Low Dose Intrarenal Infusions of PGE$_2$, PGI$_2$, and 6-Keto-PGE$_1$ Vasodilate the in Vivo Rat Kidney

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SUMMARY. The renal vascular effects of prostaglandin E$_2$ (PGE$_2$), 6-keto-PGE$_1$, and PGI$_2$ were investigated in indomethacin-pretreated rats. These prostanoids were infused directly into the left renal artery at rates ranging from 0.01 to 1.0 /ig/min, while renal blood flow and mean arterial blood pressure were constantly monitored. PGE$_2$, 6-keto-PGE$_1$, and PGI$_2$ produced reductions in mean arterial blood pressure with threshold doses of 1.0, 0.3, and 0.03 /g/min (P < 0.01), respectively, and maximal vasodepressor responses of 18.9 ± 4.3, 37.0 ± 7.8, and 58.7 ± 8.2 mm Hg (P < 0.01), respectively. In addition, all three prostanoids caused a dose-related reduction in renal vascular resistance with a threshold dose of 0.01 /g/min (P < 0.05). The maximal reductions in renal vascular resistance were 2.59 ± 0.52, 4.41 ± 1.20, and 5.29 ± 1.06 mm Hg/(ml per min) for PGE$_2$, 6-keto-PGE$_1$, and PGI$_2$ (P < 0.01), respectively. Whereas PGE$_2$ and 6-keto-PGE$_1$ produced dose-dependent increases in renal blood flow (maximal increases of 1.5 ± 0.3 and 1.0 ± 0.3 ml/min, respectively (P < 0.01), PGI$_2$ nonsignificantly increased renal blood flow at low doses and decreased renal blood flow at higher infusion rates (P < 0.01). These data indicate that the in vivo rat kidney, similar to the kidneys of other species, is vasodilated by low doses of PGE$_2$, PGI$_2$, and 6-keto-PGE$_1$.

PROSTAGLANDIN E$_2$ (PGE$_2$) is abundantly synthesized by renal tissue (Whorton et al., 1978; Hassid et al., 1979; Sraer et al., 1979). Furthermore, recent studies indicate that PGE$_2$ may participate in several physiological mechanisms in the kidney (Anderson et al., 1976; Dunn and Hood, 1977; Franco-Saenz et al., 1980), as well as in the response of the kidney to drugs (Attallah, 1979; Campbell and Zimmer, 1981) and pathophysiological states (Isakson et al., 1977; Tan et al., 1978; Dunn et al., 1978; Suzuki et al., 1980). Delineation of the precise involvement of PGE$_2$ in renal physiological, pathophysiological, and pharmacological mechanisms requires an understanding of the actions of intrarenally formed PGE$_2$ on kidney function in the in vivo situation.

With respect to the effects of PGE$_2$ on the renal vasculature, an important species difference apparently exists. In the rabbit, dog, and cat, PGE$_2$ vasodilates the renal vasculature (Malik and McGiff, 1975; Chang et al., 1975; Chapnick et al., 1976) and inhibits renal adrenergic neurotransmission (Lonigro et al., 1975; Frame and Hedqvist, 1975; Chapnick et al., 1976). In contrast, in vitro PGE$_2$ constricts the rat kidney and facilitates adrenergic neurotransmission (Malik and McGiff, 1975). Even when administered to the rat in vivo into either the abdominal aorta (Baer and McGiff, 1979) or the left ventricle of the heart (Gerber and Neis, 1979), PGE$_2$ produces renal vasoconstriction. Therefore, based upon current knowledge, involvement of PGE$_2$ in renal mechanisms in the rat must be quite different from that in other species.

However, for several reasons, previous investigations of the renal effects of PGE$_2$ in the rat kidney might not have reflected the true effects of intrarenally formed PGE$_2$ on the renal vasculature in vivo. First, basal prostaglandin production was not suppressed by cyclooxygenase inhibition before dose-response curves to exogenous prostaglandins were determined. As discussed by Horrobin (1977), this becomes particularly important when examining the effects of prostaglandins that might exhibit bell-shaped dose-response curves. Second, the most convincing vasoconstrictor responses to PGE$_2$ were demonstrated in the isolated, artificially perfused rat kidney preparation (Malik and McGiff, 1975). As previously documented with histamine (Campbell and Itskovitz, 1976), the artificially perfused kidney may respond quite differently from the blood-perfused kidney. Third, for those studies conducted in vivo, the prostaglandins were administered into either the suprarenal abdominal aorta (Baer and McGiff, 1979) or the left ventricle of the heart (Gerber and Neis, 1979), rather than directly into the renal artery. Since these approaches resulted in marked hemodynamic changes, the direct renal effects of the infused prostaglandins might have been obscured by reflex sympathetic renal vasoconstriction due to the systemic hypotension, or by the action of other vasoconstrictors such as angiotensin II.

