Effect of A Renal Prostaglandin on Distribution of Blood Flow in the Isolated Canine Kidney

By Harold D. Itskovitz, Norberto A. Terragno, and John C. McGiff

ABSTRACT

In isolated blood-perfused canine kidneys, progressive increases in renal blood flow and its fractional distribution to the inner cortex were correlated with increases in perfusate concentrations of a prostaglandin E-like (PGE-like) substance. The ratio of blood flow to the outer and inner halves of the renal cortex measured by the radioactive-microsphere method changed from 77:23 to 68:32 (P < 0.001) after 90 minutes of perfusion; simultaneously, the concentration of the PGE-like substance increased from 0.04 ng/ml to 0.59 ng/ml (P < 0.02). Thus, the greatest rate of increase in the concentration of the PGE-like substance occurred coincidently with the largest increase in inner cortical blood flow, i.e., from 30 ml/min to 69 ml/min (P < 0.001). During the two subsequent 90-minute perfusion periods, the ratio of outer cortical blood flow to inner cortical blood flow fell to 61:39, and the concentration of the PGE-like substance increased further. When indomethacin was added to the perfusate, the concentration of the PGE-like substance decreased from 1.00 ng/ml to 0.24 ng/ml (P < 0.05), and renal blood flow decreased, especially in the inner cortex where the decline was from 34% to 19% of total renal blood flow (P < 0.05). Thus, renal blood flow was redistributed after indomethacin was administered. PGE$_2$ was infused to determine whether substitution for the loss of circulating PGE-like substance could prevent the effects of indomethacin on the distribution of renal blood flow. Despite administration of exogenous PGE$_2$, renal blood flow redistributed after administration of indomethacin, and the ratio of outer cortical blood flow to inner cortical blood flow increased from 68:32 to 80:20 (P < 0.05). These results suggest that the intrarenal site of synthesis and release of PGE$_2$ determines its action as a local hormone affecting deep cortical blood flow.

KEY WORDS

- indomethacin
- renal inner cortical blood flow
- antinflammatory compounds
- renal hormones
- prostaglandin E$_2$
- local hormones
- regulation of the renal circulation
- inhibition of prostaglandin synthetase

Inhibition of renal synthesis of prostaglandins results in a reduction in renal blood flow, which is highly correlated with a decline in renal efflux of a substance that has the properties of PGE$_2$ (PGE-like substance) (1). On the basis of this finding, we have proposed that the renal circulation is supported by the continuous synthesis of a PGE compound. Furthermore, we have hypothesized that changes in the synthesis of PGE$_2$, the principal renal prostaglandin (2, 3), affect blood flow primarily to the medulla and the inner cortex. The major localization of prostaglandin synthetase to the medulla and the relative absence of prostaglandin-metabolizing enzymes within the medulla (4) suggest that the medulla and the juxtapamedullary cortex are the primary, although not necessarily the exclusive, sites of action of PGE$_2$. PGE$_2$ presumably functions as a local or tissue hormone, since PGE$_2$ and PGF$_{2\alpha}$, in contrast to PGA$_2$, are almost entirely destroyed in the lung (5).

The present study was designed to define the zonal localization within the kidney of the vascular effects of altered synthesis of renal prostaglandins and, thereby, to identify a possible determinant of the distribution of renal blood flow. Use of inhibitors of prostaglandin synthesis, such as indomethacin and meclofenamate (1), allowed the intrarenal role of prostaglandins to be studied by subtraction methods; measurements of renal blood flow and its
distribution before and after the addition of an inhibitor permitted the assessment of renal hemodynamics in the presence and the absence of endogenous prostaglandins. Furthermore, measurements of renal hemodynamics during infusions of PGE\textsubscript{2} after inhibition of prostaglandin synthesis provided insight into the separate activities of PGE\textsubscript{2} as a local hormone and a circulating hormone. The infused prostaglandin would substitute for the loss of a circulating prostaglandin but not for the loss of a prostaglandin functioning as a local hormone, since, presumably, an exogenous prostaglandin cannot mimic the intrarenal actions of the corresponding endogenous prostaglandin in terms of specific localization of activity, sequence of vascular elements affected, or concentrations that can be achieved at sites of synthesis and release.

