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Renal function and glomerular hemodynamics in male endotoxemic rats

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Renal function and glomerular hemodynamics in male endotoxemic rats. The renal effects of a single intravenous dose of two different *E. coli* lipopolysaccharides (LPS 0111:B4 and LPS 0127:B8), at the same dose of 100 μg/kg, were evaluated in euvolemic Munich-Wistar (MW) rats by whole kidney clearance techniques and micropuncture studies. Following LPS infusion, a significant decrease (8%) in mean BP was observed only in the LPS 0127:B8 treated group. Inulin clearance fell 57% (LPS 0111:B4), $P < 0.01$, and 38% (LPS 0127:B8), $P < 0.01$. Para-aminohippuric (PAH) clearance decreased 31% ($P < 0.01$) and total effective renal vascular resistance rose 70% ($P < 0.03$) in response to LPS 0111:B4. No significant change in PAH clearance was noted in the LPS 0127:B8 group. Superficial single nephron glomerular filtration rate (SNGFR) was reduced 69% (LPS 0111:B4), $P < 0.03$, and 33% (LPS 0127:B8), $P < 0.02$. Superficial glomerular plasma flow fell 48% (LPS 0111:B4), $P < 0.03$, and 24% (LPS 0127:B8), $P < 0.03$. Both lipopolysaccharides were associated with an increase in afferent arteriolar resistance (R_A) which accounted for a reduction in the glomerular capillary hydraulic pressure (P_{GC}). There was no change in the proximal tubular pressure in either group and, therefore, the net transcapillary hydraulic pressures were reduced. No measurable change in the ultrafiltration coefficient, K_f, was observed in either group. In a second set of protocols, the effect of prior administration of indomethacin or captopril on LPS 0111:B4 action was investigated. A significant decrease in BP occurred when animals were pretreated with captopril. Both indomethacin and captopril prevented the renal effects of LPS 0111:B4. The data indicate that lipopolysaccharides affect the kidney primarily by causing a selective increase in R_A. They also indicate that the nephrotoxic potential of the lipopolysaccharides studied are not equipotent. Finally, the results from indomethacin and captopril groups suggest that hormonal alterations play a role in the LPS-induced renal hemodynamic changes.

Acute renal failure is a frequent accompaniment of endotoxemia [1]. The pathophysiological events that occur in sepsis, however, are not fully understood. Hypotension, when present, may contribute to the renal failure of septicemia. The acute renal failure, however, is not entirely dependent on hypotension. Renal alterations varying from minimal impairment to acute tubular necrosis [2, 3] and bilateral cortical necrosis [4, 5] have resulted from the acute administration of bacterial endotoxins, some of them in the absence of hypotension [2, 4, 6].

The mechanisms by which endotoxin exerts its renal effects have received considerable attention. Eicosanoids [6, 7], renin and angiotensin [4, 8], renal nerves [8] and adenosine [9] have all been implicated in the genesis of the renal abnormalities of endotoxemia. Few studies, however, have investigated the renal hemodynamic changes that occur with endotoxemia. Conger, Falk and Guggenheim [4] studied the renal hemodynamic effects of a relatively high dose of lipopolysaccharide (LPS) infusion (~6 mg/kg) in postpartum Munich-Wistar rats. In this setting, the infusion of LPS uniformly resulted in bilateral cortical necrosis. Utilizing micropuncture techniques, they found that the early deterioration in inulin and PAH clearances was due to alterations in glomerular hemodynamics. It should be noted that a small but significant decrease in renal function was observed in virgin controls receiving the same dose of LPS. Unfortunately, the mechanisms related to the reduction of renal function in virgin animals were not addressed in that study, since micropuncture studies were not performed in the control group. The acute renal failure associated with endotoxemia, however, does not occur exclusively in the peripartum period. Furthermore, cortical necrosis is frequently not the histological lesion associated with sepsis. For these reasons, the present study was undertaken to evaluate glomerular hemodynamics in euvolemic male rats infused with a dose of LPS which results in ARF but not cortical necrosis [6]. Two endotoxins derived from different strains of *E. coli* were studied to determine if variations in the type of endotoxin used could explain some of the differences observed between studies.

