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, PGI2) 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 PGI2 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 PGI2 during the study. These results suggest that PGI2 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-
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 vasodilation 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 vasodilation.

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 renal 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 de-

\(^1\) Abbreviations used in this paper: CO, cardiac output; GFR, glomerular filtration rate; 6-keto-PGF\(_{1\alpha}\)-TAME, 6-keto PGF\(_{1\alpha}\)-tyrosine methyl ester hydrochloride; MAF, mean arterial pressure; PAH, para-aminohippurate; PGI\(_2\), prosta-
cyclin; PRA, plasma renin activity; PVR, peripheral vascular resistance; RBF, renal blood flow; RSR, renin secretory rate; RVR, renal vascular resistance.

nervation to learn the physiologic role of prostaglan-
dins 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 assessed using electrical renal nerve stimulation (Grass Electric-
trical 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 (Hew-
lett-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 Indocy-
ane Green dye-dilution technique (28) using a Lyons cardiac output computer (Lyons Medical Corporation, Pleas-
antville, 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 re-
mainder 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, respec-
tively.

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 sam-
ple strains 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 ex-
traction (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.).
Blood samples for plasma renin activity (PRA) were collected on ice from arterial and renal venous catheters at the midpoints of each collection period, centrifuged at 4°C, and stored at -70°C until analyzed. 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 PG12.** Arterial blood samples for assay of PG12 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-PGF1α, the stable metabolite of PG12, was determined using a radioimmunoassay procedure according to the method of Tai et al. (30, 31). In this assay, an internal standard (6-keto-PGF1α, tyrosine methylester hydrochloride (TME)) was prepared by dissolving 1 mg of 6-keto-PGF1α, (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 125I-Na (Radiochemical Center, Amersham Corp., England) in 3 μl of 0.1 NaOH to 1.5 μg (5 μl) of 6-keto-PGF1α-TME in 50 μl of 0.5 M sodium phosphate buffer, pH 7.5. The extraction of 6-keto-PGF1α 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 [3H]PGF1α-metabolite (15,14 dihydro-15-keto-PGF1α) was added for the calculation of recovery rate after extraction. The percent recoveries of the labeled PGF2α, and 6-keto-PGF1α, 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 PGF2α, metabolite and 6-keto-PGF1α 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 [3H]PGF1α metabolite was counted in a liquid scintillation counter (Beckman LS-250 Beckman Instruments, Inc., Fullerton, Calif.). A 6-keto-PGF1α 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-PGF1α, 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-PGF1α, (31). The incubation mixture (0.1 ml) contained standard or sample, diluted antiserum, 125I-6-keto-PGF1α-TME (10,000 cpm) and assay buffer, 0.1 ml each. The incubation was carried out for 2 h at room temperature. Free 125I-6-keto-PGF1α-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-PGF1α 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 isotonie solution equal in volume and toxicity 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.). The dogs 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, lyophylized *Escherichia coli* 055 lipopolysaccharide, prepared by the method of Westphal and Jann (93) 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-
Group I, n = 9
Endotoxin only
Mean 126 126 94
±SE 5 5.1 4
P NS <0.001

Group II, n = 11
PG inhibitor and endotoxin
Mean 131 136 144
±SE 4 4 4
P NS NS NS

Group III, n = 5
PG inhibitor only
Mean 141 144 147
±SE 6 3.4 4.7
P 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.

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 PGI2 (measured as the 6-keto-F1 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-PGF1 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-PGF1 alpha immediately and 1 h postendotoxin bolus. No significant changes in 6-keto-PGF1 alpha occurred in the group III dogs. The percent change in 6-keto-PGF1 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.