In the present study, we used the subtraction method to define the role of endogenous PGE\textsubscript{2} as a potential regulator of the renal circulation, particularly in terms of the distribution of blood flow within the kidney. This study was performed in isolated blood-perfused canine kidneys in which constant perfusion pressure was maintained. Isolating the kidney eliminated extrarenal sources of prostaglandins and excluded extrarenal hemodynamic and hormonal influences that might affect renal hemodynamics independently of changes in prostaglandin synthesis.

Methods
Mongrel dogs (15–20 kg) were anesthetized with sodium pentobarbital (65 mg/kg, iv). Their kidneys were removed and transferred to a Lucite perfusion chamber within 2 minutes according to previously published techniques (6). The isolated kidneys were perfused with 700–800 ml of autologous heparinized blood that was pumped in a pulsatile fashion (70 beats/min) by a Waters pump-oxygenator system. The perfusion pressure, which was monitored continuously by a Tycos manometer, was kept constant by changing the renal blood flow to maintain systolic pressure at 140 mm Hg. Blood pH, PO\textsubscript{2}, and PCO\textsubscript{2} were measured at 30-minute intervals and maintained within physiological ranges by delivering various mixtures of air, O\textsubscript{2}, and CO\textsubscript{2} to the oxygenator. The temperature of the blood was kept at 37°C by passing it through a coil immersed in a constant-temperature water bath. Replacement fluids, electrolytes, and other chemicals, including sodium, calcium, magnesium, chloride, phosphate, bicarbonate, and urea, were added to the perfusate through venous ports to maintain concentrations at physiological levels, which were verified by blood analyses. Glucose (1 mg/min), lactate (4.5 mg/min), and pyruvate (0.45 mg/min) were also added as metabolic substrates for the kidney. Regular insulin and antidiuretic hormone were infused at rates of 0.01 unit/min and 1 munit/min, respectively. The functional characteristics of the isolated kidney after 2 hours of perfusion are shown in Table 1. Renal blood flow was similar to that measured in anesthetized dogs, but glomerular filtration rate was about 30% less (7). The capacity of the isolated kidney to absorb sodium, about 98% of the filtered load, was only slightly less than that of the kidney in situ.

Total renal blood flow was measured directly by timed collections of the renal venous effluent. The intrarenal distribution of blood flow was assessed using microspheres 15–20\(\mu\)m in diameter labeled with \(^{169}\text{Yb}, \(^{86}\text{Sr}, \(^{14}\text{Ce}, \(^{51}\text{Cr}, \) or \(^{46}\text{Sc}\) (3M Company). For this purpose, we adapted the technique of McNay and Abe (8) to the isolated kidney as described recently (6). Microspheres (1–4 \(\mu\)c) were injected into the perfusion circuit just proximal to the perfusion pump 8.4 m from the kidney. This distance between the injection site and the kidney was greater than that which can be achieved in vivo and provided adequate mixing of the microspheres in the perfusate. To increase the accuracy of our results and to ensure representative sampling, at least 12 tissue specimens were obtained from four equal cortical zones of each kidney; their isotopic content was counted and mean distribution ratios and blood flows to the outer (zones C\textsubscript{1} and C\textsubscript{4}) and the inner (zones C\textsubscript{2} and C\textsubscript{4}) cortex were calculated. The above procedures were repeated two and often three times for each kidney to ensure the reproducibility of our results. The injections were made at various intervals under control conditions and immediately before and 15–30 minutes after the addition of indomethacin\(^1\) (2–5 mg) into the perfusion circuit. The indomethacin was added in bolus fashion to 17 isolated perfused kidneys. Five of these kidneys received continuous intra-arterial infusions of PGE\textsubscript{2} \(^2\) (400–1,000 ng/min) for 50 minutes before and 40 minutes after the administration of indomethacin. In three additional experiments, meclofenamate (1–2 mg) was substituted for indomethacin as an inhibitor of prostaglandin synthesis (1). In ten experiments, we obtained blood from the renal outflow of the perfusion circuit to measure prostaglandins of the E series by parallel bioassay after solvent extraction and thin-layer chromatography. The details of this method have been described previously (9).

