Dissociation of Systemic and Renal Effects in Endotoxemia

PROSTAGLANDIN INHIBITION UNCOVERS AN IMPORTANT ROLE OF RENAL NERVES

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ABSTRACT To elucidate the mechanisms responsible for systemic and renal hemodynamic changes in early endotoxemia, the roles of prostaglandins (PG) and renal nerves were investigated. Endotoxin (E, 3 μg/kg i.v.) was given to two groups of anesthetized dogs that had undergone unilateral renal denervation: Group I ($n = 9$) E only; Group II ($n = 11$) E + indomethacin (10 mg/kg i.v.) or meclofenamate (5 mg/kg i.v.). A third group of dogs (Group III, $n = 5$) received indomethacin (10 mg/kg i.v.) only. 1 h after E in group I dogs, mean arterial pressure (MAP) decreased from 126 to 94 mm Hg ($P < 0.001$), and prostacyclin (6-keto-F1α metabolite, PGI_2) increased (from 0.64 to 2.08 ng/ml, $P < 0.005$). Glomerular filtration rate (GFR) and renal blood flow (RBF) declined comparably both in innervated and denervated kidneys. In marked contrast, group II dogs had a stable MAP (136–144 mm Hg, NS) and no increase in PGI_2 levels. Plasma renin activity (0.7–2.5 ng/ml per h, $P < 0.005$) increased, and renin secretion was greater in innervated compared with denervated kidneys (255 vs. 74 U/min, $P < 0.01$) in these PG-inhibited dogs. In addition, denervated kidneys in group II dogs had a greater GFR (42 vs. 34 ml/min, $P < 0.01$) and RBF (241 vs. 182 ml/min, $P < 0.01$) than innervated kidneys after E. Group III animals had no significant changes in systemic or renal hemodynamics, plasma renin activity or PGI_2 during the study. These results suggest that PGI_2 mediates the systemic hypotension of early endotoxemia in the PG-intact animal. Moreover, PG inhibition uncovers an important effect of E to increase efferent renal nerve activity with a consequent decline in GFR and RBF independent of changes in MAP. Finally, the results demonstrate that renal nerves are important stimuli to renin secretion in early endotoxemia via pathways that are PG-independent.

INTRODUCTION

Endotoxemia induces a wide array of pathophysiologic abnormalities with multiple adverse consequences (1–3). Foremost among the important disturbances observed are systemic hypotension and renal insufficiency that may occur early in the syndrome and contribute substantially to the high morbidity and mortality seen in endotoxemia (4–6). The pathogenetic factors responsible for these early changes in endotoxemia however, are incompletely defined. For example, while sympathetic nervous system activity is markedly stimulated during the syndrome (1), the precise contribution of renal sympathetic nerves to renal hemodynamic control during early endotoxemia is unknown. Similarly, endotoxin is known to stimulate prostaglandin synthesis and release in vitro (7), but the consequences of this effect for the systemic and renal circulations are incompletely defined. Furthermore, the interrelationship between the renal sympathetic nervous and prostaglandin systems is unclear during endotoxemia, but is of critical importance in influencing renal hemodynamics during several other stressful conditions (8).

Several lines of experimental evidence have previ-

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ously linked renal sympathetic nerves and prostaglandins in the control of renal hemodynamics. Acute alterations in renal hemodynamics often result from an interplay of opposing renal vasoconstriction and vasodilation (9). Experimental maneuvers that cause renal ischemia (e.g., renal nerve stimulation, angiotensin or catecholamine infusion, hemorrhage, sodium depletion, heart failure, cirrhosis, and hypercapnia) cause enhanced renal vasoconstriction when prostaglandin synthesis is inhibited (8, 10–20). In addition, each of these vasoconstrictive stimuli markedly stimulates renal prostaglandin synthesis that leads to renal vasodilatation and opposes the vasoconstrictor input (9–20). Hence, the renal vasodilatory system mediated by intrarenal production of prostaglandins attenuates renal ischemia in the setting of an unstable systemic circulation; conversely, prostaglandin synthesis inhibition enhances renal ischemia because vasoconstrictive stimuli are unopposed by compensatory vasodilatation.

