Dependency of Renal Blood Flow on Prostaglandin Synthesis in the Dog
By Andrew J. Lonigro, Harold D. Itskovitz, Keith Crowshaw, and John C. McGiff

ABSTRACT
Inhibition of prostaglandin synthesis in chloralose-anesthetized dogs reduced renal blood flow, and this reduction closely correlated \( r = 0.92, P < 0.01 \) with a decline in the renal efflux of a substance having the properties of PGE\(_2\). We used solvent extraction and thin-layer chromatography coupled with parallel bioassay to identify and assay the PGE- and PGF-like substances (expressed as PGE\(_2\) and PGF\(_{2\alpha}\) equivalents). Either of two antiinflammatory acids, indomethacin or meclofenamate, that inhibited conversion of \(^{14}\)C-arachidonic acid to prostaglandins in renal homogenates decreased the basal concentration of a PGE-like substance in renal venous blood to \(0.06 \pm 0.02 \text{ng/ml} \) from a mean control value of \(0.34 \pm 0.10 \text{ng/ml} \) \((P < 0.01)\). This change was associated with a mean reduction in renal blood flow of 45% in spite of increased renal perfusion pressure. Femoral blood flow and cardiac output were variably and insignificantly affected. Changes in the renal efflux of a PGF-like substance induced by indomethacin were unrelated to the decline in renal blood flow. Changes in the efflux of a PGE-like substance from the femoral vascular bed were unrelated to the small and variable changes in femoral blood flow. Extrarenal factors, i.e., humoral, nervous, or cardiopulmonary factors, did not account for the decline in renal blood flow produced by the inhibitors of prostaglandin synthesis, since the inhibitors produced identical effects in the isolated blood-perfused canine kidney. We concluded that PGE\(_2\) participates in maintaining renal vascular tone which heretofore has been ascribed to autonomous, intrinsic renal arteriolar activity.

KEY WORDS
regulation of renal blood flow
antiinflammatory acids
renal prostaglandins
PGE-like substance
renal homogenate
isolated blood-perfused dog kidney
renal vascular resistance
renal vascular tone
arachidonic acid

The kidneys account for 0.4% of the body weight but receive 15-20% of the resting cardiac output—the largest allotment to any organ (1). Blood flow to an organ is dependent on the level of vascular tone, and in general resting vascular tone is determined by the net effects of neurogenic vasoconstrictor fiber discharge to the organ and metabolic activity of the organ (2). However, neither of these factors plays a large role in determining renal blood flow at rest: neurogenic vasoconstrictor activity to the kidney is negligible, and blood flow to the kidney is far in excess of the organ’s metabolic needs (2). These considerations raise the question of the mechanism(s) maintaining renal vascular tone at rest. It has been proposed (3) that renal blood flow is determined by autonomous, intrinsic activity of the renal arterioles.”

During infusion of either norepinephrine or angiotensin II, the renal venous concentration of a prostaglandin E-like (PGE-like) substance increases (4-6), and this increase is associated with blunting of the renal vasoconstrictor-antidiuretic actions of the pressor hormone. Although these observations suggest that PGE\(_2\), or a substance indistinguishable from it using physicochemical, chromatographic, and biological criteria defends renal function against excesses of pressor hormones, they do not permit assignment of a physiological role to PGE\(_2\). Nonetheless, it is difficult to overlook the basal concentration of a PGE-like substance in renal venous blood that varies from 0.12 to 0.65 ng/ml (6). Examination of possible relationships between the efflux (product of renal blood flow and renal venous concentration) of PGE\(_2\) from the

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kidney and the resting renal vascular tone is possible, since Vane (7) has shown that indomethacin and other antiinflammatory acids block synthesis of prostaglandins. We used indomethacin and meclofenamate to define a possible dependency of the renal circulation at rest on the capacity of the kidney to synthesize prostaglandins.

**Methods**

In male mongrel dogs anesthetized with morphine (2 mg/kg, sc) and chloralose (100 mg/kg, iv), the artery of the right kidney was isolated through a transabdominal incision. Anesthesia was maintained with chloralose (40 mg/kg, iv). The femoral artery and vein were isolated just below the inguinal ligament. Mean aortic blood pressure was measured with a Statham P23Db transducer, and blood flows were measured with Statham M-4001 electromagnetic flowmeters according to procedures previously described (8). Cardiac output was determined by the dye-dilution method using indocyanine green.

