Influence of Prostaglandin E₂, Indomethacin, and Reserpine on Renal Vascular Responses to Nerve Stimulation, Pressor and Depressor Hormones

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SUMMARY The effects of prostaglandin E₂ (PGE₂), indomethacin, and reserpine were evaluated in the rabbit renal vascular bed in situ under conditions of controlled blood flow. Intrarenal infusion of PGE₂, 0.03 and 0.3 fig/min, decreased responses to renal nerve stimulation, intra-arterial norepinephrine, and angiotensin. Responses to nerve stimulation were decreased to a greater extent than responses to norepinephrine. At lower concentrations the effects of PGE₂ on pressor responses and on vascular resistance could be separated. Reserpine decreased the histochemical evidence of adrenergic innervation and reduced the response to renal nerve stimulation, enhanced the response to norepinephrine, and was without effect on the response to angiotensin. Indomethacin decreased depressor responses to arachidonic acid, produced a small increase in renal vascular resistance but did not enhance renal pressor responses. The increase in renal vascular resistance after indomethacin was not modified by reserpine pretreatment. Indomethacin enhanced the renal response to bradykinin. These data show that PGE₂ possesses the ability to modulate pressor responses in the kidney. However, experiments with indomethacin suggest that endogenous prostaglandins neither modulate pressor responses nor mediate the response of the renal vascular bed to bradykinin. In addition, these data suggest that the increase in renal resistance after indomethacin is not dependent on the adrenergic nervous system.

THE CAPACITY of the rabbit renal medulla to synthesize prostaglandins is exceeded only by the seminal gland, and PGE₂ concentrations as high as 0.9 ìg/ml have been reported in renal venous blood. Prostaglandins have been shown to modulate the effects of pressor hormones and sympathetic stimulation in a variety of peripheral vascular beds, including the kidney. If endogenous prostaglandins serve to modulate pressor responses, then inhibitors of synthesis would be expected to enhance the vasoconstrictor effects of nerve stimulation and pressor hormones. However, the effects of synthesis inhibitors on responses to renal nerve stimulation are controversial because in a recent study indomethacin was reported to be without effect on the response to renal nerve stimulation in the isolated rabbit kidney whereas, in a still more recent investigation, the synthesis inhibitor was reported to enhance these responses. The effects of synthesis inhibitors on responses to nerve stimulation of the cat spleen and rat kidney also are controversial. It has also been postulated that prostaglandins may play a role in maintaining the renal vascular bed in a dilated state under resting conditions and in mediating the renal vasodilator response to bradykinin. Since the biosynthetic capacity is so great in the rabbit, this should be an ideal species in which to examine the above hypotheses. The present investigation was undertaken to examine the effects of PGE₂ and reserpine, an agent which depletes adrenergic nerves, on responses to renal nerve stimulation and pressor hormones. In addition we evaluated the effects of indomethacin in doses that decreased responses to arachidonic acid, the prostaglandin precursor, on vascular resistance and renal vascular responses to nerve stimulation and pressor and depressor hormones. In these experiments, a new technique that avoids manipulation of renal arteries and ischemia was used to maintain renal blood flow constant in situ.

Methods

Sixty-eight albino rabbits of either sex weighing 1.9-3.5 kg were anesthetized with pentobarbital sodium, 25 mg/kg, or urethane, 1 g/kg, injected into the marginal ear vein. The trachea was intubated and the left carotid artery and jugular vein were cannulated for measurement of systemic arterial pressure and iv administration of drugs. The abdominal aorta was approached through a midline incision, the superior mesenteric artery was ligated and loose ties were placed around the aorta above and below the origin of the renal arteries. The aorta was ligated below the left renal artery and a polyethylene catheter was inserted into the aorta and the tip advanced to just below the left renal artery. A catheter for withdrawal of blood was inserted into the right carotid artery and the kidneys could then be perfused with blood withdrawn from the

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Supported by U.S. Public Health Service Grants HL 15580, HL 11802, HL 19997, and HL 05969 and a grant from the American Heart Association.