Despite these advances in our understanding of mechanisms controlling renal hemodynamics during alterations in the systemic circulation, important questions remain unresolved. First, the specific ability of an ischemic factor (e.g., renal sympathetic nerves) to influence renal blood flow (RBF)[1] and increase prostaglandin synthesis has been tested in relatively few pathophysiological conditions. Second, while many stimuli and effects of renal prostaglandin production have been demonstrated, the spectrum of importance of systemically active prostaglandins (particularly prostacyclin [PGI_2]) is incompletely defined. Such a systemic effect has recently been proposed for PGI_2 in pregnancy (21) and endotoxemia (1). Further, the beneficial effect of prostaglandin inhibition on blood pressure (1, 22) during endotoxemia has not been studied with respect to the renal hemodynamic consequences of such a maneuver. Finally, the exact importance of prostaglandins in renin secretion during stimulation of the sympathetic nervous system stress has been a controversial subject (23–26).

The present studies address several of these unresolved issues by examining the in vivo relationship between renal nerves and prostaglandins in early endotoxemia. Measurements of systemic and renal hemodynamics, PGI_2 levels, and renin secretion rates were performed in animals with unilateral renal denervation to learn the physiologic role of prostaglandins in the early phase of this syndrome.

METHODS

Preparations and calculations. 24 mongrel dogs weighing between 20 and 25 kg were used in this study. On the morning of the study, the animals were anesthetized with pentobarbital (25 mg/kg), intubated, and ventilated with a Harvard respirator. (Harvard Apparatus Co., Natick, Mass.) Nonhypotensive doses of pentobarbital were subsequently given to maintain an even state of anesthesia. Polyethylene catheters were placed in both ureters and renal veins through a retroperitoneal approach. Unilateral renal denervation was performed in all dogs by severing all visible renal nerves from the renal artery and vein and applying 95% ethanol to the renal pedicle. This technique has been previously shown to result in renal tissue norepinephrine depletion (8). An equal number of right and left kidneys were denervated in the study. The completeness of renal denervation was also verified using electrical renal nerve stimulation (Grass Electrical Stimulator, Grass Instrument Co., Quincy, Mass.). Experiments were performed in which the RBF response to a standard ischemic electrical stimulation (20 Hz, 20 V, 1.5 ms, and 2.5 mA) of the renal nerves before and after denervation was tested (27). Before denervation, electrical stimulation decreased RBF significantly (290 ± 21 to 201 ± 14 ml/min, $P < 0.001$); after denervation, stimulation of the severed renal nerves did not change RBF (278 ± 16 to 262 ± 14 ml/min, NS).

A brachial artery catheter was inserted and connected to a Statham pressure transducer (Statham Instruments, Inc., Oxnard, Calif.) and Hewlett-Packard 7712 recorder (Hewlett-Packard Co., Palo Alto, Calif.) to monitor arterial blood pressure. A right atrial catheter was placed via the jugular vein for measurement of cardiac output (CO) by the Indocyanine Green dye-dilution technique (28) using a Lyons cardiac output computer (Lyons Medical Corporation, Pleasantville, N. Y.). Midway through the surgical preparations, a 0.5% sodium chloride infusion was begun through a peripheral vein at a rate of 10 ml/min (Harvard infusion pump setting 0.1, Harvard Apparatus Co.) to replace fluid losses and achieve stable urine flows. This infusion was continued for 60 min; after a stable urine flow rate was obtained, the infusion was reduced to equal urine flow rates for the remainder of the experiment. At the conclusion of the surgical preparations, a solution containing a sufficient concentration of inulin and para-aminohippurate (PAH) was infused (0.5 ml/min) into a peripheral vein to maintain the plasma levels of these substances at 15–20 mg/dl and 2–3 ml/dl, respectively.

All animals stabilized for 1 h after surgery. Urine samples for inulin and PAH were obtained during three to four, 5–10-min collections during each collection period. Blood samples for inulin and PAH were obtained at the midpoint of each urine collection. Urine and blood samples for inulin and PAH were analyzed on a Technicon II autoanalyzer (Technicon Instruments, Corp., Tarrytown, N. Y.). Standard calculations were used for inulin clearance (GFR), PAH extraction (RBF), and renal vascular resistance (RVR) (29). CO was performed in duplicate during each clearance period. Peripheral vascular resistance was calculated by dividing the mean arterial blood pressure (MAP) by the CO. Arterial blood gases were measured in duplicate at the midpoint of each period on an automated analyzer (pH-165 analyzer Corning Medical, Corning Glass Works, Medfield, Mass.).