PGE- and PGF-like substances were assayed in 100 ml of blood collected from either the femoral or the renal vein immediately before and 15 minutes after intravenous administration of either indomethacin or meclofenamate (2 mg/kg). Dextran was infused intravenously at the same rate at which the blood was removed. Venous blood for assay was collected directly in ethyl alcohol, which precipitated proteins and cells. Arterial blood, obtained under identical conditions and processed in the same manner as venous blood in six experiments, contained no detectable PGE- or PGF-like substances. The ethanol-blood mixture was filtered and evaporated, and the acidic lipids were separated from the neutral lipids by procedures described previously (5, 6). The acidic lipids were purified by thin-layer chromatography on silica gel layers with a chloroform-methanol-acetic acid solvent system. Eluates from the prostaglandin zones of thin-layer chromatographic plates were reconstituted in Krebs solution, and 0.1-ml volumes were tested in vitro for their content of prostaglandinlike substances by a parallel pharmacological assay. Three assay organs—rat stomach strip, rat colon, and chick rectum—were superfused in series according to the technique described by Vane (9). The sensitivity of our in vitro method for assay of prostaglandins was 0.02 and 0.04 ng/ml blood for PGE- and PGF-like substances, respectively, assayed as the corresponding PGE\textsubscript{2} and PGF\textsubscript{2\alpha} equivalents (6).

The precision of this assay procedure expressed as the coefficient of variation was 9.2% for replicate determinations of the content of PGE- and PGF-like substances in the eluates. The concentrations of prostaglandinlike substances were not corrected for losses incurred on extraction and chromatographic purification. In eight experiments, we recovered 40 ± 8% of PGE\textsubscript{2} after the addition of 0.15—5.0 µg of authentic PGE\textsubscript{2} to arterial blood to give concentrations of 0.4—10 ng/ml blood. These methods do not allow definitive chemical identification of prostaglandins, but when solvent extraction and thin-layer chromatography are coupled with parallel bioassay on two or more tissues identification is believed to be "almost certain" (10).

To show that indomethacin and meclofenamate under these experimental conditions inhibited synthesis of renal prostaglandins, in two dogs one kidney was removed prior to and the other 30 minutes after administration of either antiinflammatory agent. A sample of the renal medulla (2 g) was homogenized in 20 ml of sodium phosphate buffer, pH 8.0, containing 2 mM glutathione and 0.5 mM (8.85 mmole) of 1\textsuperscript{4}C-arachidonic acid and incubated for 1 hour (11). A portion of the total lipid extract (1/10) was separated on Merck silica gel G-plates in a solvent system consisting of chloroform-methanol-acetic acid (90:5:5, by volume). The radioactive products were localized, and radioactivity was estimated using a Packard radiochromatogram scanner equipped with a disc integrator. Using untreated dog kidneys, we detected \textsuperscript{14}C-labeled compounds indistinguishable from PGE\textsubscript{2} (17% yield) and PGF\textsubscript{2\alpha} (10% yield) (Fig. 1). In contrast, homogenates of medullas from the experimental kidneys were unable to convert \textsuperscript{14}C-arachidonic acid to prostaglandins, representing complete inhibition of synthesis.

A method of isolated renal perfusion described previously (12) was modified by substituting a Waters pulsatile pump (64 pulsations/min) and a membrane oxygenator for the Blue膜 pump and the King oxygenator. Kidneys were obtained from male mongrel dogs weighing approximately 20 kg. The perfusate consisted of 700—800 ml of autologous dog blood. Renal blood flow was measured directly by timed collections of renal venous effluent. Systolic renal perfusion pressure was maintained at 140 mm Hg, and diastolic pressure was kept between 110 and 116 mm Hg.

Indomethacin was dissolved in 10 ml of absolute ethanol and diluted with Krebs solution to give a final concentration of 1 mg/ml for intravenous injection. Control solutions containing identical concentrations of ethanol and Krebs solution did not affect renal blood flow when they were administered intravenously. Meclofenamate as the sodium salt was diluted in Krebs solution to a final concentration of 1 mg/ml.

Changes in concentrations of PGE- and PGF-like substances were tested for statistical significance by the t-test based on paired observations or by the sign test. Dunnett's procedure was used to determine significant differences between control and 5-, 10-, and 15-minute values postindomethacin for blood pressure, cardiac output, renal blood flow, and femoral blood flow. The significance of the relationship between changes in efflux of PGE- or PGF-like substances and changes in renal blood flow was determined by calculating correlation coefficients. A P value of 0.05 or less was considered statistically significant. Statistical analyses were performed according to methods described by Steel and Torrie (13).