Since knowledge concerning the vascular effect of PGE$_2$ in the rat is prerequisite to understanding the involvement of PGE$_2$ in renal processes in this species, we decided to investigate further the vascular effects of PGE$_2$ in the rat kidney. In these studies, PGE$_2$ and—for comparison—PGI$_2$ and 6-keto-PGE$_1$ were
infused over a 100-fold dose range directly into the renal artery of indomethacin-pretreated animals, while renal blood flow was constantly monitored with an electromagnetic flow probe. Our studies indicate that, like other species, the rat renal vasculature is dilated by these prostaglandins.

Methods

Male Wistar rats (400–500 g) obtained from Harlan Labs were used in all experiments. Rats were maintained on a diet of Wayne Lab-Blox containing 170 mEq Na/kg and 246 mEq K/kg and were given tap water ad libitum. Under pentobarbital anaesthesia (50 mg/kg, ip), the trachea was cannulated and polyethylene cannulae (PE50) were inserted into the left femoral artery and vein. Body temperature was maintained at 37°C by a thermostatically controlled heating pad (Yellow Springs Instrument Co.) connected to a rectal temperature monitor. A noncannulating electromagnetic flow probe (Statham Instruments) 1.0 min in diameter was positioned around the left renal artery for renal blood flow (RBF) measurements. The electromagnetic flow probe was electronically zeroed, and the renal artery was momentarily occluded to validate the electronic balancing. Mean arterial blood pressure (MABP) was measured from the femoral artery line via a Hewlett-Packard pressure transducer and was recorded on a Hewlett-Packard Physiograph (model 7768A). A 30-gauge needle connected to a section of Silastic tubing was carefully inserted into the aorta so that the tip of the needle was positioned approximately 1–2 mm into the left renal artery. An intrarenal 0.9% saline infusion (80 μl/min) to maintain patency of the renal cannula then was begun, as was a supplementary infusion of 0.9% saline (40 μl/min) into the femoral vein. Finally, the abdomen was covered with saline-moistened gauze, and indomethacin (10 mg/kg) suspended in olive oil was administered subcutaneously.

Thirty minutes after the indomethacin treatment, a cumulative dose-response curve to either PGE₂, PGI₂, or 6-keto-PGE₁ was obtained by infusing either 0 (vehicle), 0.01, 0.03, 0.1, 0.3, or 1 μg/min of the prostanooids into the renal artery for 5 minutes at each dose (infusion rate was 80 μl/min). For statistical comparisons, the RBF and MABP were recorded 5 minutes into each infusion. In order to avoid treatment interactions, only a single dose-response curve to a single prostaglandin was performed in each rat. To evaluate the effect of vehicle (glycine buffer) alone on renal resistance, in three rats vehicle only was infused throughout the treatment period (i.e., 30 minutes).

Stock solutions of PGE₂, 6-keto-PGE₁, and PGI₂ (2 mg/ml) were stored in ethanol, acetone, and ethanol saturated with Na₂CO₃, respectively, at −20°C. Immediately before the experiment, a small volume of these stock solutions was appropriately diluted in a glycine-NaCl buffer (pH 10.6) and placed on ice.

All values are expressed as the mean ± SEM. The dose-response curves for each prostaglandin were analyzed by a model III 2-factor analysis of variance without replication in which the random factor was the animal and the fixed factor the dose (Zar, 1974). A Dunnett’s test for multiple comparisons to a control was then used to determine which doses produced statistically significant changes in MABP, RBF, or renal resistance. A P value of <0.05 was used as the criterion of significance.

Results

The baseline values for MABP, RBF, and renal resistance of each treatment group are in Table 1. As shown in Figure 1, when infused intrarenally, all three prostaglandins elicited a dose-dependent reduction in MABP with the order of potency being PGI₂ > 6-keto-PGE₁ > PGE₂. Although PGI₂ lowered MABP at doses as low as 0.03 μg/min, neither PGE₂ nor 6-keto-PGE₁ significantly altered MABP until the dosage was increased to 1.0 and 0.3 μg/min, respectively. Both PGE₂ and 6-keto-PGE₁ produced significant increases in RBF at doses ≥0.03 μg/min, while PGI₂ caused a nonsignificant increase in RBF at low doses and a significant reduction of RBF at the highest dose (Fig. 2). A representative tracing depicting the time course of PGE₁-induced increases in RBF is shown in Figure 3. Each prostanooid reduced renal vascular resistance (Fig. 4), and the reduction in renal resistance was significant at doses as low as 0.01 μg/min. Assuming that 1.0 μg/min produced a near-maximal decline in renal resistance for each prostanooid, PGE₂, PGI₂, and 6-keto-PGE₁ produced half-maximal effects with infusion rates of 20, 23, and 47 ng/min, respectively. Although PGI₂ and 6-keto-PGE₁ elicited similar maximal changes in renal resistance (−5.29 ± 1.06 vs. −4.41 ± 1.20 mm Hg/(ml per min)), the maximal vasodilatory response to PGE₂ was somewhat less (−2.59 ± 0.52 mm Hg/(ml per min), P < 0.05, when compared to PGI₂ maximal response). It is important to recognize that both PGE₂ and 6-keto-PGE₁ produced increases in RBF and reductions in resistance with doses that did not affect MABP. On the other hand, the doses of PGI₂ required to reduce renal resistance also reduced MABP.