[1] *Abbreviations used in this paper*: CO, cardiac output; GFR, glomerular filtration rate; 6-keto-$PFG_{1\alpha}$-TME, 6-keto $PGF_{1\alpha}$-tyrosine methylester hydrochloride; MAP, mean arterial pressure; PAH, para-aminohippurate; PGI_2, prostacyclin; PRA, plasma renin activity; PVR, peripheral vascular resistance; RBF, renal blood flow; RSR, renin secretory rate; RVR, renal vascular resistance.

Blood samples for plasma renin activity (PRA) were collected on ice from arterial and renal venous catheters at the midpoint of each collection period, centrifuged at 4°C, and then stored at −70°C until assayed. Duplicate samples were obtained in most collection periods and samples were analyzed by radioimmunoassay using standard reagents (E. R. Squibb & Sons, Inc., Princeton, N. J.).

Assay for PGI_2. Arterial blood samples for assay of PGI_2 concentration were obtained in duplicate and placed in chilled heparinized tubes containing 10 μg of indomethacin/ml of blood. The plasma concentration of 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) was determined using a radioimmunoassay procedure according to the method of Tai et al. (30, 31). In this assay, an internal standard (6-keto-$PGF_{1\alpha}$-tyrosine methylester hydrochloride (TME)) was prepared by disolving 1 mg of 6-keto-$PGF_{1\alpha}$ (Upjohn Co., Kalamazoo, Mich.) in 0.2 ml of dimethylformamide at 0°C with 2 μl of triethylamine. Radioiodination of this TME-conjugate was then performed by adding 300 μCi of ^{125}I-Na (Radiochemical Center, Amersham Corp., England) in 3 μl of 0.1 NaOH to 1.5 μg (5 μl) of 6-keto-$PGF_{1\alpha}$-TME in 50 μl of 0.5 M sodium phosphate buffer, pH 7.5. The extraction of 6-keto-$PGF_{1\alpha}$ from plasma was performed twice with a triple volume of ethylacetate after acidifying to pH 3.0 with 1 N HCl. 10,000 cpm of [^{3}H]$PGF_{2\alpha}$-metabolite (13,14 dihydro-15-keto-$PGF_{2\alpha}$) was added for the calculation of recovery rate after extraction. The percent recoveries of the labeled $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ metabolites were verified as comparable (59.95±1.87 vs. 62.12±1.44%, respectively). The dry residue was dissolved in 1.0 ml of solvent I (benzene/ethylacetate 60:40) and transferred to a preactivated silicic acid column in which 0.5 g of silicic acid was suspended in 3 ml of solvent I. Before transferring the sample, each column was washed with 4 ml of solvent II (benzene/ethylacetate/methanol, 60:40:20) and then with 3 ml of solvent I. The $PGF_{2\alpha}$-metabolite and 6-keto-$PGF_{1\alpha}$ were eluted in 4 ml of solvent II. The eluate was evaporated and reconstituted with 1 ml of assay buffer (0.05 M sodium phosphate buffer pH 7.4 containing 0.9% NaCl and 0.1% bovine gamma globulin) and frozen at −20°C until use for radioimmunoassay. A small volume of sample was removed and the [^{3}H]$PGF_{2\alpha}$ metabolite was counted in a liquid scintillation counter (Beckman LS-250 Beckman Instruments, Inc., Fullerton, Calif.). A 6-keto-$PGF_{1\alpha}$ standard solution was prepared serially after being diluted with assay buffer from 10 pg/0.1 ml to 4,000 pg/0.1 ml. Anti-6-keto-$PGF_{1\alpha}$ rabbit serum (provided by Dr. H. H. Tai, University of Kentucky, Lexington, Ky.) obtained 40% of initial binding at the final concentration of 1:20,000. This antiserum is specific for 6-keto-$PGF_{1\alpha}$ (31). The incubation mixture (0.4 ml) contained standard or sample, diluted antiserum, ^{125}I-6-keto-$PGF_{1\alpha}$-TME (10,000 cpm) and assay buffer, 0.1 ml each. The incubation was carried out for 2 h at room temperature. Free ^{125}I-6-keto-$PGF_{1\alpha}$-TME was separated from the fractions bound to the antibody with gamma globulin-coated charcoal. Both supernatant and charcoal were separately counted in a gamma counts (Beckman Biogamma TM, Beckman Instruments, Inc.). The counts per minute of charcoal background was <5%. The bound-to-total ratio was calculated for each sample and the concentration of each sample was determined from a standard curve. The lower limit of assay sensitivity is 75 pg/ml. Finally, the concentration of 6-keto-$PGF_{1\alpha}$ was corrected by dividing by recovery rate. The recovery rate averaged 57% and the intraassay coefficient of variation (in 15 identical tubes) was 12%. The interassay coefficient of variation averaged 15%. All samples and standards were assayed in duplicate.

