS-Mil} \) 27 ± 14 min\(^{-1} \); ΔHR\(_{LPS-Ro} \) −26 ± 15 min\(^{-1} \)). None of the PDE inhibitors affected MAP, HR, or GFR in the vehicle-treated rats.

Renal Water and Sodium Handling (Fig. 2). LPS caused a significant fall in urine flow rate (V). Neither milrinone nor Ro-20-1724 pretreatment changed the LPS-induced antidiuresis. In the vehicle-treated rats, Ro-20-1724 but not milrinone produced a diuretic response in the first hours of infusion. LPS infusion had an immediate antinatriuretic effect and PDE inhibitor treatment had no effect on LPS-induced changes in sodium excretion rate (U\(_{NaV} \)). However,
in the vehicle-treated rats PDE inhibitors produced an immediate natriuretic response, which was most pronounced in the Ro-20-1724-treated rats. The same pattern was found when sodium excretion was expressed in fractional terms (\(FE_{\text{Na}}\)).

**Tubular Lithium Handling (Fig. 3).** LPS caused a significant fall in \(C_{\text{Li}}\) and \(FE_{\text{Li}}\), suggesting a reduced delivery of tubular fluid out of the proximal tubules. Pretreatment with the PDE inhibitors had no effect on \(C_{\text{Li}}\) in the LPS rats, but both milrinone- and Ro-20-1724 reversed the decline in \(FE_{\text{Li}}\) toward the end of the experiment. Both PDE inhibitors increased \(C_{\text{Li}}\) and \(FE_{\text{Li}}\) in the vehicle-treated rats.

The fractional distal sodium excretion as evaluated by \(C_{\text{Na}}/C_{\text{Li}}\) fell throughout the study period in all the three vehicle-treated groups as well as in the LPS-treated rats not receiving PDE inhibitors. In the LPS-Ro group, \(C_{\text{Na}}/C_{\text{Li}}\) was stable throughout, whereas the LPS-Milrinone group showed a significant increase in the fractional distal sodium excretion.

**Plasma Lactate and TNF-\(\alpha\) (Table 1).** LPS induced marked increases in plasma concentrations of lactate and TNF-\(\alpha\). Pretreatment with milrinone or Ro-20-1724 enhanced LPS-induced increases in TNF-\(\alpha\), and Ro-20-1724 also exacerbated the increase in p-lactate.

**Arterial Blood Gases, pH, and Hematocrit (Table 1).** LPS increased \(P_a\text{O}_2\) and both milrinone and in particular Ro-20-1724 further increased \(P_a\text{O}_2\). Similarly, Ro-20-1724 increased \(P_a\text{O}_2\) in the vehicle-treated rats. This effect of LPS and Ro-20-1724 on oxygenation was associated with hyperventilation as indicated by the concomitant decrease in \(P_a\text{CO}_2\) found in all the LPS groups and in the Vehicle-Ro group.

**Plasma AVP (Table 2).** Plasma AVP concentration was significantly increased in all three LPS-treated groups compared with the vehicle-treated groups at the end of the clearance study on day 1.

**Mortality.** In the first 24 h after administration of LPS, 4 of 14 LPS rats pretreated with Ro-20-1724 died, whereas only one LPS-treated rats not receiving PDE inhibitor and one LPS rat pretreated with milrinone died.

**Renal Function Study, Day 2**

**MAP and GFR (Fig. 4).** MAP was preserved in the LPS-treated rats. However, GFR was still decreased in all three LPS-treated groups. GFR was significantly decreased in the vehicle-mil rats, whereas GFR was unchanged in the Vehicle-Ro rats.

**Renal Water and Sodium Handling (Figs. 5 and 6).** \(C_{\text{Li}}\) and \(FE_{\text{Li}}\) were significantly decreased in the LPS-treated rats, suggesting an increase in proximal tubular reabsorption. However, both the urine flow rate (\(V\)) and \(U_{\text{Na}}V\) were unchanged in the LPS-treated rats, suggesting a compensatory decrease in distal sodium and water reabsorption. In fact, the fractional distal water excretion \((V/CLi)\) was markedly increased in the LPS-treated rats and a similar pattern was seen in the \(C_{\text{Na}}/C_{\text{Li}}\), even though it only reached statis-
tical significance in the LPS-Mil group. Treatment with both PDE inhibitors had no effect on V, U$_{Na}$V or renal lithium handling in the LPS-treated rats, whereas both V and U$_{Na}$V were significantly increased in vehicle rats treated with either milrinone or Ro-20-1724. Furthermore, C$_{Li}$ and FE$_{Li}$ were increased in the Vehicle-Mil rats.