It is possible that pretreatment of the animals with indomethacin qualitatively changed the renal vascular response to exogenous PGE₂. To determine whether or not this was the case, we obtained a cumulative dose-response curve to exogenous PGE₂ in four different rats not pretreated with indomethacin. As shown in Table 2, in these animals PGE₂ significantly increased renal blood flow and decreased renal vascular resistance, without significantly affecting mean arterial blood pressure.

The glycine buffer alone, when infused over the entire time course of the experiment, had no affect on MABP (Fig. 1), but caused a decline in RBF (Fig. 2) and, therefore, an increase in renal resistance (Fig. 3). Since the buffer increased renal resistance, the decreases in renal resistance produced by infusions of the prostanooids were not due to a vehicle or time effect.

Discussion

These studies indicate that, when infused intrarenally in vivo at doses ranging from 0.01 to 1.0 μg/min, PGE₂, PGI₂, and 6-keto-PGE₁ are vasodilators in the rat kidney. This would suggest, therefore, that the direct vascular response of the in vivo rat kidney to prostaglandins is qualitatively similar to the response observed in other species.

These data are in contrast to earlier evidence which indicates that, unlike other species, the rat kidney is vasoconstricted by exogenous PGE₂. In the isolated, artificially perfused rat kidney, PGE₂ is unquestiona-
Jackson et al. / Prostaglandins on Rat Renal Blood Flow

TABLE 1
Baseline Values for Mean Arterial Blood Pressure, Renal Blood Flow, and Renal Resistance

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Renal blood flow (ml/min)</th>
<th>Renal resistance [mm Hg/(ml per min)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₂ (7)*</td>
<td>116 ± 9.4</td>
<td>9.8 ± 1.3</td>
<td>12.6 ± 1.6</td>
</tr>
<tr>
<td>PGI₂ (7)*</td>
<td>125 ± 7.4</td>
<td>7.0 ± 0.4</td>
<td>17.9 ± 1.0</td>
</tr>
<tr>
<td>6-keto-PGE₁ (7)*</td>
<td>122 ± 9.9</td>
<td>7.6 ± 0.6</td>
<td>16.1 ± 1.3</td>
</tr>
<tr>
<td>Vehicle (3)*</td>
<td>128 ± 9.3</td>
<td>8.1 ± 1.7</td>
<td>16.7 ± 2.3</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM. * Indicates sample size.

FIGURE 1. The influence of PGE₂, PGI₂, and 6-keto-PGE₁ on mean arterial blood pressure when infused directly into the rat renal artery. Values express mean ± SEM.

FIGURE 2. Changes in renal blood flow induced by intrarenal infusions of PGE₂, PGI₂, and 6-keto-PGE₁ in the rat. Values express mean ± SEM.

masked any vasodilatory response. Indeed, in this same study, similarly hypotensive doses of PGI₂ failed to alter renal vascular resistance. Gerber and Nies (1979), using a radioactive microsphere technique, demonstrated that left ventricular bolus injections of PGE₂ produced renal vasoconstriction, a response which was unaffected by ganglionic blockade with hexamethonium. These investigators concluded that PGE₂ is a direct renal vasoconstrictor. However, the possibility of production or release of vasoconstrictors other than catecholamines, such as angiotensin II, was not eliminated. In addition, in this study only a single dose of PGE₂ was used.

In contrast to these earlier reports, our studies indicate that low dose intrarenal infusions of PGE₂ decrease renal vascular resistance. The major technical differences between our study and the aforementioned reports were: (1) the site of prostaglandin infusion (i.e., intrarenal vs. nonintrarenal), (2) the doses of PGE₂ (0.01–1.0 µg/min vs. 2–20 µg/min), and (3) pretreatment of the animals with a cyclooxygenase inhibitor.

The intrarenal site of PGE₂ administration allowed assessment of the renal vascular effects of PGE₂ at doses which did not produce systemic hemodynamic changes. Along these lines, it is particularly relevant to note that doses of PGE₂ ranging from 0.01 to 0.3 µg/min significantly altered RBF and renal vascular resistance without affecting MAPR. Therefore, the decrease in renal resistance induced by PGE₂ at these doses was neither significantly attenuated by reflex sympathetic activity nor accentuated by renal autoregulatory processes.