\(^1\) Kindly supplied by Merck, Sharp and Dohme.
\(^2\) Kindly provided by the Upjohn Company.

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**TABLE 1**

**Functional Characteristics of the Isolated Kidney**

<table>
<thead>
<tr>
<th>Glomerular</th>
<th>Urinary osmolality/ plasma osmolality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal blood flow (ml/min)</td>
<td>Urine flow (ml/min)</td>
</tr>
<tr>
<td>148 ± 15</td>
<td>20 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± se for ten isolated kidneys obtained after 120 minutes of perfusion. The glomerular filtration rate was determined by creatinine clearance.
Differences between values for consecutive perfusion intervals were determined by the t-test for unpaired observations. Differences between values obtained before and after the administration of indomethacin were determined by Student's t-test for paired observations; \( P < 0.05 \) was considered statistically significant. Statistical analyses were performed according to methods described by Steel and Torrie (10).

Results

Changes in Blood Flow and Its Cortical Distribution in the Isolated Blood-Perfused Kidney: Temporal Dependency and Relationship to Changes in the Concentration of the PGE-Like Substance

Table 2 shows the relationship between changes in renal blood flow, its distribution within the cortex, and the perfusate concentration of the PGE-like substance during four successive 90-minute periods, i.e., the elapsed time after in vitro renal perfusion was begun. The initial levels of blood flow were relatively low in the isolated kidney, although its fractional distribution between the outer and the inner cortex was within the range reported previously for in vivo studies (8). Despite the maintenance of constant perfusion pressure, renal blood flow increased during the first 3 hours of perfusion but achieved stable levels for the final 3 hours (perfusion time 181–360 minutes). Although blood flow increased to both the outer and the inner cortex, the inner cortex received a larger fraction, i.e., renal blood flow was redistributed. Thus, within 3 hours after renal perfusion was begun, highly significant increases in the fraction of blood flow to the inner cortex were measured compared with those measured within the first 90 minutes, namely, 23–32% \( (P < 0.001) \). Although inner cortical blood flow increased to the greatest degree between 91 minutes and 180 minutes, a further significant increase in inner cortical blood flow to 39% of total renal blood flow occurred during the final period of perfusion (271–360 minutes). Perfusate concentrations of the PGE-like substance increased during the first 270 minutes of perfusion of the isolated kidney; the major increment occurred between 91 minutes and 180 minutes when the PGE-like substance increased almost fifteenfold, from 0.04 ng/ml to 0.59 ng/ml, relative to the preceding period. The latter was associated with the greatest increase in inner cortical blood flow.

Effects of Inhibitors of Prostaglandin Synthesis in the Isolated Blood-Perfused Kidney

Inhibition of prostaglandin synthesis decreased renal blood flow, primarily the fraction to the inner cortex. In each of twelve experiments (Fig. 1), injection of indomethacin into the blood perfusion circuit decreased renal blood flow. The mean