Experimental protocol

The experimental protocol was divided into several clearance periods, lasting 15–20 min each. The periods were as follows:

Control period. Base-line clearance measurements.

Prostaglandin inhibitor infusion. The three groups of dogs were given one of the following solutions 15 min before the beginning of this period: group I (n = 9) received a blank bicarbonate isotonic solution equal in volume and tonicity to the prostaglandin inhibitor solutions; group II (n = 11) received one of two chemically dissimilar prostaglandin synthesis inhibitors: either indomethacin (n = 6, 10 mg/kg i.v.) or meclofenamate (n = 5, 5 mg/kg i.v.). These doses have previously been associated with prostaglandin synthesis inhibition (8, 32). Because the results obtained with these two inhibitors were virtually identical, the results have been combined into one group of animals; group III (n = 5) received the prostaglandin synthesis inhibitor indomethacin (10 mg/kg i.v.).

Endotoxin infusion. The three groups of dogs were treated with endotoxin or saline as follows 5 min before the beginning of this period: groups I and II received an intravenous injection of 3 μg/kg of purified, lyophilyzed *Escherichia coli* 055 lipopolysaccharide, prepared by the method of Westphal and Jann (33) by Dr. Munford (University of Texas Southwestern Medical School). This preparation contained <1% protein and nucleic acid contamination. In a series of eight preliminary studies, this dose reliably reduced systemic blood pressure by 20–30 mm Hg 1 h after injection; group III dogs received only a blank saline injection during this period and served as a prostaglandin synthesis inhibitor control. Thus, only group II dogs received both the prostaglandin synthesis inhibitor and the endotoxin; group I dogs received endotoxin only; and group III dogs received the prostaglandin synthesis inhibitor only.

Postendotoxin. This period commenced 60 min after the endotoxin injection in all groups of dogs.

There were no detectable alterations of renal or systemic hemodynamics immediately following the endotoxin infusion, and the prostaglandin inhibitor and endotoxin infusion periods have been consolidated and reported as a single "postinfusion" period in Results.

Statistics

Statistical analysis was performed using an analysis of variance procedure when making comparisons between periods or groups (34). Student's paired t test was used when comparing an innervated to denervated kidneys. The data are reported as the mean±1 SEM and a P value of <0.05 was considered significant.

RESULTS

Effects of endotoxin on systemic hemodynamics (Table I). MAP fell 25% 60 min following endotoxin (126±5.1 to 94±4 mm Hg, $P < 0.001$) in group I dogs. This reduction in MAP was characterized by a significant fall in CO (from 2.95±0.45 to 2.25±0.31 liter/min, $P < 0.05$) and an absence of an increase in peripheral vascular resistance (PVR, 46.6±8 to 44.9±8.7 mm Hg/liter/min, NS). In marked contrast, group II dogs, which received either of two prostaglandin in-

TABLE I
Effects of Endotoxin on Systemic Hemodynamics

		MAP			CO			PVR		
		Cont	PI	PE	Cont	PI	PE	Cont	PI	PE
		mmHg			*liter/min*			*mmHg/liter/min*		
Group I, $n = 9$										
Endotoxin only	Mean	126	126	94	3.14	2.95	2.25	42.3	46.6	44.9
	±SE	5	5.1	4	0.42	0.45	0.31	6.1	8	8.7
	P		NS	<0.001		NS	<0.05		NS	NS
Group II, $n = 11$										
PG inhibitor	Mean	131	136	144	3.34	3.24	2.16	41.9	45.2	70*
and endotoxin	±SE	4	4	4	0.35	0.35	0.16	4.1	4.4	6.5
	P		NS	NS		NS	<0.001		NS	<0.001
Group III, $n = 5$										
PG inhibitor	Mean	141	144	147	3.18	2.81	2.74	45.3	52.4	53.6
only	±SE	6	3.4	4.7	0.35	0.17	0.23	5.9	3.1	11.5
	P		NS	NS		NS	NS		NS	NS