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### TABLE II

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>GFR C (ml/min)</th>
<th>RBF C (ml/min)</th>
<th>RVR C (mm Hg/ml/min)</th>
<th>GFR PI (ml/min)</th>
<th>RBF PI (ml/min)</th>
<th>RVR PI (mm Hg/ml/min)</th>
<th>GFR PE (ml/min)</th>
<th>RBF PE (ml/min)</th>
<th>RVR PE (mm Hg/ml/min)</th>
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<tr>
<td>I</td>
<td>Endotoxin only</td>
<td>Mean 44.3</td>
<td>Mean 426</td>
<td>Mean 0.44</td>
<td>Mean 42.2</td>
<td>Mean 275</td>
<td>Mean 0.45</td>
<td>Mean 32.6</td>
<td>Mean 266</td>
<td>Mean 0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±SE 4</td>
<td>±SE 26</td>
<td>P NS</td>
<td>±SE 4</td>
<td>±SE 35</td>
<td>P NS</td>
<td>±SE 5</td>
<td>±SE 47</td>
<td>P NS</td>
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<tr>
<td></td>
<td>Denervated</td>
<td>Mean 45.2</td>
<td>Mean 304</td>
<td>Mean 0.49</td>
<td>Mean 40</td>
<td>Mean 260</td>
<td>Mean 0.50</td>
<td>Mean 29.5</td>
<td>Mean 245</td>
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<td></td>
<td></td>
<td>±SE 3.6</td>
<td>±SE 38</td>
<td>P NS</td>
<td>±SE 2</td>
<td>±SE 40</td>
<td>P NS</td>
<td>±SE 4</td>
<td>±SE 29</td>
<td>P NS</td>
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<td>Mean 45.2</td>
<td>Mean 377</td>
<td>Mean 41.2</td>
<td>Mean 281</td>
<td>Mean 0.38</td>
<td>Mean 33.9</td>
<td>Mean 182</td>
<td>Mean 0.81</td>
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<tr>
<td></td>
<td></td>
<td>±SE 2.6</td>
<td>±SE 45</td>
<td>P NS</td>
<td>±SE 3.9</td>
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<td>P NS</td>
<td>±SE 3.1</td>
<td>±SE 23</td>
<td>P NS</td>
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<td>Mean 42.5</td>
<td>Mean 337</td>
<td>Mean 0.35</td>
<td>Mean 41.9</td>
<td>Mean 279</td>
<td>Mean 0.49</td>
<td>Mean 42.2*</td>
<td>Mean 241*</td>
<td>Mean 0.61*</td>
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<tr>
<td></td>
<td></td>
<td>±SE 2</td>
<td>±SE 33</td>
<td>P NS</td>
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<td>P NS</td>
<td>±SE 2.6</td>
<td>±SE 24</td>
<td>P NS</td>
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<td>III</td>
<td>PG inhibitor only</td>
<td>Innervated</td>
<td>Mean 43.3</td>
<td>Mean 364</td>
<td>Mean 39.5</td>
<td>Mean 326</td>
<td>Mean 0.37</td>
<td>Mean 38.6</td>
<td>Mean 288</td>
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<td>±SE 2.8</td>
<td>±SE 36</td>
<td>P NS</td>
<td>±SE 4.3</td>
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<td>±SE 2.7</td>
<td>±SE 11</td>
<td>P NS</td>
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<tr>
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<td>Denervated</td>
<td>Mean 42.9</td>
<td>Mean 355</td>
<td>Mean 0.39</td>
<td>Mean 43</td>
<td>Mean 327</td>
<td>Mean 0.43</td>
<td>Mean 42.1</td>
<td>Mean 292</td>
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<td>±SE 0.9</td>
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<td>±SE 17</td>
<td>P NS</td>
<td>±SE 2.3</td>
<td>±SE 31</td>
<td>P NS</td>
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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_{1α}*

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>C (ng/ml)</th>
<th>PI (ng/ml)</th>
<th>PE (ng/ml)</th>
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</tr>
<tr>
<td>I</td>
<td>Endotoxin only</td>
<td>Mean 0.64</td>
<td>1.26</td>
<td>2.08*</td>
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<tr>
<td></td>
<td></td>
<td>±SE 0.05</td>
<td>0.24</td>
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<td></td>
<td></td>
<td>P &lt;0.05</td>
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<td>&lt;0.05</td>
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<tr>
<td>II</td>
<td>PG inhibitor and endotoxin</td>
<td>Mean 0.76</td>
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<td>0.76</td>
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<td></td>
<td></td>
<td>±SE 0.08</td>
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<td>Mean 0.79</td>
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<td>±SE 0.10</td>
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<td></td>
<td></td>
<td>P NS</td>
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</table>

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 $U_{Na}V$

<table>
<thead>
<tr>
<th>Group</th>
<th>PRA</th>
<th>RSR</th>
<th>$U_{Na}V$</th>
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<tr>
<td></td>
<td>C</td>
<td>PI</td>
<td>PE</td>
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<td></td>
<td>ng/ml/h</td>
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<td>Group I</td>
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<tr>
<td>Endotoxin only</td>
<td>Mean 1.15 1.34 3.92</td>
<td>Innervated Mean 155 139 464</td>
<td>118 125 32</td>
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<tr>
<td></td>
<td>±SE 0.37 0.46 0.81</td>
<td>±SE 51 94 89</td>
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<td></td>
<td>P NS</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>±SE 59 59 191</td>
<td>P NS &lt;0.05</td>
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<td>Group II</td>
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<tr>
<td>PG inhibitor and endotoxin</td>
<td>Mean 0.77 0.66 2.52</td>
<td>Innervated Mean 135 58 255*</td>
<td>93 126 43</td>
</tr>
<tr>
<td></td>
<td>±SE 0.17 0.14 0.58</td>
<td>±SE 59 20 59</td>
<td>P NS &lt;0.05</td>
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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 meclofenamate. 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 PGI2 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 PGI2 (as measured by the PGI2 metabolite 6-keto PGF1α) noted in group I dogs in the postendotoxin period correlated with percent decrease in MAP. Thus, the results suggest that PGI2 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 PGI2 is modified, however, by the reported salutary effects of postendotoxin PGI2 infusions on cardiac performance, platelet aggregation, and microcirculation integrity (1, 40). The postendotoxin levels of 6-keto-PGF1α observed in this study are similar to earlier results (22, 39). The lack of a detectable reduction in 6-keto-PGF1α 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, pO2, and pCO2 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 PGI₂ 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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