**Results**

**IN SITU KIDNEY**

Blood flow to the experimental kidney prior to the administration of either indomethacin or meclofenamate was 198 ± 22 (se) ml/min, i.e., 3.7
Radiochromatograms of prostaglandins biosynthesized from 1-14C-arachidonic acid in homogenates of renal medulla obtained from a dog before (a) and after (b) treatment with meclofenamate. The chromatographic mobilities of the biosynthesized PGF₃₀ and PGE₁ in the control experiment were indistinguishable from those obtained for authentic PGF₂₀ and PGE₁ compounds separated on identical TLC plates. Note the absence of PGF₂₀ and PGE₁ peaks after meclofenamate treatment. M is a radioactive marker spotted 1 cm from the end of the 20-cm TLC plate.

Mean aortic blood pressure increased from a mean of 99 ± 9 to 127 ± 6 mm Hg (P < 0.01). Decreases in renal blood flow and reductions in renal efflux of a PGE-like substance produced by indomethacin and meclofenamate were highly correlated (r = 0.92, P < 0.01) (Fig. 2). In contrast, in the five experiments in which PGF-like substances were measured, decreases in their efflux were unrelated to reductions in renal blood flow (r = 0.46, P > 0.05). Femoral blood flow and cardiac output were not significantly affected (Table 1). Changes in efflux of a PGE-like substance from the femoral bed were unrelated to the small and variable changes in femoral blood flow. However, the preindomethacin concentration of a PGE-like substance in femoral venous blood (0.04 ± 0.02 ng/ml) was considerably less than that in renal venous blood.

**ISOLATED KIDNEY**

In three experiments, 50 ml of blood was obtained before and after administration of 5 mg of indomethacin into the perfusion circuit of an isolated dog kidney, and the blood was assayed for prostaglandins. Fifteen minutes after injection of indomethacin, the concentration of a PGE-like substance in the venous effluent of the isolated kidney decreased to 0.35 ± 0.31 ng/ml from a mean control value of 1.51 ± 0.80 ng/ml. Concomitantly, renal blood flow fell from 184 ± 16 to 135 ± 3 ml/min in the face of constant perfusion pressure (mean 122 ± 1 mm Hg).
TABLE 1
Changes in Mean Aortic Blood Pressure and Renal Blood Flow Produced by Indomethacin (Six Experiments) or Meclofenamate (Three Experiments) in Nine Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Time after drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5 Minutes</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>99 ± 9</td>
<td>114 ± 5</td>
</tr>
<tr>
<td>CO (liters/min)</td>
<td>2.72 ± 0.21</td>
<td>2.72 ± 0.27</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>196 ± 22</td>
<td>143 = 26*</td>
</tr>
<tr>
<td>FBF (ml/min)</td>
<td>49 ± 9</td>
<td>47 ± 7</td>
</tr>
</tbody>
</table>

All values are means ± SE. MABP = mean arterial blood pressure and RBF = renal blood flow. Femoral blood flow (FBF) and cardiac output (CO) were measured in six and seven of these experiments, respectively.

*Significantly different from control using Dunnett’s procedure (two-tailed), P < 0.01. Absence of an asterisk indicates that P > 0.05.

Discussion

These results suggest that PGE₂ of renal origin is a determinant of resting blood flow to the kidneys. After inhibition of prostaglandin synthesis, the decrease in renal blood flow was closely correlated with the reduced efflux of a PGE-like substance from the kidney. A similar relationship did not exist between changes in femoral blood flow and changes in efflux of a PGE-like substance from the hindquarter. Furthermore, changes in renal efflux of a PGF-like substance did not correlate with reductions in renal blood flow. PGF₂α, unlike PGE₂, does not dilate renal blood vessels (15). A reduction in renal blood flow of itself cannot account for a decreased renal efflux of a PGE-like substance, since renal vasoconstrictor stimuli increase its release from the kidney (4-6). Renal blood flow decreased despite variable changes in cardiac output and increases in blood pressure. Thus, changes in renal blood flow cannot be ascribed to either changes in cardiac function or decreases in renal perfusion pressure.