Although in this investigation we limited the dose...
of PGE₂ to 1.0 μg/min, in several pilot studies we attempted to infuse 3.0 μg/min of PGE₂ into the rat kidney. We found that the animals became hemodynamically unstable and the renal responses were quite variable i.e., some kidneys constricted, while others dilated. However, even though our contention that PGE₂ is a renal vasodilator in the rat kidney applies only to infusion rates of ≤1.0 μg/min, it is unlikely that the effects of higher infusion rates would be biologically relevant. Even our highest dose of 1.0 μg/min would mimic a renal PGE₂ production rate of 2.88 mg/day (1.0 μg/min per kidney × 1440 min per day × 2 kidneys), which is approximately 27,000 times the daily renal excretion rate of PGE₂ in the rat (Campbell et al., 1979).

Pretreatment of the rats with indomethacin allowed assessment of the renal vascular effects of PGE₂ during suppression of endogenous PGE₂ production. This approach allowed construction of a dose-response curve to exogenous PGE₂ starting at a baseline of near zero concentration of prostaglandins in the biophase. This is important in that, if the concentrations of endogenous prostaglandins in the biophase are high, the addition of exogenous prostaglandins may produce no further effect, or in some situations a response opposite to that produced by biologically relevant concentrations of the prostaglandins. However, we infused PGE₂ into the renal artery of rats not pretreated with indomethacin and noted changes in RBF, MABP, and renal resistance that were qualitatively similar to those changes observed in indomethacin-treated animals. Therefore, in retrospect, pre-

**Figure 3.** A representative tracing which illustrates the time course of PGE₂-induced increases in renal blood flow in the rat.

**Figure 4.** Changes in renal resistance produced by intrarenal infusions of PGE₂, PGI₂, and 6-keto-PGE₁ in the rat. Values express mean ± SEM.
treatment of the animals with indomethacin in this study was probably an unnecessary, yet logical, precaution.

Like PGE₂, we found PGI₂ and 6-keto-PGE₁ also to be potent renal vasodilators in the rat. These results are in accord with previously published studies (Baer and McGiff, 1979; Baer et al., 1979; Quilley et al., 1979), although the decreases in renal vascular resistance induced by PGI₂ and 6-keto-PGE₁ reported previously were somewhat smaller. Interestingly, in an earlier report we found that in the nonfiltering, β-adrenoreceptor-blocked dog kidney, 6-keto-PGE₁ was more potent than PGI₂ as a renal vasodilator (Jackson et al., 1981). In the current study, we found PGI₂ and 6-keto-PGE₁ to be approximately equipotent. However, unlike 6-keto-PGE₁ and PGE₂, the renal vasodilatory and systemic vasodepressor effects of PGI₂ could not be separated. That is to say, any dose of PGI₂ that produced a decline in renal resistance also caused systemic hypotension. Therefore, it is difficult to estimate what percentage of the decline in renal resistance induced by PGI₂ was autoregulatory in origin, what percentage was the result of a direct effect of PGI₂ on the renal vasculature, and to what extent the renal vasodilation was attenuated by reflex activation of the sympatho-adrenal axis.

In conclusion, PGE₂, PGI₂, and 6-keto-PGE₁ when infused intrarenally at doses ranging from 0.01 to 1.0 μg/min are potent vasodilators of the in vivo rat kidney. This would suggest that in the in vivo production of these prostanooids within the kidney of the rat might participate in renal mechanisms which produce a decrease in renal vascular tone.

Table 2

<table>
<thead>
<tr>
<th>Dose (μg/min)</th>
<th>0</th>
<th>0.01</th>
<th>0.03</th>
<th>0.1</th>
<th>0.3</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal blood flow (ml/min)</td>
<td>8.8 ± 2.7</td>
<td>9.4 ± 2.6</td>
<td>10.0 ± 2.7*</td>
<td>10.2 ± 2.9*</td>
<td>10.6 ± 3.1t</td>
<td>11.3 ± 3.1t</td>
</tr>
<tr>
<td>Renal resistance (mm Hg/ml per min)</td>
<td>18.7 ± 6.1</td>
<td>16.8 ± 5.3</td>
<td>15.6 ± 4.6</td>
<td>14.2 ± 4.1</td>
<td>12.7 ± 3.0*</td>
<td>11.4 ± 2.2*</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>125 ± 11</td>
<td>125 ± 11</td>
<td>125 ± 9</td>
<td>118 ± 11</td>
<td>110 ± 9</td>
<td>111 ± 9</td>
</tr>
</tbody>
</table>

Values indicate mean ± SEM (n = 4). * P < 0.05 compared to control (2 way—ANOVA); t P < 0.01 compared to control (2 way—ANOVA).

References


INDEX TERMS: Prostaglandin E₂ • Kidney vasodilator

Prostacyclin • 6-Keto-PGE₁
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