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
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<tbody>
<tr>
<td><strong>Time-Related Changes in Blood Flow, Its Distribution, and Blood Concentrations of the PGE-Like Substance in the Isolated Canine Kidney</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Perfusion time (minutes)</th>
<th>Total RBF (ml/min)</th>
<th>Total RBF (%)</th>
<th>Blood flow (ml/min)</th>
<th>Blood flow (ml/min)</th>
<th>PGE-like substance (ng/ml blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-90</td>
<td>133 ± 9</td>
<td>77 ± 2</td>
<td>23 ± 2</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>91-180</td>
<td>214 ± 9</td>
<td>68 ± 1</td>
<td>32 ± 1</td>
<td>0.59 ± 0.16</td>
</tr>
<tr>
<td>3</td>
<td>181-270</td>
<td>234 ± 15</td>
<td>81 ± 6</td>
<td>35 ± 2</td>
<td>1.42 ± 0.55</td>
</tr>
<tr>
<td>4</td>
<td>271-360</td>
<td>218 ± 24</td>
<td>84 ± 10</td>
<td>39 ± 2</td>
<td>1.32 ± 0.49</td>
</tr>
<tr>
<td>( \text{P}^\dagger )</td>
<td>( \text{&lt;0.001} )</td>
<td>( \text{&lt;0.001} )</td>
<td>( \text{&lt;0.001} )</td>
<td>( \text{&lt;0.001} )</td>
<td>( \text{&lt;0.02} )</td>
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<td>( \text{P}^\ddagger )</td>
<td>( \text{&lt;0.05} )</td>
<td>( \text{&lt;0.025} )</td>
<td>( \text{&lt;0.025} )</td>
<td></td>
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</tr>
</tbody>
</table>

All values are means ± SE. RBF = renal blood flow and N = number of experiments.

\* 1–4 indicate sequential 90-minute perfusion intervals.

\dagger Significant differences (P values) are indicated by the numbers in parentheses at the bottom of the table. Numbers within each pair of parentheses are not significantly different from each other. For example, (1) (2, 3, & 4) indicates that values for intervals 2, 3, and 4 are not different from each other but are all significantly different from that for interval 1.
PGE₂ AND RENAL BLOOD FLOW DISTRIBUTION

Percent Inner Cortical Blood Flow

CONTROL

INDOMETHACIN

-- = mean

EARLY LATE PRE POST

FIGURE 1

Effect of indomethacin on fractional distribution of inner cortical blood flow in isolated kidneys. In any one experiment, the distribution of cortical blood flow was measured twice (early and late control or pre- and post-indomethacin) at intervals ranging from 10 minutes to 270 minutes by the radioactive-microsphere method. Increased fractional blood flow to the inner zone of the cortex, which progresses with time, was reversed by indomethacin.

Decline was from 190 ± 21 (SE) ml/min to 156 ± 18 ml/min \( (P < 0.05) \). The decrease in total renal blood flow produced by indomethacin was due primarily to a reduction in inner cortical blood flow from a mean of 55 ± 9 to 26 ± 5 ml/min \( (P < 0.001) \). Outer cortical blood flow was not significantly affected; it was 135 ml/min before indomethacin was administered and 129 ml/min after injection of indomethacin. Decreases in the fractional blood flow to the inner cortex produced by indomethacin contrast with the time-dependent increases (control of Fig. 1) which occur when indomethacin is withheld. Thus, redistribution of blood flow in the isolated kidney occurred in response to indomethacin; the ratio of outer cortical blood flow to inner cortical blood flow increased from a mean of 72:28 to 84:16 \( (P < 0.001) \). In contrast, when indomethacin was not given, an increase in the fractional blood flow to the inner cortex occurred; the ratio of outer cortical blood flow to inner cortical blood flow decreased from a mean of 76:24 to 66:34 in the control experiments of Figure 1 \( (P < 0.001) \).

Since there was a time-dependent increase in inner cortical blood flow, unless indomethacin was administered (Table 2 and control of Fig. 1), the magnitude of the reduction in fractional blood flow to the inner cortex produced by indomethacin was related to the perfusion time at which the inhibitor was given (Fig. 2). Administration of indomethacin at perfusion times of 50–100 minutes in five experiments reduced fractional blood flow to the inner cortex from a mean of 22% to 14% of total renal blood flow \( (P < 0.02) \). At perfusion times of 150–280 minutes when inner cortical blood flow was at or approaching its highest level (Table 2), administration of indomethacin reduced fractional blood flow to the inner cortex by a greater amount, i.e., from a mean of 32% to 18% \( (P < 0.005) \) of total renal blood flow (Fig. 2). Thus, the magnitude of reduction in inner cortical blood flow produced by indomethacin was determined by the perfusion time at which the inhibitor was given. In three experiments in which meclofenamate instead of
indomethacin was added to the perfusate, inner cortical blood flow decreased from a mean of 30 ± 3% to 15 ± 6% of total renal blood flow, which was comparable to the mean reduction in the same component of renal blood flow produced by indomethacin (from 28% to 16% of total renal blood flow).