Cont, control period; PI, Postinfusion period; PE, 60-min postendotoxin period; PG, prostaglandin.
* Significantly greater than PE values in groups I and III, $P < 0.01$.

hibitors before endotoxin, had a stable MAP 60 min postendotoxin (136±4 to 144±4 mm Hg, NS). This stability of MAP resulted from a rise in PVR (45.2±4.4 to 70±6.5 mm Hg/liter/min, $P < 0.001$) concomitant with a fall in CO (3.24±0.35 to 2.16±0.16 liter/min, $P < 0.001$). There were no significant changes in group III animals (prostaglandin synthesis inhibitor only).

Effects of endotoxin on renal hemodynamics (Table II). The marked decline in MAP 60 min after endotoxin in group I dogs was attended by a decline in glomerular filtration rate (GFR) without a change in RBF in both innervated and denervated kidneys. Renal vascular resistance (RVR) remained unchanged in innervated and denervated kidneys in response to the decline in perfusion pressure.

In group II dogs pretreated with either indomethacin or meclofenemate, postendotoxin differences between the innervated and denervated kidneys were noted despite the fact that systemic MAP was unchanged. GFR decreased significantly in innervated kidneys (from 41.2±3.9 to 33.9±3.1 ml/min, $P < 0.05$) but remained unchanged in the contralateral denervated kidneys (41.9±3 to 42.2±2.6 ml/min, NS) following endotoxin. The postendotoxin GFR was significantly greater in denervated kidneys compared with innervated kidneys ($P < 0.01$) in this group. Similarly, postendotoxin RBF was significantly lower in innervated compared with denervated kidneys (182±23 vs. 241±24 ml/min, $P < 0.001$). The significant increase in postendotoxin RVR in innervated kidneys was attenuated by renal denervation (0.81±0.18 vs. 0.61±0.10 mm Hg/ml per min, $P < 0.01$).

Group III dogs, which received the prostaglandin synthesis inhibitor alone, had a stable GFR during the period of observation. An insignificant decrease in RBF occurred during the experiment.

Effects of endotoxin on arterial prostaglandin (Table III). Arterial levels of PGI_2 (measured as the 6-keto-$F_{1\alpha}$ metabolite) increased significantly 15 min after the endotoxin bolus in group I dogs (0.64±0.05 to 1.26±0.24 ng/ml). 1 h after the endotoxin, 6-keto-$PGF_{1\alpha}$ had further increased to 2.08±0.38 ng/ml ($P < 0.005$). Pretreatment of group II animals with indomethacin or meclofenamate effectively prevented the increase in 6-keto-$PGF_{1\alpha}$ immediately and 1 h postendotoxin bolus. No significant changes in 6-keto-$PGF_{1\alpha}$ occurred in the group III dogs. The percent change in 6-keto-$PGF_{1\alpha}$ between the postinfusion and the postendotoxin periods correlated significantly with the percent change in mean blood pressure for each group of animals ($r = -0.824$, $P < 0.001$).

Effects of endotoxin on plasma renin activity (PRA) and renin secretory rate (RSR) (Table IV). PRA increased significantly in all group I dogs (1.34±0.46 to 3.92±0.81 ng/ml per h, $P < 0.001$) in association with the measured decline in MAP after endotoxin. This increase was accounted for by an increase in RSR from both innervated (139±94 to 464±89 ng/ml per h per min, $P < 0.05$) and denervated kidneys (38±59 to 362±191 ng/ml per h per min, $P < 0.05$). Despite maintenance of a stable MAP and pretreatment with a prostaglandin synthesis inhibitor in group II dogs, PRA again increased significantly after endotoxin (0.66±0.14 to 2.52±0.58 ng/ml

TABLE II
Effects of Endotoxin on Renal Hemodynamics, Comparison of Innervated vs. Denervated Kidneys