Since previous studies have reported that plasma renin activity is elevated in endotoxemia [8, 10] and that administration of a competitive angiotensin II inhibitor may in part protect the kidney [4], the renal effect of LPS in the presence of captopril was assessed. Finally, cyclo-oxygenase products such as thromboxane A_2, prostaglandin E_2 and I_2, are elevated in endotoxemia [4, 9, 14], while cyclo-oxygenase inhibition has been consistently associated with improvement of the systemic hemodynamics and increased survival in endotoxemia [11–14]. Thus, the effect of indomethacin was also investigated in the present model.

Methods

General

Studies were performed on five groups of male Munich-Wistar rats weighing 230 to 330 g. Each rat was allowed free

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access to water and standard rat chow until the morning of the study.

Rats were anesthetized with Inactin (BIK, Germany), 100 mg/kg i.p., placed on a thermostatically controlled table, and their temperature monitored with an electronic thermometer (Simpson Co., Chicago, Illinois, USA). Following tracheostomy, the left femoral artery was catheterized with a PE-50 polyethylene tubing and approximately 60 μl of arterial blood was collected for baseline hematocrit (Hct) and protein (C_A) determinations. This arterial catheter was used for blood sampling and monitoring of mean femoral arterial pressure. Polyethylene catheters were also inserted into the right and left jugular veins. An infusion of an inulin and para-aminohippuric acid (PAH) containing saline solution at 1.2 ml per hour (Harvard Co., South Natick, USA) was started in the right jugular vein and continued throughout the entire experiment. Surgical losses were replaced via the left jugular vein with isoncotic rat serum at a rate calculated to maintain the animals euvolemic [15]. Laparotomy was performed through a median incision and a PE-10 polyethylene catheter was introduced into the left ureter for collection of timed urine samples.

Following an equilibration period of 45 minutes, control collections were begun. Two timed urine samples and two blood samples of approximately 60 μl each were collected for Hct, C_A, and plasma and urinary determinations of inulin, PAH, sodium and potassium.

At the end of the control period, 100 μg/kg body wt of either *E. coli* 0111:B4 LPS (Difco Labs, Detroit, Michigan, USA) or *E. coli* 0127:B8 (Sigma Chemical Co., St. Louis, Missouri, USA) was given as an intravenous bolus. In the control group, an equivalent volume of saline (vehicle) was substituted for endotoxin. Following a second equilibration period of 50 minutes to allow a maximal effect of LPS [6], repeat collections were performed.

Micropuncture studies

In Groups 2 and 3, animals were set up as above. In addition, the posterior face of the left kidney was cautiously detached from its retroperitoneal fat and supported on a plastic shovel. A plastic holder was placed to minimize transmission of diaphragmatic movements to the kidney. Exactly timed (1 to 3 min) samples were collected from a minimum of three proximal convoluted tubules for determination of flow rate and inulin concentration and calculation of single nephron glomerular filtration rate (SNGFR). Coincident with these collections, samples of femoral arterial blood were obtained in each period for determination of Hct, total protein, inulin and PAH concentrations.

Measurements of hydraulic pressures were obtained from punctures of glomerular capillaries (P_{GC}), proximal tubules (P_T), and efferent arterioles (P_{EA}) with 2 to 4 μm pipets containing 2.0 M sodium chloride connected to a continuous recording servonull-transducer system (IPM, San Diego, California, USA). Hydraulic output from the servo-system was channeled via an electronic transducer (Statham Instruments Division, Gould Inc., Hato Rey, Puerto Rico) to the second channel of the recorder.