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Received April 5, 1976; accepted for publication January 18, 1977.
thoracic aorta. A Sigmamotor pump (model T-8 or TM-35) was used to maintain blood flow constant. After administration of heparin sodium, 1,000 U/kg, iv, perfusion was started and the aorta was ligated above the renal axis to form an "aortic pouch" which isolated the kidneys from the systemic circulation. This procedure permitted isolation and perfusion of the kidneys without manipulation of the renal arteries and required no period of renal ischemia. Perfusion pressure was measured from a side arm on the perfusion catheter between the pump and the aortic pouch. The flow rate was adjusted so that renal perfusion pressure approximated systemic arterial pressure and was not changed during the experiment. The flow rate averaged 36 ml/min or 2.7 ml/min per g of kidney. Urine flow averaged 8 ml/hour in the control period. Histological examination of the kidney revealed little or no pathology and then only a small amount of congestion. Systemic arterial pressure was recorded from a brachial artery in some experiments, and all pressures were measured with Statham P23AC transducers and recorded on a Grass polygraph (model V or VII). The renal nerves were carefully isolated from both renal arteries and placed on shielded Palmer electrodes. The renal nerves were stimulated with rectangular pulses, 5 msec in duration and of supramaximal voltage at 3, 10, and 30 cycles/sec for a period of 30 sec with an Electronics and Medicine or Grass stimulators. PGE₂ and PGF₂α (Upjohn) were dissolved in 100% ethyl alcohol and stored in a freezer. On the day of use an aliquot was diluted to the appropriate concentration with 0.9% saline solution and infused into the perfusion circuit at a rate of 0.2 ml/min with a Harvard infusion pump. Bradykinin triacetate (Sigma) angiotensin II amide (Hypertensin, Ciba, dose calculated in terms of base) were injected into the renal perfusion circuit in small volumes. Arachidonic acid, 99% pure, (Sigma or NuCheck) and indomethacin (Merck) were prepared as sodium salts in 1% sodium carbonate in saline and were injected iv. Norepinephrine (l-norepinephrine hydrochloride, Sigma) and nitroglycerin (Lilly) were injected in terms of base. Lactated Ringer's solution was infused into the jugular vein at a rate of 0.1–0.2 ml/min for the duration of the experiment.

For experiments with reserpine, animals were given an ip injection of reserpine phosphate (Ciba), 1 mg/kg, dissolved in 20% propylene glycol in saline, 18 hours prior to the experiment. Smaller doses of the anesthetic were used for rabbits receiving reserpine and in these rabbits blood flow averaged 38 ml/min or 3.2 ml/min per g and urine flow averaged 10 ml/hour.

Fluorescent histochemical examination was carried out on renal interlobar arteries from control and reserpine pretreated animals. The arteries were opened up longitudinally and stretched out on a glass slide. The tissues were dried overnight under vacuum and were treated for 1 hour with formaldehyde vapor at 80°C and viewed with a fluorescent microscope.

All data were analyzed by the methods described by Snedecor and Cochran for paired and grouped comparison. All values were expressed as mean ± SEM and a P value of less than 0.05 was considered significant.

Results

INFLUENCE OF PGE₂ ON THE RENAL VASCULAR BED

The effects of PGE₂ on renal vascular responses to renal nerve stimulation and pressor hormones were studied in two groups of rabbits. Dose-response curves for the pressor hormones and a frequency-response curve for renal nerve stimulation were determined before, during, and 15–30 minutes after infusion of PGE₂ into the renal perfusion circuit. Renal nerve stimulation in the range of 3–30 cycles/sec and intra-arterial norepinephrine and angiotensin (0.3 and 1 μg) resulted in frequency and dose-related increases in renal arterial perfusion pressure. Since blood flow was held constant with a pump, the increase in perfusion pressure reflected an increase in renal vascular resistance. The response of the renal vascular bed to nerve stimulation and pressor hormones was consistent over the period of time in which these experiments were carried out. The infusion of PGE₂ into the renal perfusion circuit at a rate of 0.3 μg/min decreased renal vasoconstrictor responses to norepinephrine, renal nerve stimulation, and angiotensin (Fig. 1A). Responses to noradrenergic were decreased to about 40% of control whereas responses to nerve stimulation were reduced to approximately 20% of control (Fig. 1A). Responses to nerve stimulation and the pressor hormones returned toward control values after the termination of the PGE₂ infusion; however, responses to renal nerve stimulation still were significantly less than control 30 minutes after the infusion (Fig. 1A). During infusion of PGE₂, 0.3 μg/min, there was a significant reduction in renal perfusion pressure which was well maintained during the 30-minute infusion period; however, no changes in aortic pressure were observed (Fig. 2, bottom).