			GFR			RBF			RVR		
			C	PI	PE	C	PI	PE	C	PI	PE
				ml/min			*ml/min*			*mm Hg/ml/min*	
Group I											
Endotoxin only	Innervated	Mean	44.3	42.2	32.6	296	275	266	0.44	0.45	0.35
		±SE	4	4	5	26	35	47	0.10	0.11	0.09
		P		NS	<0.05		NS	NS		NS	NS
	Denervated	Mean	45.2	40	29.5	304	260	245	0.49	0.50	0.38
		±SE	3.6	2	4	38	40	29	0.11	0.11	0.15
		P		NS	<0.05		NS	NS		NS	NS
Group II											
PG inhibitor and endotoxin	Innervated	Mean	45.2	41.2	33.9	377	281	182	0.38	0.50	0.81
		±SE	2.6	3.9	3.1	45	35	23	0.06	0.07	0.18
		P		NS	<0.05		NS	<0.01		NS	<0.01
	Denervated	Mean	42.5	41.9	42.2*	337	279	241*	0.35	0.49	0.61*
		±SE	2	3	2.6	33	27	24	0.04	0.06	0.10
		P		NS	NS		NS	NS		NS	NS
Group III											
PG inhibitor only	Innervated	Mean	43.3	39.5	38.6	364	326	288	0.37	0.47	0.52
		±SE	2.8	4.3	2.7	36	21	11	0.03	0.03	0.02
		P		NS	NS		NS	NS		<0.05	NS
	Denervated	Mean	42.9	43	42.1	355	327	292	0.39	0.43	0.48
		±SE	0.9	2.4	2.3	13	17	31	0.03	0.04	0.06
		P		NS	NS		NS	NS		NS	NS

C, control period; PI, postinfusion period; PE, 60-min postendotoxin period; PG, prostaglandin.
* Significantly different from contralateral kidneys, $P < 0.01$.

TABLE III
Effects of Endotoxin on 6-keto-$F_{1\alpha}$

		C	PI	PE
			ng/ml	
Group I				
Endotoxin only	Mean	0.64	1.26	2.08*
	±SE	0.05	0.24	0.38
	P		<0.05	<0.05
Group II				
PG inhibitor and endotoxin	Mean	0.76	0.71	0.76
	±SE	0.08	0.04	0.09
	P		NS	NS
Group III				
PG inhibitor only	Mean	0.79	0.72	0.77
	±SE	0.10	0.03	0.06
	P		NS	NS

C, control period; PI, postinfusion period; PE, 60-min postendotoxin period; PG, prostaglandin.
* Significantly greater than PE values in groups II and III, $P < 0.01$.

TABLE IV
Effects of Endotoxin on PRA, RSR, and $U_{Na}V$

		PRA					RSR			$U_{Na}V$		
		C	PI	PE			C	PI	PE	C	PI	PE
		ng/ml/h					*ng/ml/h per min*			*meq/min*		
Group I												
Endotoxin only	Mean	1.15	1.34	3.92	Innervated	Mean	155	139	464	118	125	32
	±SE	0.37	0.46	0.81		±SE	61	94	89	25	28	10
	P		NS	<0.001		*P*		NS	<0.05		NS	<0.001
					Denervated	Mean	39	38	362	234*	232*	65
						±SE	59	59	191	41	43	24
						P		NS	<0.05		NS	<0.001
Group II												
PG inhibitor	Mean	0.77	0.66	2.52	Innervated	Mean	135	58	255*	93	126	43
and endotoxin	±SE	0.17	0.14	0.58		±SE	59	20	59	36	51	16
	P		NS	<0.005		*P*		NS	<0.05		NS	<0.05
					Denervated	Mean	65	12	74	148	168	68
						±SE	35	9.5	25	29	40	21
						P		NS	NS		NS	<0.001
Group III												
PG inhibitor	Mean	1.36	1.57	1.30	Innervated	Mean	82	45	30	118	145	84
only	±SE	0.64	1.01	0.53		±SE	33	10	18	42	51	26
	P		NS	NS		*P*		NS	NS		NS	NS
					Denervated	Mean	16	123	24	134	153	72
						±SE	86	103	13	47	58	9
						P		NS	NS		NS	NS

C, Control period; PI, postinfusion period; PE, 60-min postendotoxin period; PG, prostaglandin; $U_{Na}V$, whole kidney excretion rate of sodium.
* Significantly greater than contralateral kidney, $P < 0.01$.

per h, $P < 0.005$). In contrast to group I dogs, this increase resulted primarily from an increased renin secretion by innervated kidneys (58±20 to 255±59 ng/ml per h per min, $P < 0.05$) as renal denervation effectively blunted the increase in RSR (12±9.5 to 74±25 ng/ml per h per min, NS). Both PRA and RSR were unchanged in the group III animals treated with the prostaglandin synthesis inhibitor alone. Urinary sodium excretion declined postendotoxin in the group I and group II animals, but was not different in innervated vs. denervated kidneys during the postendotoxin period. No significant changes in sodium excretion were observed in the group III dogs.