**Plasma AVP (Table 2).** Like on day 1, plasma AVP levels were significantly increased in all three LPS-treated groups compared with the vehicle-treated groups at the end of the clearance study on day 2. Moreover, the plasma AVP concentration in the LPS-Ro group was significantly higher than in the LPS group.

**Effect of LPS on Renal Expression of AQP1 and AQP2 (Fig. 7).** Fig. 7A show an immunoblot of membrane fractions (20 μg of protein/lane) from whole kidney preparations. The affinity-purified anti-AQP1 protein antibody recognizes the 29-kDa band, corresponding to the AQP1 protein. Densitometry of all samples (Fig. 7B) revealed that AQP1 expression was unchanged in the LPS-treated rats (vehicle, 100 ± 6% versus LPS, 71 ± 13%, N.S.). Figure 7C shows another immunoblot of membrane fractions (20 μg of protein/lane) from whole kidney preparations. The affinity-purified anti-AQP2 protein antibody recognizes the 29-kDa and the 35- to 50-kDa band, corresponding to nonglycosylated and glycosylated AQP2 protein, respectively. Densitometry of all samples (Fig. 7D) revealed that AQP2 expression was unchanged in the LPS-treated rats despite the significant increase in plasma AVP found in these rats (vehicle, 100 ± 8% versus LPS, 84 ± 4%, N.S.).

**Effect of LPS on Renal Expression of Na$^{+}$,K$^{+}$-ATPase and BSC1 (Fig. 8).** Fig. 8A show an immunoblot of membrane fractions (20 μg of protein/lane) from whole kidney preparations. The affinity-purified monoclonal anti-Na$^{+}$,K$^{+}$-ATPase protein antibody recognizes the 96-kDa band corresponding to the Na$^{+}$,K$^{+}$-ATPase protein. Densitometry of all samples (Fig. 8B) revealed that Na$^{+}$,K$^{+}$-ATPase expression was unchanged in the LPS-treated rats (vehicle, 100 ± 8% versus LPS, 85 ± 4%, N.S.). Figure 8C shows one more immunoblot of membrane fractions (20 μg of protein/lane) from whole kidney preparations. The affinity-purified anti-BSC1 protein antibody recognizes a broad band around 161-kDa corresponding to the bumetanide-sensitive type-1 Na$^{+}$-K$^{+}$-2Cl$^{-}$ cotransporter BSC1 protein exclusively expressed in the thick ascending limb of Henles and in the macula densa. Densitometry of all samples (Fig. 8D) revealed that the LPS treatment increased the expression of BSC1 (vehicle, 100 ± 13% versus LPS, 160 ± 14%, P < 0.05).

**Discussion**

**LPS-Induced Changes in Renal Function.** Septic shock is a major cause of ARF. The mechanism responsible for the renal injury is complex and only partly explained, but different lines of evidence exist to indicate that the reduction of GFR in sepsis is secondary to selective preglomerular vasoconstriction and hypoperfusion (Lugon et al., 1989; Camussi et al., 1998). In our model, infusion of LPS over 1 h immediately caused a significant reduction in GFR that was sustained on day 2. Noteworthy, the fall in GFR occurred without significant changes in MAP. This finding is in agreement with results from Schwartz et al. (1997) showing that LPS induces a significant fall in GFR without a reduction in
blood pressure in conscious rats, whereas in anesthetized rats LPS induces a concomitant fall in GFR and blood pressure. The reason for this difference between conscious and anesthetized rat models is uncertain. It is well known that anesthesia and acute surgery have profound effects of neurohumoral systems and stress hormones (Schaller et al., 1985). Furthermore, surgical stress and anesthesia activate inducible nitric-oxide synthase (Losonczy et al., 1997) and circulating levels of TNF-α (Beno and Kimura, 1999). In the present study LPS infusion induced a marked tachycardic response, indicating a generalized baroreceptor-mediated sympathetic nerve activation to counter the LPS-mediated vasodilator response. It is well described that baroreflex mechanisms regulating blood pressure are blunted in anesthetized animals (Carter et al., 1986). An acute hypotensive response to LPS found in anesthetized animals models could therefore be explained by insufficient sympathetic nerve activation. This emphasizes that renal function studies in experimental animal models whenever possible should be made in conscious animals.