In a second series of animals in which the infusion rate was decreased to 0.03 μg/min, PGE₂ had no significant effect on renal perfusion pressure (Fig. 2, top). Although PGE₂ at the lower concentration did not alter renal vascular resistance, renal vasoconstrictor responses to norepinephrine, nerve stimulation, and angiotensin were decreased significantly (Fig. 1B). The reduction in response to renal nerve stimulation on a percentage basis was significantly greater than the reduction in response to norepinephrine. Renal responses to norepinephrine, nerve stimulation (3 and 10 cycles/sec), and angiotensin were not significantly different from control 30 minutes after the end of the PGE₂ infusion (Fig. 1B).

The effects of PGF₂α on responses to nerve stimulation and pressor hormones were studied in another group of seven rabbits. PGF₂α, when infused into the kidney at rates of 0.3 and 1.0 μg/min, had no consistent effect on renal vascular responses to norepinephrine, renal nerve stimulation, or angiotensin.

EFFECTS OF ARACHIDONIC ACID, INDOMETHACIN, AND RESERPINE

The effects of arachidonic acid, the precursor for prostaglandins E₂ and F₂α, were evaluated. Arachidonic acid produced dose-dependent decreases in systemic arterial pressure when injected iv in doses of 100, 300, and 500 μg (Fig. 3C). Arachidonic acid also produced dose-depend-
ent decreases in renal pressure when injected into the renal perfusion circuit in doses of 30, 100, and 300 μg (Fig. 3A). The reduction in renal perfusion pressure in response to the midrange (100 μg) dose of arachidonic acid was decreased significantly 15-30 minutes after administration of indomethacin, 2.5 mg/kg, iv (Fig. 3B). The renal dilator response to the prostaglandin precursor was reduced in terms of both the absolute (mm Hg) and percent decrease in pressure and the response duration (T½) was decreased markedly (Fig. 3B). In five of these rabbits, renal dilator responses to PGE₂, 30 and 100 ng, were not decreased after indomethacin. The reduction in systemic arterial pressure in response to the prostaglandin precursor was decreased significantly 15-30 minutes after indomethacin 2.5 mg/kg, iv (Fig. 3C). The reduction in systemic arterial pressure in response to nitroglycerin was not modified after indomethacin (Fig. 3C).

In another series of rabbits, we studied the effects of a dose of indomethacin that diminished depressor responses to arachidonic acid on responses to pressor hormones and nerve stimulation. Renal vasoconstrictor responses to norepinephrine, renal nerve stimulation at 3 cycles/sec, and the low dose of angiotensin were not modified 15-30 minutes after indomethacin, 2.5 mg/kg, iv (Fig. 4A). However, renal responses to nerve stimulation at 10 and 30 cycles/sec and to the high dose of angiotensin were decreased significantly after indomethacin (Fig. 4A). Indomethacin increased resistance to flow in the renal vascular bed (Fig. 4B). The increase in renal perfusion pressure developed slowly over a 30-minute period; however, indomethacin was without significant effect on aortic pressure (Fig. 4B). Renal perfusion pressure did not change over the 30-minute period when the saline sodium carbonate vehicle for indomethacin was administered.