The afferent arteriolar oncotic pressure (π_A) and efferent arteriolar oncotic pressure (π_E) were estimated from the plasma protein concentrations of femoral artery (C_A) and superficial efferent arterioles (C_E) as previously described [16]. These estimations permit calculation of colloid osmotic pressures, single nephron filtration fraction (SNFF), glomerular capillary plasma flow ($\dot{Q}_A$), afferent (R_A), efferent (R_E), total ($R_T = R_A + R_E$) arteriolar resistance, and the glomerular capillary ultrafiltration coefficient (K_f) using equations described elsewhere [17, 18].

Characterization of groups

Group 1, control ($N = 6$), received an injection of saline (1 ml/kg) at the end of the first period to evaluate the effects of time, vehicle and procedures on renal function.

Group 2 ($N = 12$) received a single dose of intravenous LPS 0111:B4, 100 μg/kg in saline (100 μg/ml) at the end of the first period enabling evaluation of the effects of LPS on renal function and glomerular hemodynamics. Complete micropuncture data were collected in six animals of this group.

Group 3 ($N = 8$) underwent a protocol identical to that of Group 2 except LPS 0127:B8 was injected rather than LPS 0111:B4.

Group 4 ($N = 6$) was designed to assess the effect of pretreatment with an inhibitor of cyclo-oxygenase on the renal effects of LPS. Animals received an i.v. bolus of indomethacin (2 mg/kg), immediately following catheterization of the left jugular vein. As in Group 2, they also received LPS 0111:B4 at the end of the first period.

Group 5 ($N = 6$), was designed to investigate the effect of pretreatment with captopril, a converting enzyme inhibitor, on the renal action of LPS. Animals received a continuous infusion of captopril (Squibb, Princeton, New Jersey, USA; 2 mg/kg per hr) which was begun at the time of the left jugular vein catheterization and continued throughout both experimental periods. LPS (0111:B4) was infused at the end of the initial period, as in Group 2.

Analytical procedures

The concentration of inulin in the tubule fluid was measured by the microfluorescence method of Vurek and Pegram [19]. Inulin and PAH concentrations in plasma and urine were determined by the macroanthrone method of Fuhr, Kaczmarczyk and Kruttgen [20] and by the method of Smith et al [21], respectively. Protein concentrations in femoral artery plasma for all groups were determined by refractometry and corrected according to a curve constructed by the method of Lowry modified by Schachterle [22]. In Group 2, C_A was determined by the ultracolorimetric method of Viets et al [23] used for C_E to permit adequate comparison. Sodium and potassium concentrations in plasma and urine were measured by flame photometry (Tecnov, Sao Paulo, Brazil). Plasma activity of renin was determined by the method of Vieira et al [24].

Glomerular filtration rate (GFR) was estimated by inulin clearance and effective renal plasma flow (ERPF) by PAH clearance. Total effective renal vascular resistance (ERVR) was calculated as the BP/ERPF ratio. Values of GFR and ERPF were corrected for kidney weight.

Statistical analysis

Data from the first and second periods in each group were compared using a signed rank test. The Rank Sum Test was used for comparing data between groups 2 and 3. Changes

Table 1. Summary of whole animal data

	Body weight	Kidney weight	BP *mm Hg*		Hct %		C_A *g/dl*	
	g		1	2	1	2	1	2
Group 1 Control	277 ± 14	1.08 ± 0.06	104 ± 3	106 ± 3	46 ± 1	46 ± 1	5.8 ± 0.1	5.8 ± 0.1
Group 2 LPS 0111:B4	266 ± 5	1.01 ± 0.03	109 ± 3	102 ± 3	47 ± 1	48 ± 1	5.5 ± 0.1	5.4 ± 0.1
Group 3 LPS 0127:B8	265 ± 6	1.03 ± 0.05	104 ± 3	96 ± 2[a]	45 ± 1	46 ± 1	5.3 ± 0.1	5.2 ± 0.1
Group 4 Indomethacin and LPS 0111:B4	267 ± 5	1.04 ± 0.03	111 ± 5	115 ± 5	48 ± 1	49 ± 1	5.5 ± 0.1	5.3 ± 0.2[a]
Group 5 Captopril and LPS 0111:B4	282 ± 12	1.12 ± 0.06	102 ± 4	90 ± 4[a]	47 ± 1	47 ± 1	5.7 ± 0.2	5.5 ± 0.1