We measured concentrations of PGE-like substance in the perfusate before and after inhibition of prostaglandin synthesis in seven experiments. The inhibitor was given at perfusion times from 2-6 hours (average 4 hours), i.e., when inner cortical blood flow was relatively stable (Table 2), and it decreased concentrations of PGE-like substance in the seven experiments from a mean of 1.00 ± 37 ng/ml to 0.24 ± 0.13 ng/ml (P < 0.05). Concomitantly, renal blood flow was reduced from 227 ± 20 ml/min to 145 ± 19 ml/min primarily because of a decrease in its inner cortical fraction from 34 ± 1% to 19 ± 5% of total renal blood flow (P < 0.05).

**ATTEMPTED MODIFICATION OF THE RENAL HEMODYNAMIC EFFECTS OF INDOMETHACIN BY PGE₂**

In five experiments in which indomethacin was omitted, infusion of PGE₂ at rates of 400-1,000 ng/min increased renal blood flow by 13% from a mean control value of 157 ± 29 ml/min (P < 0.02). Simultaneously, the fraction of inner cortical blood flow increased from 22 ± 3% to 28 ± 3% (P < 0.05); this response of the isolated kidney does not differ from its response to other vasodilator agents (11). In each of six additional experiments, PGE₂ was infused at rates of 400-1,000 ng/min for 50 minutes before and 40 minutes after the administration of indomethacin to determine if exogenous PGE₂ could prevent the decrease in inner cortical blood flow that followed inhibition of prostaglandin synthesis with indomethacin. In these experiments (Fig. 3), renal blood flow was maintained at relatively stable levels until indomethacin was administered. However, despite infusion of PGE₂, significant reductions in renal blood flow resulted, as they did in the previous series when PGE₂ was not infused. The most important observation was that the decrease in renal blood flow produced by indomethacin during infusion of PGE₂, measured 15 minutes after indomethacin was administered, was accounted for almost entirely by a decrease in its inner cortical component, which fell from 32% to 20% of total renal blood flow (P < 0.05) (Fig. 3). Exogenous PGE₂ did not prevent the major hemodynamic effect of indomethacin, i.e., decreased
blood flow to the inner cortex. Furthermore, these effects of indomethacin could not be attributed to blockade of the renal vasodilator action of PGE$_2$. Thus, when PGE$_2$ was infused at rates of 300–1,200 ng/min, after the effect of indomethacin was fully established in six experiments, renal blood flow increased by 54% from a control value of 122 ± 7 ml/min ($P < 0.02$).