Reserpine, an agent which causes depletion of catecholamines from adrenergic nerves, abolished the formaldehyde-induced fluorescence in renal arteries, when given in a dose of 1 mg/kg, ip, 18 hours before the experiment (Fig. 5A-B). Reserpine greatly reduced the response of the renal vascular bed to renal nerve stimulation but enhanced the response to norepinephrine and was without significant effect on the response to angiotensin (Table 1). The rise in renal vascular resistance in response to indomethacin, 2.5 mg/kg, iv, was not significantly different in control rabbits and in rabbits pretreated with reserpine, 1 mg/kg, ip, 18 hours prior to the experiment.

**EFFECT OF INDOMETHACIN ON DILATOR RESPONSES**

The effects of indomethacin on renal vasodilator responses to bradykinin and nitroglycerin were also evaluated. Responses to the vasodilators were compared before
and 15–30 minutes after indomethacin, 2.5 mg/kg, iv. The decreases in renal arterial perfusion pressure in response to intra-arterial bradykinin and nitroglycerin were significantly greater than control after administration of indomethacin (Fig. 6). The decrease in perfusion pressure in response to both dilator agents was significantly greater whether the responses were compared in terms of the absolute (mm Hg) decrease or percent decrease in renal perfusion pressure (Fig. 6).

Discussion

Results of this study show that PGE₂ has the ability to inhibit vasoconstrictor responses to renal nerve stimulation in the rabbit renal vascular bed. The reduction in response to nerve stimulation was greater than the decrease in response to norepinephrine; this suggests that PGE₂ may act on adrenergic terminals to inhibit release of transmitter and on vascular smooth muscle to decrease responsiveness to norepinephrine. These data are in agreement with results of studies on the isolated perfused rabbit kidney in which PGE₂ inhibited responses to nerve stimulation and the release of tritium-labeled norepinephrine. These results are also in agreement with data obtained for a variety of sympathetically innervated organs in a number of species. The present study extends previous work on the isolated rabbit kidney by showing that PGE₂ also attenuates renal vasoconstrictor responses to angiotensin, that for the in situ preparation renal arterial concentrations as low as 1 ng/ml decrease pressor responses and that the effects of PGE₂ on renal vascular resistance and pressor responses can be separated. The present data provide support for the hypothesis that prostaglandins may modulate the effects of pressor systems in the kidney.

Because renal nerve stimulation and pressor hormones release large quantities of prostaglandins and PGE₂, the major renal prostaglandin has the capacity to modulate renal pressor responses, then inhibitors of prostaglandin synthesis should increase responses to renal nerve stimulation and pressor hormones. However, the effects in the rabbit of inhibitors of prostaglandin synthesis on renal pressor responses are unclear. For example, in the studies of Malik and McGiff, indomethacin enhanced the response to nerve stimulation in the isolated rabbit kidney whereas in the studies of Needleman et al., on the same species, responses to renal nerve stimulation and the release of catecholamines were not affected by the synthe-
Inhibited synthesis on responses to pressor hormones and nerve stimulation was observed. In a more recent study, both indomethacin and meclofenamate, two chemically dissimilar inhibitors, decreased responses in the isolated cat spleen. However, differences in experimental design may be involved since in the present study the kidney was autoperfused in vivo and the pressor hormones were injected, whereas in the other study, the kidney was isolated and the hormones were infused. In the present study, the prostaglandin precursor, arachidonic acid, produced dose-dependent decreases in systemic arterial pressure and renal perfusion pressure and these responses were decreased markedly by indomethacin, whereas dilator responses to PGEndo and nitroglycerin were not diminished. These data suggest that the precursor is transformed into dilator substances in the systemic vascular bed and the kidney and that biosynthesis of these substances, presumably prostaglandins, is inhibited by indomethacin.

The failure of indomethacin to enhance responses to nerve stimulation in the rabbit kidney in situ in doses adequate to decrease responses to arachidonic acid does not provide support for the hypothesis that endogenous prostaglandins serve to modulate pressor responses in the rabbit renal vascular bed and are consistent with the studies of Needleman et al. The observed decreases in response to nerve stimulation and angiotensin may be due to an effect of indomethacin unrelated to prostaglandin synthesis since indomethacin has many actions.