Values are expressed as the mean ± SEM. Abbreviations are: 1, values at first period; 2, values at second period; BP, blood pressure; Hct, hematocrit; C_A, plasma protein concentration of femoral artery.
[a] $P < 0.05$ vs. values at first period

Table 2. Summary of several measures of whole kidney function

	GFR	ERPF	ERVR	$\dot{V}$	$U_{Na}\dot{V}$	FE_{Na}	$U_K\dot{V}$	FE_K
	ml/min		*mm Hg · min/ml*	*μl/min*	*μEq/min*	%	*μEq/min*	%
Group 1								
Control 1	0.96 ± 0.07	3.38 ± 0.15	31.14 ± 1.57	2.6 ± 0.2	0.45 ± 0.08	0.30 ± 0.04	1.07 ± 0.14	49.68 ± 7.83
Control 2	1.14 ± 0.05	3.65 ± 0.22	29.56 ± 1.92	3.0 ± 0.2	0.63 ± 0.12	0.35 ± 0.05	1.46 ± 0.21	60.35 ± 16.48
Group 2								
Control	0.91 ± 0.03	3.09 ± 0.14	36.31 ± 2.41	3.2 ± 0.5	0.88 ± 0.17	0.59 ± 0.11	1.22 ± 0.12	62.00 ± 9.95
LPS 0111:B4	0.39 ± 0.05[a]	2.13 ± 0.29[a]	63.07 ± 11.65[a]	3.1 ± 0.3	0.21 ± 0.03[a]	0.38 ± 0.05	0.35 ± 0.05[a]	53.98 ± 6.76
Group 3								
Control	1.04 ± 0.07	3.48 ± 0.27	30.95 ± 2.43	3.5 ± 0.6	0.90 ± 0.22	0.47 ± 0.14	1.24 ± 0.18	46.36 ± 8.98
LPS 0127:B8	0.61 ± 0.04[a]	3.01 ± 0.36	35.71 ± 5.16	4.7 ± 0.8	0.43 ± 0.12	0.45 ± 0.12	0.68 ± 0.09[a]	35.82 ± 4.35
Group 4								
Indomethacin	1.00 ± 0.09	3.53 ± 0.38	35.67 ± 4.98	2.8 ± 0.4	0.68 ± 0.17	0.43 ± 0.09	0.98 ± 0.17	50.70 ± 10.71
Indomethacin + LPS	1.03 ± 0.16	4.26 ± 0.79	38.35 ± 5.32	4.9 ± 1.4	1.09 ± 0.45	0.49 ± 0.15	1.06 ± 0.31	43.10 ± 11.37
Group 5								
Captopril	0.94 ± 0.05	3.89 ± 0.43	38.25 ± 3.78	3.3 ± 0.4	0.89 ± 0.24	0.58 ± 0.15	1.50 ± 0.20	93.15 ± 13.73
Captopril + LPS	0.90 ± 0.14	5.02 ± 0.75	19.25 ± 1.94	4.2 ± 0.7	0.58 ± 0.22	0.35 ± 0.11	0.95 ± 0.26	68.18 ± 19.58

Values are expressed as the mean ± SEM. Abbreviations are: GFR, glomerular filtration rate; ERPF, effective renal plasma flow; ERVR, effective renal vascular resistance; $\dot{V}$, urine flow rate; $U_{Na}\dot{V}$, urinary excretion of sodium; FE_{Na}, fractional excretion of sodium; $U_K\dot{V}$, urinary excretion of potassium; and FE_K, fractional excretion of potassium.
[a] $P < 0.05$ vs. values at first period

among groups 1, 2, 4, and 5 were evaluated by a Kruskal-Wallis test complemented by a Dunn variation.