Effects of endotoxin on changes in blood gases. Arterial blood gases were unchanged during the observation period in group I dogs (pH 7.4±0.03 before endotoxin to 7.43±0.03 pH units after endotoxin, NS). Arterial pH declined slightly (from 7.41±0.02 to 7.39±0.02 pH units, $P < 0.02$) in group II dogs that received the prostaglandin synthesis inhibitors. Blood pH was stable in the group III dogs during the experimental period (pH 7.40±0.02 before endotoxin to 7.39±0.03 after endotoxin, NS). Both pO_2 and pCO_2 were comparable in all three groups before endotoxin, and did not change during the observation period.

DISCUSSION

The present series of experiments were performed to elucidate several of the mechanisms that influence renal function during early endotoxemia. Specifically, the importance of changes in the systemic circulation, the sympathetic nervous system, and prostaglandins were investigated as each pertains to renal hemodynamics. An investigation of sympathetically mediated renin release was also incorporated into the design of the study.

The effects of endotoxin on systemic hemodynamics were sharply modified by pretreatment of the animals with a prostaglandin synthesis inhibitor as demon-

strated by the striking difference in MAP between group I and group II dogs 60 min after endotoxin. Animals with intact prostaglandin synthesis had a 25% fall in MAP (126–94 mm Hg), which was completely abolished by prior treatment with indomethacin or meclofenemate. One important factor in the hypotension following endotoxin was the reduction in CO, an observation noted in previous studies (1, 35–38). However, the similar depression of cardiac function in groups I and II yet maintenance of MAP in group II indicates that another factor was operant. The reason for maintenance of MAP in group II appeared to be prevention of the vasodilator effects of PGI_2 achieved by prior treatment with indomethacin or meclofenamate, which allowed PVR to increase significantly. Further, the similar reductions in CO in groups I and II imply that endotoxin may cause a decrease in CO directly, independently of a prostaglandin-dependent mechanism. An increase in venous pooling and a decrease in venous return may also contribute to the decrease in CO after endotoxin. Moreover, the percent increase in PGI_2 (as measured by the PGI_2 metabolite 6-keto $PGF_{1\alpha}$) noted in group I dogs in the postendotoxin period correlated with percent decrease in MAP. Thus, the results suggest that PGI_2 contributes to the hypotension of early endotoxemia by blocking compensatory increases in PVR. A similar conclusion has been recently made by Harris et al. (39) from experiments in a baboon endotoxic shock model. Any adverse impact of this early hypotensive effect of endogenous PGI_2 is modified, however, by the reported salutary effects of postendotoxin PGI_2 infusions on cardiac performance, platelet aggregation, and microcirculation integrity (1, 40). The postendotoxin levels of 6-keto-$PGF_{1\alpha}$ observed in this study are similar to earlier results (22, 39). The lack of a detectable reduction in 6-keto-$PGF_{1\alpha}$ levels after indomethacin in group III dogs is likely due to diminished sensitivity of the assay below 0.6–0.8 ng/ml. This was so since the lower limit of assay sensitivity was 75 pg/tube and the dilution factor was 1:10.