It has been shown that LPS stimulates the formation of cytokines, chemokines, and platelet-activating factor in glomerular mesangial cells expressing CD14 receptors (Camussi et al., 1995; Schlondorff et al., 1997), and tubular epithelial cells produce chemokines when stimulated by LPS (Schlondorff et al., 1997). Moreover, LPS increases the circulating levels of angiotensin II, norepinephrine, eicosanoids, cytokines, endothelin, and nitric oxide as well as the formation of oxygen radicals (Camussi et al., 1998). Several of these mechanisms may be responsible for the initial LPS-induced fall in GFR in conscious rats. In this study, increased plasma levels of TNF-α induced by LPS coincided with the initial fall in GFR. Recently, studies in a murine model of sepsis indicated a deleterious effect of TNF-α in the early

**TABLE 2**

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<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Vehicle-Mil</th>
<th>Vehicle-Ro</th>
<th>LPS</th>
<th>LPS-Mil</th>
<th>LPS-Ro</th>
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<tbody>
<tr>
<td>p-AVP day 1 (pg/ml)</td>
<td>0.98 ± 0.31</td>
<td>1.05 ± 0.66</td>
<td>1.20 ± 0.27</td>
<td>19.68 ± 9.31*</td>
<td>12.35 ± 4.25*</td>
<td>26.14 ± 7.41*</td>
</tr>
<tr>
<td>p-AVP day 2 (pg/ml)</td>
<td>0.64 ± 0.14</td>
<td>0.49 ± 0.11</td>
<td>0.76 ± 0.15</td>
<td>3.48 ± 1.95*</td>
<td>5.14 ± 2.28*</td>
<td>6.63 ± 1.89*</td>
</tr>
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p, plasma.
* P < 0.05 versus vehicle on day 1 and day 2, respectively.
† P < 0.05 versus LPS on day 2.
renal dysfunction independent of other inflammatory pathways and of systemic hypotension (Knotek et al., 2001). Further studies are warranted to examine the initial mechanisms responsible for the development of LPS-induced acute renal failure in conscious rats.

**LPS Induced Changes in Tubular Water and Sodium Handling.** The present study shows that the LPS-induced fall in GFR is paralleled by a decreased delivery of tubular fluid out of the proximal tubules (decreased $C_{Li}$). Therefore, the decreases in GFR and proximal tubular outflow may account for the oliguria and antinatriuresis seen in the first hours after LPS administration. On day 2, however, marked increases in fractional excretions of sodium and water had developed in spite of a sustained decrease in proximal tubular outflow ($C_{Li}$). As a consequence, increased fractional distal excretion of Na$^+$ ($C_{Na}/C_{Li}$) and water ($V/C_{Li}$) were found in the LPS-treated rats, suggesting a delayed LPS-induced impairment in distal tubular reabsorption.

The impaired distal water reabsorption in the LPS-treated rats was present despite significantly increased plasma levels of AVP. AVP regulates water permeability in the renal collecting ducts (CDs) by increasing the expression and plasma membrane targeting of the membrane-bound water channel AQP2 (Nielsen et al., 1999). In addition to its actions on CD water permeability, AVP also stimulates the expression of BSC1 and sodium reabsorption in the TAL by a V$_2$ receptor-mediated mechanism (Kim et al., 1999). AVP-regulated CD water reabsorption therefore depends on 1) expression and membrane targeting of AQP2 in CD principal cells, and 2) the magnitude of the cortico-medullary osmotic gradient generated by sodium reabsorption in the TAL. Immunoblotting of whole kidney homogenates revealed that the expression of BSC1 was markedly increased in the LPS-treated rats, suggesting that rats with LPS-induced in contrast to ischemia-induced ARF (Kwon et al., 2000) have the capacity to increase the cortico-medullary osmotic gradient secondary to increased Na$^+$ reabsorption in the TAL. However, despite increased plasma levels of AVP the AQP2 expression was unchanged in the LPS rats. This finding indicates that LPS-treated rat develops a relative escape from the effect of AVP in the collecting ducts. In septic conditions associated with a decrease in GFR and high circulating AVP levels, this escape may serve to protect against water intoxication by reducing the transepithelial water reabsorption in the collecting ducts secondary to an increased cortico-medullary osmotic gradient. A similar AVP escape mechanism has been described in a number of conditions with increased plasma AVP levels as liver cirrhosis (Jonassen et al., 1998, 2000) and continuous infusion of the AVP V$_2$ receptor agonist 1-desamino-8-D-arginine vasopressin to water-loaded rats (Ecelbarger et al., 1997). The mechanisms behind the AVP escape phenomenon are not fully understood, but it has been demonstrated that cAMP accumulation in response to V$_2$ receptor stimulation is significantly decreased in isolated collecting ducts (Ecelbarger et al., 1998).

The expression of AQP1 and Na$^+$,K$^+$-ATPase was unchanged in the LPS-treated rats. Recent reports have shown that ischemia induced ARF is associated with an almost ubiquitous down-regulation of all major renal tubular sodium and water transporters (Fernandez-Llama et al., 1999; Kwon et al., 1999, 2000). Together, these findings suggest that the tubular damage in LPS-induced ARF is less ubiquitous than in ischemia-induced ARF. The mechanisms responsible for
these differences in tubular damage in different models are unknown.