Statistical significance was considered as $P < 0.05$. Results are expressed as mean ± SE.

Results

The results of the whole animal data in the five groups are summarized in Table 1. All groups had similar body weights. Significant decreases of 8 and 12% were observed in the mean arterial pressure in the second period of Groups 3 ($P < 0.04$) and 5 ($P < 0.04$), respectively. A small but statistically significant reduction in C_A was observed in Group 4.

Renal action of LPS

Table 2 summarizes parameters of whole kidney function. All studied parameters remained constant throughout the experiment in the control group (Group 1). Treatment with *E. coli* 0111:B4 LPS (Group 2) induced a 57% decline in GFR ($P < 0.01$) and a 31% decrease in PAH clearance ($P < 0.01$) in association with a 74% increase in total renal vascular resistance ($P < 0.03$). The sodium and potassium excretion rates were reduced 76% ($P < 0.01$) and 71% ($P < 0.01$), respectively, but no alteration was found in the urinary flow rate. In contrast, *E. coli* 0127:B8 infusion (Group 3) reduced the GFR only 38% ($P < 0.01$) without significantly affecting either the PAH clearance or effective renal vascular resistance. The urinary excretion rate of potassium declined 45% ($P < 0.02$). There was also a tendency for Na excretion to fall but this did not reach statistical significance. As in Group 2, the urinary flow rate remained unchanged (Table 2). The percent changes induced by the two LPS's in inulin clearance and PAH clearance were statistically different, 57% versus 38% ($P < 0.05$), and 31% versus 14% ($P < 0.04$), respectively.

Micropuncture data obtained in six animals of Group 2 and seven animals of Group 3 are depicted in Table 3. In Group 2, SNGFR, $\dot{Q}_A$ and SNFF were all significantly reduced by *E. coli* 0111:B4 LPS infusion, 69% ($P < 0.03$), 48% ($P < 0.03$), and 37% ($P < 0.03$), respectively. Since P_{GC} declined from 44 ± 1 to 36

Table 3. Glomerular hemodynamic measurements before and after LPS administration—Groups 2 and 3

	SNGFR	$\dot{Q}_A$		P_{GC}	P_T	ΔP	R_A	R_E	R_T	C_A	C_E	π_A	π_E		K_f
	nl/min		SNFF	*mm Hg*			$\times 10^{10}$ *dyn · sec · cm*$^{-5}$			*g/dl*		*mm Hg*		$\pi_{E/\Delta P}$	*nl/sec · mm Hg*
Group 2 LPS 0111:B4															
Before	35.48	102.55	0.35	44	12	32	2.79	1.52	4.31	5.4	8.3	17.54	33.82	1.06	0.086
	±3.40	±7.49	±0.01	±1	±1	±1	±0.19	±0.15	±0.31	±0.1	±0.2	±0.39	±1.14	±0.06	±0.010
After	10.83[a]	52.87[a]	0.22[a]	36[a]	11	25[a]	5.62[a]	2.07	7.69[a]	5.4	6.9[a]	17.22	25.50[a]	1.05	0.080
	±1.75	±9.23	±0.03	±2	±1	±2	±0.86	±0.31	±1.10	±0.1	±0.2	±0.46	±1.33	±0.10	±0.028
Group 3 LPS 0127:B8															
Before	34.17	97.50	0.35	45	16	29	2.62	1.43	4.04	5.0	7.7	15.59	30.36	1.03	0.082
	±2.34	±4.56	±0.03	±1	±1	±1	±0.20	±0.19	±0.37	±0.2	±0.3	±1.04	±1.61	±0.03	±0.005
After	22.76[a]	74.58[a]	0.30[a]	35[a]	12	23[a]	3.90[a]	1.47	5.37[a]	5.0	7.2[a]	15.38	26.80[a]	1.19	0.089
	±3.48	±8.28	±0.02	±1	±2	±1	±0.72	±0.21	±0.89	±0.1	±0.2	±0.46	±1.27	±0.09	±0.011