The interpretation that prostaglandins participate in the support of resting renal blood flow was considerably strengthened by the results obtained from the isolated kidney; extrarenal factors, i.e., humoral, venous, or cardiac factors, could not have contributed to the reduction of renal blood flow which followed blockade of prostaglandin synthesis in the isolated preparation. The likelihood that decreased renal blood flow was due to an effect of the drug unrelated to inhibition of synthesis of prostaglandins is remote, since (1) identical results were obtained with two chemically dissimilar

TABLE 2
Changes in Renal Blood Flow and Concentrations of PGE- and PGF-like Substances in Renal Venous Blood Produced by Indomethacin or Meclofenamate in Nine Dogs

<table>
<thead>
<tr>
<th>Dog</th>
<th>Mean aortic blood pressure (mm Hg)</th>
<th>Renal blood flow (ml/min)</th>
<th>Prostaglandin concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>90</td>
<td>120</td>
<td>0.06</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>150</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>105</td>
<td>115</td>
<td>0.23</td>
</tr>
<tr>
<td>4</td>
<td>135</td>
<td>160</td>
<td>0.23</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>115</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>105</td>
<td>125</td>
<td>0.87</td>
</tr>
<tr>
<td>7*</td>
<td>65</td>
<td>100</td>
<td>0.49</td>
</tr>
<tr>
<td>8*</td>
<td>135</td>
<td>125</td>
<td>0.06</td>
</tr>
<tr>
<td>9*</td>
<td>105</td>
<td>135</td>
<td>0.78</td>
</tr>
<tr>
<td>Mean</td>
<td>99</td>
<td>127</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Experimental values were obtained 15 minutes after administration of either indomethacin or meclofenamate. In experiments 1-4, concentrations of a PGF-like substance were not determined. ND = not detected (below threshold of sensitivity [0.02 and 0.04 ng/ml for PGE- and PGF-like substances, respectively] and considered to be zero).

*Meclofenamate was given in place of indomethacin.
inhibitors of prostaglandin synthesis, indomethacin and meclofenamate, and (2) a mechanism operating uniquely in the renal vasculature would have to be postulated because femoral blood flow was unaffected. Furthermore, we obtained biochemical confirmation for inhibition of prostaglandin synthesis: arachidonic acid, the precursor of PGE\(_2\) and PGF\(_{2\alpha}\), was not converted to prostaglandins in renal medullary homogenates of dogs treated with indomethacin or meclofenamate.

We have not identified the renal vascular elements affected by the consequences of inhibition of prostaglandin synthesis. However, a consideration of the renal distribution of prostaglandin synthetase, which is exclusively medullary (16), and of the major degrading enzyme, prostaglandin dehydrogenase, which is primarily cortical (17), suggests that prostaglandins released from the renal medulla affect medullary blood vessels and possibly vessels of the juxtamedullary cortex after traversing the vasa recta. It is impossible to account for the magnitude of the decrease in renal blood flow (mean 45%) in terms of changes in medullary and juxtamedullary blood flow, although the vascular architecture of the canine kidney in which nephrons having long loops of Henle predominate (14, p 1457) might, in part, determine these changes. Extrapolation of these results to man would be unjustified, since nephrons having long loops of Henle constitute less than 20% of all nephrons (14, p 1457). In addition, a cortical site of action of renal prostaglandins should not be discounted, since a variant route of transport of prostaglandins to the cortex via tubular fluid can be inferred from the identification of PGE\(_2\) in human urine (18). The recent finding that renal autoregulation depends on prostaglandins (19) urges consideration of possible renal cortical site(s) of action of these agents. Furthermore, other intrarenal humoral systems, i.e., kallikrein-kinin or renin-angiotensin, might be affected by inhibition of prostaglandin synthetase. Secondary changes in the activity of either of these humoral systems might influence renal blood flow. Finally, we cannot make any statement regarding a renal medullary site of action of these agents. The recent finding that renal autoregulation depends on prostaglandins (19) urges consideration of possible renal cortical site(s) of action of these agents. Furthermore, other intrarenal humoral systems, i.e., kallikrein-kinin or renin-angiotensin, might be affected by inhibition of prostaglandin synthetase. Secondary changes in the activity of either of these humoral systems might influence renal blood flow. Finally, we cannot make any statement regarding a renal medullary site of action of these agents. We thank A. Nasjletti, J. Strand, and L. Hebert for their useful suggestions. We thank D. Pacholeczyk, M. Boenjak, and P. Welsh for their assistance.

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