The Hemodynamic Effects of Prostaglandins in the Rat

Evidence for Important Species Variation in Renovascular Responses

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SUMMARY The hemodynamic effects of prostaglandins E₂, F₂α, D₂, and I₂, and of indomethacin and arachidonic acid were studied in Sprague-Dawley rats, by means of the radioactive microsphere technique. In contrast to effects in other species, PGE₂, PGD₂, and arachidonic acid were renal vasoconstrictors in the rat, although PGE₂ and arachidonic acid reduced total vascular resistance. PGF₂α and indomethacin had no effect on the renal vasculature, but PGI₂ produced renal and systemic vasodilation. These data indicate that if prostaglandin-mediated renal vasodilation occurs in the rat, PGI₂ may be the substance responsible. In view of the species differences in renal vascular responses to the prostaglandins, the rat may not be an appropriate model for study of the prostaglandin system as it relates to other species.

One of the postulated physiological roles for prostaglandins is that they are locally produced vasodilator substances that can modulate vasoconstrictor influences and thereby maintain organ blood flow (Staszewska-Barczak and Vane, 1975). In this regard, there is considerable evidence that the endogenously produced prostaglandins are renal vasodilators in several species, including man, and may be important in maintaining renal blood flow during an infusion of vasoconstrictor drugs (Aiken and Vane, 1973; Swain et al., 1975), renal nerve stimulation (Dunham and Zimmerman, 1970), hemorrhagic hypotension (Data et al., 1976), the hepatorenal syndrome (Zipser et al., 1977), and possibly chronic renal disease (Berg, 1977; Kimberly and Plotz, 1977). The one species that has been reported to have anomalous responses to prostaglandins is the laboratory rat. Instead of producing vasodilation, prostaglandin PGE₂ or its precursor, arachidonic acid, is vasoconstrictor when infused into the isolated rat kidney or mesenteric vasculature (Malik and McGiff, 1975; Malik et al., 1976). In addition, PGE₂ enhances and indomethacin decreases the vasoconstriction of isolated mesenteric and renal vascular beds produced by stimulation of sympathetic nerves or infusion of norepinephrine or angiotensin II, responses opposite to those in other species. The differences might be an artifact of the in vitro preparation; however, in a similar in vitro preparation from the rabbit, PGE₂ and arachidonic acid had the expected vasodilator properties and inhibited the vasoconstriction produced by sympathetic nerve stimulation.

Relatively little has been studied in the intact animal to confirm or refute the findings for isolated rat vascular beds. Finn and Arendshorst (1976) reported that inhibition of prostaglandin synthesis with indomethacin or meclofenamate in Sprague-Dawley rats had little effect on renal blood flow measured electromagnetically, but the vasoconstrictor response to angiotensin II was enhanced. Mimran et al. (1975) reported that indomethacin decreased renal blood flow of Wistar rats. Diising et al. (1977) found that indomethacin reduced renal blood flow in Sprague-Dawley rats that had expanded plasma volumes, but not in control rats. These in vivo studies imply that endogenous prostaglandins are, if anything, renal vasodilators rather than vasoconstrictors, and suggest that the findings for the isolated rat vascular beds may be an artifact of the in vitro preparation.

Because of the continuing controversy (Dunn, 1976) and because the spontaneously hypertensive rat is an important model of human disease, we have investigated the vascular effects of indomethacin, arachidonic acid, PGE₂, PGD₂, PGI₂, and PGF₂α in the anesthetized rat, using the radioactive microsphere technique.