Values are expressed as mean ± sem. Abbreviations are: SNGFR, single nephron glomerular filtration rate; $\dot{Q}_A$, glomerular plasma flow; SNFF, single nephron filtration fraction; P_{GC}, glomerular capillary hydraulic pressure; P_T, proximal tubular hydraulic pressure; ΔP, transcapillary hydraulic pressure; R, arteriole resistance; C, plasma protein concentration; π, plasma oncotic pressure and K_f, glomerular capillary ultrafiltration coefficient. Subscripts are: A, afferent and E, efferent.

[a] $P < 0.05$ vs. before LPS

± 2 mm Hg ($P < 0.03$) and P_T did not change, ΔP, the net hydrostatic pressure decreased from 32 ± 1 to 25 ± 2 mm Hg ($P < 0.03$). A 101% increase was observed in R_A ($P < 0.03$), but R_E remained unchanged (Table 3). Mean values for C_A and, thus π_A, were similar before and after LPS administration. Consequent to the reduction in SNFF, however, C_E and thus, π_E decreased significantly ($P < 0.03$). Despite euvolemic conditions, filtration disequilibrium ($\pi_E/\Delta P < 1$) was obtained in both periods in only two rats. Two other animals were at filtration equilibrium in both periods. Of the two remaining rats, one changed from equilibrium to disequilibrium, the reverse being observed with the other. When the data are considered together, there is no indication that a reduction in K_f plays a role in LPS induced decrease in SNGFR.

Less severe alterations were observed in the glomerular hemodynamics after infusion of LPS 0127:B8 (Group 3). Mean SNGFR, $\dot{Q}_A$ and SNFF were reduced 33% ($P < 0.02$), 24% ($P < 0.03$) and 14% ($P < 0.02$), respectively. P_{GC} fell from 45 ± 1 to 35 ± 1 mm Hg ($P < 0.02$) leading to a reduction in ΔP, from 29 ± 1 to 23 ± 1 mm Hg ($P < 0.02$). A 49% increase was noted in R_A ($P < 0.05$). Most of the animals were at filtration equilibrium, the calculated K_f representing a minimum in six rats prior to LPS and in five rats after LPS administration. With this limitation, no data to support a role for K_f in the pathogenesis of endotoxin-induced acute renal failure were found.

Effects of pretreatment with indomethacin and captopril

Rats pretreated with indomethacin (Group 4) did not demonstrate any significant change in BP (Table 1). In the group pretreated with captopril, LPS 0111:B4 infusion caused a 12% reduction in BP ($P < 0.04$). Both experimental maneuvers, however, were effective in preventing LPS 0111:B4 actions on GFR, ERPF, ERVR, $U_{Na}V$ and U_KV (Table 2).

Plasma renin activity was measured at the end of experiments in three animals of Group 2 (LPS 0111:B4), and 4 animals of Group 4 (indomethacin + LPS 0111:B4). Mean values were ten times higher in Group 2 (2.39 ± 0.09 ng · ml^{-1} hr^{-1}) versus Group 4 (0.23 ± 0.08 ng · ml^{-1} hr^{-1}), $P < 0.04$, suggesting that the inhibition of prostaglandin synthesis may have secondarily affected the renin-angiotensin system.

Discussion

Endotoxin is felt to be a mediator of sepsis-induced acute renal failure. Recent data indicate that endotoxins reduce renal function by modifications in renal hemodynamics independent of changes in arterial pressure [2, 4, 6]. In agreement with these studies, the reduction in BP following LPS infusion in the present study cannot account for the renal findings. Indeed, the decrease in GFR was greater with LPS 0111:B4 which was not associated with a significant effect on the BP.