The relationship between endotoxin and prostaglandins was also examined in the kidney where an interplay of vasoconstrictor and vasodilator factors dictate kidney function, particularly during systemic hypotension and high sympathetic activity (10–20). The decrease in MAP in prostaglandin intact group I dogs was associated with a decrease in GFR but not RBF in both innervated and denervated kidneys. Thus, as long as the vasodilator influence of prostaglandins was present, no detectable effect of renal sympathetic nerves on GFR and RBF was noted. However, when prostaglandin synthesis was inhibited (group II) both GFR (24%) and RBF (32%) were significantly greater in denervated kidneys compared with innervated kidneys 1 h after endotoxin. The reductions in GFR and RBF in prostaglandin-inhibited dogs were accompanied by a significant increase in renal vascular resistance in innervated kidneys not observed in the denervated kidneys. Interestingly, these changes in renal hemodynamics occurred in the absence of a decrease in MAP. Therefore these results provide evidence that the renal sympathetic nervous system is activated in endotoxemia despite an unchanging MAP. This sympathetic input into the kidney is detectable only during prostaglandin synthesis inhibition. Several possible mechanisms exist for the sympathetic nervous system activation. The decrease in CO observed in both groups receiving endotoxin may have reflexively activated the efferent sympathetic nervous system via a change in arterial baroreceptor tone (41, 42). In group I animals, the fall in systemic blood pressure would be expected to activate arterial baroreceptors and thereby lead to an increase in efferent sympathetic activity. In view of the stable blood pressure in group II dogs, the reduction in CO may have activated left atrial receptors, decreased vagal tone, and increased sympathetic activity. Alternatively, the possibility that endotoxin may cross the blood-brain barrier and directly elicit central nervous system responses also exists (43, 44). The increases in sympathetic activity were, however, dissociated from marked changes in pH, pO_2, and pCO_2 in these studies. Thus, the beneficial effects of MAP protection with prostaglandin synthesis inhibition after endotoxin are balanced by a failure of compensatory renal vasodilation. The long-term effects of this dissociative response are unknown, although a recent study demonstrated no improvement in survival when prostaglandin synthesis was inhibited following endotoxin (1); renal function was not specifically assessed in prior experiments of this type (1, 39).

Another important aspect of the results is the mechanism of renin release following endotoxin. An increase in PRA and renin secretion (from both innervated and denervated kidneys) occurred in prostaglandin-intact dogs (group I) following hypotension and endotoxin. In these dogs the magnitude of the increase in renin secretion from innervated and denervated kidneys were similar. This finding was predictable since multiple pathways to renin secretion (arterial baroreceptor, sympathetic nerves in innervated kidneys, and macula densa) are likely to be activated during a hypotensive stress of this type (23–26). Urinary sodium excretion was also comparable in this group in innervated and denervated kidneys suggesting that sodium delivery to the distal nephron was similar, and thus that the macula densa stimulation was comparable. In view of the continuing controversy regarding the physiologic role prostaglandins play in

sympathetically mediated renin release (16, 23–26), the renin secretion results in the prostaglandin synthesis-inhibited dogs of group II are of particular interest. In group II animals renin secretion increased in innervated kidneys and accounted for the significant rise in PRA. This increase in PRA and RSR occurred without a decrease in MAP and equivalent decrements in urinary sodium excretion from innervated and denervated kidneys. Interestingly, renal denervation abolished the increase in renin secretion in this group of animals. Thus, in this group of animals, efferent sympathetic nerves to the kidney provided the primary pathway for renin release, a pathway that was not affected by prostaglandin synthesis inhibition. This sympathetic pathway to renin release likely involves the activation of β-1 receptors located in proximity to juxtaglomerular cells (45, 46). Hence, the results strongly imply that this sympathetically mediated increase in renin secretion does not require intact prostaglandin synthesis, a conclusion supported by other studies of endogenous sympathetic stimuli to renin release (16). The postendotoxin renin secretion rate in these innervated kidneys was not significantly different from that observed in the prostaglandin intact (group I) dogs. Species differences or the intensity and type of sympathetic stimulation in prior studies may account for differing conclusions regarding this pathway to renin secretion (23–26). Thus, while it should be acknowledged that the baroreceptor (23, 26, 47) and macula densa (48) pathways to renin release may be prostaglandin-dependent in the dog, the present results clearly show that renin secretion may occur in response to enhanced endogenous renal sympathetic nerve activity via pathways independent of the prostaglandin system.

In summary, the results of these experiments identify a systemic effect of PGI_2 to influence the hypotension of early endotoxemia. The data also imply that the sympathetic nervous system plays an important renal ischemic role early in this syndrome and is opposed in vivo by the compensatory vasodilation of prostaglandins. Finally, renin secretion postendotoxin occurs via several pathways when MAP declines, but primarily via renal sympathetic nerves when MAP is unchanged. This renal sympathetic nerve pathway to renin release operates independently of prostaglandin inhibition in the dog.

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