**Effects of PDE Inhibitors in LPS-Induced Acute Renal Failure.** There is increasing evidence to indicate that cAMP-sensitive PDE isozymes play an important role in several pathophysiological processes in the kidneys, including ARF, and a number of studies have examined the effect of PDE3 and PDE4 inhibitors on the development of ARF in animal models of LPS-induced SIRS. Studies by Begany et al. (1996) and Carcillo et al. (1996) have shown that pretreatment with Ro-20-1724 in the same dose as used in the present study significantly attenuated the LPS-induced fall in renal blood flow, renal vascular resistance, and GFR in anesthetized rats, and recently Thomas et al. (2001) showed that 3-day treatment with Ro-20-1724 attenuated the fall in GFR and renal blood flow found in anesthetized rats with multiple organ dysfunction syndrome due to zymosan treatment. In contrast to these findings in anesthetized rats, the present study performed in conscious rats showed that pretreatment with either milrinone or Ro-20-1724 significantly enhanced the initial LPS-induced fall in GFR. In the Ro-20-1724-treated rats this effect on GFR was associated with a marked fall in MAP. Both PDE inhibitors completely blocked the LPS induced tachycardia, suggesting that PDE3 or PDE4

![Fig. 6.](image1)

![Fig. 7.](image2)

Fig. 6. V, V/GFR, and V/C_Li during the clearance experiment on day 2 (expressed as the average of four consecutive 1-h clearance periods). □ vehicle-treated rats; ■ LPS-treated rats. Data are means ± S.E.M. *P < 0.05 versus vehicle; †P < 0.05 versus LPS.

Fig. 7. Immunoblots of membrane fractions from whole kidney homogenates prepared from female Wistar rats. The rats were either vehicle-treated (150 mM glucose) or treated for 1 h with LPS the day before the kidneys were removed and prepared for immunoblotting. A, immunoblot was reacted with affinity-purified anti-AQP1 and reveal a 29-kDa band. B, densitometry performed on all rats. C, immunoblot was reacted with affinity-purified anti-AQP1 and reveal 29-kDa and 35- to 50-kDa AQP2 bands. D, densitometry performed on all rats. Samples from the LPS-treated rats were expressed relative to the mean expression in the corresponding material from vehicle-treated rats. Data are means ± S.E.M. *P < 0.05 versus vehicle.
inhibition attenuate LPS-induced generalized sympathetic nerve activation. Together, these findings strongly indicate that pretreatment with PDE3 or PDE4 inhibitors exacerbate both systemic and renal dysfunction induced by LPS in conscious rats.

**Effects of PDE Inhibitors on LPS-Induced TNF-α Generation.** It is well known that LPS stimulates generation of TNF-α, which plays a major role as mediator of the LPS-induced inflammatory response. Some studies indicate that drugs with the ability to increase intracellular levels of cAMP, such as PDE4 inhibitors or β₂-adrenergic agonists, suppress LPS-induced TNF-α release from mononuclear cells such as macrophages (Goncalves et al., 1998). The mechanisms behind this effect of increased cAMP levels are not fully understood, but it has been suggested that cAMP-activated protein kinase A has the potential to inhibit the proinflammatory NF-κB pathway, possibly through competition between activated cAMP-responsive element binding protein and NF-κB for a limited number of nuclear cAMP-responsive element binding protein-binding protein coactivators (Parry and Mackman, 1997; Farmer and Pugin, 2000). On the other hand, it has been shown that the catalytic subunit of PKA phosphorylates the p65 subunit of NF-κB, which seems to be essential for efficient transcriptional activity of NF-κB (Zhong et al., 1998). In line with this, increased intracellular levels of cAMP were recently demonstrated to intensify inflammatory pathways in a cytokine-challenged human intestinal epithelial cell line (Cavichi and Whittle, 1999). In the present study, both milrinone and Ro-20-1724 significantly increased the LPS-induced TNF-α generation, suggesting that PDE inhibitors in vivo exacerbate the systemic inflammatory response induced by intravenous LPS infusion.

In summary, PDE inhibitor pretreatment significantly exacerbated the LPS-induced ARF in conscious rats. The mechanisms behind this unwanted effect of PDE inhibitors are unknown, but PDE inhibitor treatment significantly increased LPS-induced TNF-α production and blocked the LPS-induced tachycardic response. Furthermore, the present data show that rats with LPS-induced acute renal failure develop a relative escape from AVP in the collecting ducts, which in septic conditions may serve to protect against water intoxication.

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