Conger et al [4] investigated the renal hemodynamic effects of *E. coli* 026B LPS, ~6 mg/kg, in hydropenic female rats in the postpartum period. They reported a fourfold increase in R_A and a twofold increase in R_E. Their findings, however, resulted from a combination of factors unique to the postpartum period. In their study the animals developed cortical necrosis, a lesion which is rarely observed with sepsis. Furthermore, virgin controls receiving the same dose of LPS had a small but significant decrease in their renal function. The present set of studies was undertaken to determine if alterations in intrarenal hemodynamics are important in a more general model of endotoxin-induced acute renal failure. Male rats were investigated to avoid any possible influence of pregnancy or the estral cycle on the sensitivity to bacterial endotoxins. A dose of endotoxin which does not cause cortical necrosis was selected [6]. To determine if variations in the type of endotoxin would explain some of the differences between studies, endotoxins derived from two different strains of *E. coli* were used.

The infusion of LPS significantly altered both the whole kidney function and the single nephron function. The parameters obtained from the whole kidney studies revealed that LPS 0111:B4 reduced both the inulin clearance and the PAH clearance, and increased the total effective renal vascular resistance. LPS 0127:B8 also decreased inulin clearance but did not significantly affect either the PAH clearance or the renal vascular resistance. It should be noted, however, that consistent with reports of others [2, 3, 6] LPS 0111:B4 decreased the inulin clearance proportionately more than the PAH clearance (57% vs. 31%, $P < 0.01$). Considering the smaller reduction in the inulin clearance by LPS 0127:B8, therefore, the absence of a

significant alteration in either the PAH clearance or the total renal vascular resistance in this group is not as surprising. The infusion of both lipopolysaccharides reduced the urinary excretion of sodium and potassium to the same degree as the GFR resulting in no net change in the fractional excretion of these cations. The decreased potassium excretion was interpreted as probably determined by a reduction of distal delivery of sodium.

Overall, the results of the whole kidney function studies and those of the micropuncture studies are quite consistent. The micropuncture studies revealed that in superficial nephrons, both lipopolysaccharides significantly reduced the SNGFR and the glomerular plasma flow, and significantly increased the total arteriolar resistance. A comparison of the micropuncture data and the whole kidney data suggests, however, that the reduction in plasma flow might be more accentuated in superficial than in deep nephrons. For instance, if only the animals which had undergone micropuncture are considered, LPS 0111:B4 reduced PAH clearance 38% while, in superficial nephrons, glomerular plasma flow was decreased 48%. No statistically significant difference was evident, however. It should be noted that a previous study by Kikeri et al [3] reported that PAH extraction was decreased when a 53% reduction in GFR followed LPS infusion, suggesting that PAH clearance, in this circumstance, may underestimate the renal plasma flow. Since single nephron plasma flow is a direct calculation it would not be affected by alterations in PAH extraction. The reduction in the whole renal plasma flow in the LPS 0111:B4 group might therefore be less than the calculated 38% and perhaps significantly less than the 48% reduction in the glomerular plasma flow of superficial nephrons. No decrease in PAH clearance, however, was found in the LPS 0127:B8 group, suggesting that PAH extraction was not substantially changed when only a modest impairment in GFR was present. Despite this, a 24% reduction was observed in the glomerular plasma flow of superficial nephrons, again indicating that a greater reduction in the perfusion of superficial nephrons might take place in endotoxemia.

Although the renal effects of LPS 0111:B4 were more marked than those from LPS 0127:B8, their effects on renal parameters were qualitatively similar. The present findings suggest that in relatively low dose lipopolysaccharides exert their renal effects primarily by increasing afferent arteriolar resistance. This leads to a reduction in the glomerular capillary hydraulic pressure which, in the presence of an unaltered tubular pressure, results in a significant decrease in glomerular transcapillary hydraulic pressure. In contrast to the findings in postpartum rats [4], no change was observed in the efferent arteriolar resistance. In agreement with that report, however, no measurable change in the ultrafiltration coefficient, K_f, was detected in our series, though the presence of filtration equilibrium in a number of the animals precludes any definitive conclusion in this regard. The decrease in glomerular plasma flow and SNGFR seems to be entirely accounted for by the increase in R_A.