Methods

Male Sprague-Dawley rats weighing 300–550 g were anesthetized with pentobarbital, 35 mg/kg, intraperitoneally, and polyethylene catheters (PE...
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50, Clay Adams) were placed into the left ventricle via the right carotid artery and in the left femoral artery. Hemodynamic measurements were made with the radioactive microsphere technique (McDevitt and Nies, 1976) during the baseline state and 1 minute after injection of a bolus (0.1 ml/100 g body weight) of test substance into the left ventricle. One minute was the time when the effects, if any, of the test substances on systemic arterial pressure were maximal. The following substances were injected in separate groups of rats: sodium arachidonate, 10^{-3} g/kg; PGE_2, 10^{-7} g/kg; PGD_2, 10^{-6} g/kg; PGI_2, 3 \times 10^{-7} g/kg; and PGF_2\alpha, 10^{-6} g/kg. The doses used in this study were selected on the basis of preliminary dose-response studies in the rat. A dose was used that consistently produced systemic hemodynamic effects up to an arbitrary maximum dose of 10^{-2} g/kg for the primary prostaglandins and 10^{-3} g/kg for arachidonate. PGE_2, PGD_2, PGI_2 and arachidonic acid were tested without any pretreatment of the rats. Arachidonic acid, PGE_2, and PGI_2 were restested, and PGF_2\alpha was tested in rats with ganglionic blockade produced by hexamethonium, so that the direct vascular effects of these prostaglandins would not be obscured by baroreceptor-mediated activation of autonomic reflexes. PGD_2 at the 10^{-6} g/kg dose did not affect blood pressure and so was not restested with ganglionic blockade. PGF_2\alpha was tested only after administration of hexamethonium because it produced a complex, biphasic pressor response in rats without ganglionic blockade, which did not allow the use of the radioactive microsphere method. After hexamethonium, PGE_2 produced a smooth, consistent pressor response. The dose of hexamethonium, 10 mg/kg, was sufficient to block the pressor response to bilateral carotid artery occlusion and was given 3 minutes prior to the first microsphere injection, so that the baseline values reflect the effects of hexamethonium. In a final group of rats without ganglionic blockade, the effects of indomethacin were determined by injecting the second batch of microspheres 30 minutes after indomethacin, 8 mg/kg.

Following the second microsphere injection, the rats were killed with potassium chloride, and the brain, liver, kidneys, and small intestine were removed for radioactive counting in a gamma scintillation spectrometer (Packard Instruments). The details of the methods we used for determination of cardiac output and organ blood flow in the rat have been published (McDevitt and Nies, 1976). Briefly, 60,000–80,000 microspheres (15 ± 3 μm; 3M Co.) labeled with ^{44}Ce or ^{85}Sr were injected into the left ventricle with a total volume of 0.8 ml of saline over 20 sec. Simultaneously, 0.8 ml of arterial blood was drawn from a femoral artery over 1 minute as a reference sample. Cardiac output was calculated by multiplying the reference sample withdrawal rate by the radioactivity injected and dividing by the reference sample radioactivity. Organ blood flow was determined by multiplying the cardiac output by the fractional distribution of the cardiac output to the organ. The method of Rudolph and Heymann (1967) was used to determine the amount of radioactivity contributed by one nuclide in the presence of another nuclide.

Prostaglandins E_2, D_2 and I_2 (kindly provided by Dr. John E. Pike, The Upjohn Co.) and arachidonic acid (Nuchek Prep, Inc.) were injected as the sodium salt, and PGF_2\alpha was obtained as the tromethamine salt (The Upjohn Co.). The PGI_2 salt was injected at pH 8.5 to avoid hydrolysis that occurs rapidly at neutral pH. The buffer at pH 8.5 was injected as a control prior to the first microsphere injection. The other prostaglandins were injected at neutral pH, and arachidonate was injected at pH 8.0. Indomethacin was dissolved in sodium carbonate buffer and the pH adjusted to 8.5.

The statistical significance of the changes in systemic hemodynamics and organ blood flow produced by the test substances was determined by Student's t-test for paired observations. Because the changes in vascular resistance of the various organs did not appear to be normally distributed, the Wilcoxon signed rank test was used for these data.