**Discussion**

In the present experiments, concentrations of the PGE-like substance in the blood perfusing the isolated kidney increased during the first 3–4 hours of perfusion and were associated with a progressive increase in renal blood flow, particularly the fraction to the inner cortex. Since renal prostaglandins are not stored, an increase in concentrations of the PGE-like substance in the blood perfusing the isolated kidney denotes continuous prostaglandin synthesis by the kidney (12). The contribution of platelets to blood levels of the PGE-like substance is presumably small, since arteriovenous differences across the kidney in situ exclude a blood element as a major source of prostaglandins; the concentration of the PGE-like substance in renal venous effluent is greater than fifteenfold that in arterial blood (1). The marked reductions in perfusate concentrations of the PGE-like substance within 30 minutes of inhibition of prostaglandin synthetase by either indomethacin or meclofenamate indicate the importance of continuous synthesis in sustaining these high levels. The greatest rate of increase in renal blood flow and its inner cortical component occurred when the rate of increase in the perfusate concentration of the PGE-like substance was most rapid. The most important demonstration in the present experiments that shows the effect of the PGE-like substance on the fractional distribution of renal blood flow was the striking reversal by either indomethacin or meclofenamate of the time-dependent augmentation of inner cortical blood flow. Increasing inner cortical blood flow was associated with rising levels of the PGE-like substance, whereas reductions in blood flow to this zone measured after indomethacin and meclofenamate were administered were associated with decreasing levels of PGE-like substance. The renal hemodynamic response to indomethacin probably did not result from a direct vascular action of the drug, because (a) the effect of the drug at its highest concentration was not observed immediately after its injection. Rather, reduction of renal blood flow was delayed for 10–30 minutes and became apparent only after an effective inhibition of prostaglandin synthesis, as indicated by measurements of the PGE-like substance in the renal perfusate. Also, the effect of indomethacin was not due to blockade of the renovascular effects of endogenous PGE$_2$, because indomethacin did not diminish the renal vasodilator action of exogenous PGE$_2$. (b) We have previously obtained biochemical confirmation of the inhibition of prostaglandin synthesis by indomethacin in the canine kidney: arachidonic acid is not converted to PGE$_2$ in renomedullary homogenates (1). (c) Indomethacin does not affect blood flow in those regions with low rates of prostaglandin synthesis (1). (d) Meclofenamate, another antiinflammatory drug that is chemically dissimilar from indomethacin, also decreased renal blood flow primarily by reducing its inner cortical fraction. (e) Indomethacin possesses a direct renal vasodilator action (13). Aiken and Vane (14) have also concluded that the hemodynamic effects of indomethacin are a consequence of its ability to inhibit prostaglandin synthetase.

The results of the experiments in which indomethacin was administered during infusion of PGE$_2$ indicate that the loss of the local action of endogenous PGE$_2$ was the predominant factor in determining the effect of inhibition of prosta-

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**Figure 4**

Distribution of prostaglandin-synthesizing and degradative enzymes in the kidney. Synthesis in the medulla and degradation in the cortex favor high medullary concentrations of prostaglandins where they might act as local tissue hormones to regulate medullary vascular resistance and thereby inner cortical and medullary blood flow. Interrelationship of inner cortical and medullary circulations are shown in the diagram (from Fourman and Moffat [15]).
glandin synthesis on the distribution of renal blood flow. When indomethacin was added during continuous infusions of PGE₂ at rates which achieved blood concentrations greater than 2 ng/ml, thereby exceeding circulating endogenous levels of the PGE-like substance, reductions in inner cortical blood flow still occurred, but outer cortical blood flow was much less affected. Thus, the level of inner cortical blood flow appears to be greatly influenced by local renal concentrations of vasodilator prostaglandins.

A possible explanation for the decreased blood flow to the inner cortex produced by indomethacin might be derived by considering the anatomy of the renal circulation and loci of synthesis and degradation of prostaglandins in the kidney (Fig. 4). Prostaglandins are formed mainly in the renal medulla, but the major degradative enzyme, 15-OH-prostaglandin dehydrogenase, is located primarily in the renal cortex (4). This anatomic arrangement ensures high renomedullary concentrations of prostaglandins and favors the action of prostaglandins at these sites as local renal hormones. The efferent arterioles of the inner cortex, but not those of the outer cortex, extend into the medulla and give rise to the vasa recta (15). Thus, the inner cortical and the medullary circulations are interrelated and might be subject to the same vasodilator effects of PGE₂ acting as a local hormone. In this regard, the influences which affect medullary synthesis of PGE₂, as indomethacin does in the present study, might preferentially alter inner cortical blood flow as well as medullary blood flow. Moreover, this effect of indomethacin does not require the experimental conditions of the isolated kidney, because it has been demonstrated in situ previously (16). In addition, the inner cortex not only might be affected by prostaglandins as a result of local synthesis (4) but also might be accessible to medullary prostaglandins after they traverse either the loop of Henle or the vasa recta (17).

Acknowledgment

We thank John Stemper, Bernard McMillan, Jean Brock, and David Pacholczyk for technical assistance. We are grateful to Mr. James Strand for his assistance and many valuable suggestions. We thank Kathy Houde and Jane Priebe for secretarial assistance.

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Circ Res. 1974;34:770-776
doi: 10.1161/01.RES.34.6.770

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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