Additional studies utilizing whole clearance techniques were performed to assess the effect of prior administration of indomethacin and captopril on the renal effects of the LPS 0111:B4. A significant modification of the degree of renal impairment was found with both of these agents. Indomethacin pretreatment completely prevented the reduction in the inulin clearance, PAH clearance, sodium and potassium excretion. Since cyclo-oxygenase inhibition is thought to impair the renal function of hypoperfused kidneys [25, 26, 27], these findings were unexpected. In support of the present observations, however, there are several intriguing reports of indomethacin-associated increase in survival in animal models of sepsis [11, 13, 28]. There are, in addition, reports that cyclo-oxygenase inhibition can be protective against the LPS-induced blood flow reduction in the denervated kidney of dogs [8] and in the liver and kidney of rats [29]. Cyclo-oxygenase inhibition is expected to result in a decrease in both vasodilatory and vasoconstrictive derivatives of arachidonic acid metabolism. Our present results suggest that vasodilatory prostaglandins may not be the only system to influence renal hemodynamics in endotoxemia. Inhibition of vasoconstrictive cyclo-oxygenase product synthesis like thromboxane A_2 may have had beneficial effects. Consistent with this view, Badr, Kelly and Brenner [6] reported that a selective inhibitor of thromboxane A_2 attenuated the renal effects of endotoxin. It is also possible that inhibition of prostaglandin-dependent angiotensin II production may have played a protective role. The latter is consistent with the lower plasma renin activity in animals receiving indomethacin in the present study. Reduced renin production following indomethacin was also reported by Heinrich et al [8] in the denervated kidney of dogs whose glomerular filtration rate was maintained after endotoxin administration.

Pretreatment of animals with captopril also prevented the LPS-induced decrease in inulin clearance, PAH clearance, and sodium and potassium excretion despite an accentuation in the hypotensive effect of LPS. Since captopril can potentially interfere with kinin catabolism as well as angiotensin II production [30] it is not possible to draw any definitive conclusion regarding the mechanism responsible for this protection, from our studies. Considering, however, that Conger et al [4] reported that the renal effects of LPS were partially prevented by a competitive angiotensin II inhibitor, it is reasonable to conclude that at least part of the protection offered by captopril might be due to the inhibition of angiotensin II production. The present observations suggest that angiotensin II, which is thought to act mainly in efferent arterioles [31, 32], may have an important afferent arteriolar effect in the presence of LPS.

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Appendix. Abbreviations

BP	blood pressure
C_A	plasma protein concentration of femoral artery
C_E	plasma protein concentration of star vessel
FE_{Na}	fractional excretion of sodium
FE_K	fractional excretion of potassium
GFR	glomerular filtration rate

Hct	hematocrit
K_F	glomerular capillary ultrafiltration coefficient
LPS	lipopolysaccharide
P_{EA}	star vessel hydraulic pressure
P_{GC}	glomerular capillary hydraulic pressure
P_T	proximal tubular hydraulic pressure
ΔP	transcapillary hydraulic pressure ($\Delta P = P_{GC} - P_T$)
π_A	femoral artery oncotic pressure
π_E	star vessel oncotic pressure
PAH	para-aminohippurate
$\dot{Q}_A$	glomerular plasma flow
R_A	afferent arteriole resistance
R_E	efferent arteriole resistance
ERPF	effective renal plasma flow
ERVR	effective renal vascular resistance
SNFF	single nephron filtration fraction
SNGFR	single nephron GFR
$U_{Na}\dot{V}$	urinary excretion of sodium
$U_K\dot{V}$	urinary excretion of potassium

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