Results

Systemic hemodynamic effects of the substances tested are presented in Table 1. PGE_2, PGI_2, and sodium arachidonate produced a fall in arterial pressure at the dose given, whereas PGD_2 and indomethacin had no effect on pressure. After hexamethonium, PGE_2 and PGI_2 were vasodepressor, PGF_2\alpha was pressor, and arachidonic acid produced no consistent effect. Cardiac output was not changed by any of the test substances except for a small increase produced by PGE_2 given during ganglionic blockade with hexamethonium.

The effects on regional blood flow are shown in Table 2. In the rats with intact autonomic reflexes, arachidonic acid, PGE_2, PGI_2, and PGD_2 reduced renal blood flow. Indomethacin reduced and PGI_2 increased blood flow to the small intestines. After ganglionic blockade, the pattern of responses was generally the same, but the arachidonic acid effect on renal blood flow was less consistent and did not reach significance. The calculated total and regional vascular resistances of the kidney, small intestine, and brain are shown in Figure 1 and Table 3. Arachidonic acid significantly decreased the total peripheral resistance and cerebral vascular resistance but increased renal vascular resistance. After hexamethonium, the effects of arachidonic acid were not significant. These effects of arachidonate in the rats with intact autonomic function were closely paralleled by PGE_2. The effects of PGE_2 were unchanged by ganglionic blockade. PGD_2 also increased renal vascular resistance, although it had no effect on total peripheral resistance. PGI_2 was
the only substance that consistently reduced renal vascular resistance, along with a reduction of total vascular resistance and intestinal vascular resistance, and the effect was not altered by hexamethonium. PGF$_{2a}$, on the other hand, increased total peripheral resistance but did not significantly alter the resistance of any of the organs examined. Indomethacin had no significant effect on systemic or regional vascular resistances.

### Discussion

The rat is an important experimental model for the study of a variety of processes, such as prostaglandins, and as such it is important to ascertain that this species is representative of other species. Although some information is available regarding the effects of prostaglandins on the systemic and regional hemodynamics in the rat, the present study is the first systematic attempt to examine in vivo the vascular effects of the primary prostaglandins, arachidonic acid and indomethacin. Our data confirm findings in vitro that the response of the renal vasculature in the rat differs from that of other species. In accord with the reports of Malik and McGiff (1975) and Armstrong et al. (1976), we found that arachidonic acid and PGE$_2$ produced renal vasoconstriction, an effect also shared with PGD$_2$ (Fig. 1). These substances all produce renal vasodilation in the dog and rabbit (Chang et al., 1975; Larsson and Anggärd, 1974). The vasoconstriction is unlikely to be caused by a sympathetic reflex, since the pattern of responses to the prostaglandins was not changed by ganglionic blockade.

The only rat renal vasodilator we found was PGI$_2$ which also has a similar action in the dog (Gerber et al., 1978) but has not been tested previously in the rat. The implications of our findings are that the rat may not be a suitable model for the study of the renal effects of prostaglandins as they relate to other mammalian species. Some investigators have suggested that certain forms of hypertension in the rat may be caused by an excess of prostaglandin in the kidney as the result of a defect in prostaglandin catabolism (Armstrong et al., 1976; Pace-Asciak, 1976). The increased prostaglandin, it is argued, would result in renal vasoconstriction, which might lead to hypertension. Our data do not refute this hypothesis.

In contrast to the effects on the renal vasculature of the rat, the prostaglandins produced effects on systemic and on intestinal vascular resistance of the...
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The percent change in renal vascular resistance produced by arachidonic acid (AA), PGE$_2$ (E$_2$), PGD$_2$ (D$_2$), PGI$_2$ (I$_2$), PGF$_2\alpha$ (F$_2\alpha$), and indomethacin (INDO). The bars labeled HEXAMETHONIUM represent groups of rats pretreated with hexamethonium, 10 mg/kg, 3 minutes prior to the baseline measurements.

The changes found in total vascular resistance cannot be accounted for entirely by changes of the regional resistance in the organs that we examined. It is likely that PGE$_2$, PGI$_2$, and arachidonic acid produced vasodilation and PGF$_2\alpha$ produced vasoconstriction in the muscle vasculature, which accounts for much of the unmeasured vascular resistance in these rats. Blood flow to the brain was little altered by any of the substances tested.

Interestingly, indomethacin had little effect on systemic or regional hemodynamics, causing only a small decrease in intestinal blood flow. Similar findings have been reported for the unanesthetized (Swain et al., 1975) or lightly anesthetized dog (Terrygno et al., 1977). Finn and Arendshorst (1976) found no change in renal blood flow in the rat after indomethacin or meclofenamate. However, Mimran et al. (1975) reported that indomethacin raised arterial pressure and reduced renal blood flow in animals that were sodium depleted. Both these investigators suggested, however, that renal vasoconstriction produced by angiotensin II was enhanced by inhibition of prostaglandin synthesis in the rat. These findings and the findings of Düsing et al. (1977) that indomethacin reduced renal blood flow in volume-expanded rats imply that, in some circumstances, the rat kidney must be capable of making a renal vasodilator prostaglandin whose production is blocked by indomethacin. Although the nature of this prostaglandin is unknown, the only candidate, of the substances we tested, is PGI$_2$. PGI$_2$ has been shown to be produced by human and rabbit renal cortical microsomes (Whorton et al., 1978) and has been postulated to be an important product accounting for much of the vascular effects of arachidonic acid in the dog (Fitzpatrick et al., 1978). Although arachidonic acid infusion in the rat was more closely mimicked by PGE$_2$, it is possible that PGI$_2$ is more important in physiological circumstances in controlling the renal circulation. If there is a mechanism for renal vasodilation related to prostaglandin formation that is common to the rat and other species, then it cannot be related to PGE$_2$, PGD$_2$, or PGF$_2\alpha$, but may be related to PGI$_2$ production.

**Table 3: Percent Change in Resistance in Vascular Beds**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Intestinal</th>
<th>Cerebral</th>
<th>Renal</th>
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<tbody>
<tr>
<td><strong>AA</strong></td>
<td>7</td>
<td>-20 ± 4*</td>
<td>-13 ± 16</td>
<td>-35 ± 6*</td>
</tr>
<tr>
<td><strong>PGE$_2$</strong></td>
<td>8</td>
<td>-18 ± 7†</td>
<td>-23 ± 7*</td>
<td>-15 ± 6†</td>
</tr>
<tr>
<td><strong>PGI$_2$</strong></td>
<td>9</td>
<td>-42 ± 3*</td>
<td>-44 ± 5*</td>
<td>-45 ± 4*</td>
</tr>
<tr>
<td><strong>PGD$_2$</strong></td>
<td>9</td>
<td>4 ± 9</td>
<td>6 ± 11</td>
<td>5 ± 7</td>
</tr>
<tr>
<td><strong>INDO</strong></td>
<td>7</td>
<td>-2 ± 3</td>
<td>15 ± 9</td>
<td>17 ± 12</td>
</tr>
</tbody>
</table>

*After hexamethonium*

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</thead>
<tbody>
<tr>
<td><strong>AA</strong></td>
<td>9</td>
<td>32 ± 19</td>
<td>77 ± 36</td>
<td>-7 ± 15</td>
</tr>
<tr>
<td><strong>PGE$_2$</strong></td>
<td>7</td>
<td>-22 ± 4*</td>
<td>-24 ± 7†</td>
<td>-12 ± 7</td>
</tr>
<tr>
<td><strong>PGI$_2$</strong></td>
<td>7</td>
<td>-33 ± 5*</td>
<td>-44 ± 8*</td>
<td>-20 ± 15</td>
</tr>
<tr>
<td><strong>PGF$_2\alpha$</strong></td>
<td>6</td>
<td>47 ± 15*</td>
<td>26 ± 12</td>
<td>2 ± 8</td>
</tr>
</tbody>
</table>

AA = sodium arachidonate; INDO = indomethacin, n = number of rats studied.

* P < 0.01, † P < 0.05.
References


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