In addition to a role in modulating responses to vasoconstrictor hormones and renal nerve stimulation, it has been postulated that endogenous prostaglandins may mediate the response to bradykinin in the renal vascular bed. This hypothesis is based on the observation that bradykinin releases PGEndo from the kidney whereas edoisin in equidilator doses had no effect on the output of this prostaglandin. Results of experiments on the rat skeletal muscle vascular bed are in accord with this hypothesis that vasodilator responses to bradykinin were attenuated by inhibitors of prostaglandin synthesis. If the renal vasodilator response to bradykinin is mediated by endogenous prostaglandins, then the response of the renal vascular bed to the peptide hormone should be attenuated by inhibitors of prostaglandin synthesis since indomethacin has many actions.

We studied the effects of a dose of indomethacin reported to inhibit synthesis on responses to pressor hormones and nerve stimulation. The synthesis inhibitor did not enhance responses to angiotensin, norepinephrine, or nerve stimulation in the in situ autoperfused rabbit kidney. In fact, responses to renal nerve stimulation and to higher doses of angiotensin were decreased. These results are similar to results of studies on the rat kidney in which both indomethacin and meclofenamate decreased responses to renal nerve stimulation. These data are not in agreement with the studies of Gagnon et al. on the isolated rabbit kidney perfused with Krebs' solution. However, differences in experimental design may be involved since in the present study the kidney was autoperfused in vivo and the pressor hormones were injected, whereas in the other study, the kidney was isolated and the hormones were infused. In the present study, the prostaglandin precursor, arachidonic acid, produced dose-dependent decreases in systemic arterial pressure and renal perfusion pressure and these responses were decreased markedly by indomethacin, whereas dilator responses to PGEndo and nitroglycerin were not diminished. These data suggest that the precursor is transformed into dilator substances in the systemic vascular bed and the kidney and that biosynthesis of these substances, presumably prostaglandins, is inhibited by indomethacin. The failure of indomethacin to enhance responses to nerve stimulation in the rabbit kidney in situ in doses adequate to decrease responses to arachidonic acid does not provide support for the hypothesis that endogenous prostaglandins serve to modulate pressor responses in the rabbit renal vascular bed and are consistent with the studies of Needleman et al. The observed decreases in response to nerve stimulation and angiotensin may be due to an effect of indomethacin unrelated to prostaglandin synthesis since indomethacin has many actions.

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### Table 1: Effects of Reserpine on Responses to Norepinephrine, Nerve Stimulation, and Angiotensin in the Rabbit Renal Vascular Bed

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine (0.3 µg ia)</th>
<th>Nerve stimulation (10 cycles/sec)</th>
<th>Angiotensin (0.3 µg ia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 20)</td>
<td>43.2 ± 4.2</td>
<td>76.2 ± 8.3</td>
<td>53.4 ± 5.7</td>
</tr>
<tr>
<td>Reserpine* (n = 5)</td>
<td>108.2 ± 27†</td>
<td>8.0 ± 4.1†</td>
<td>62.0 ± 10.8</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

* Reserpine, 1 mg/kg ip, was given 18 hours prior to experiment.

† P < 0.05, when compared to control, grouped analysis.
bradykinin and nitroglycerin were in fact increased in both absolute terms and on a percentage basis. These findings suggest that indomethacin may increase the responsiveness of the vascular bed to dilator substances. These data do not support the hypothesis that the renal vasodilator response to bradykinin is dependent on prostaglandin synthesis, although the increase in responsiveness to dilator hormones after indomethacin may mask an inhibitory effect on the response to bradykinin. The failure of indomethacin to antagonize responses to this peptide does not appear to be species dependent since similar observations have been made for the feline renal vascular bed and canine superficial veins.

It has been postulated that in the resting state maintenance of renal blood flow is dependent on prostaglandin synthesis. This hypothesis is based on the finding that indomethacin decreases renal vascular resistance in the dog and these changes are correlated with a decrease in efflux of PGE-like material in renal venous blood. Results of the present study show that indomethacin increases resistance to flow in the rabbit kidney and are in agreement with results obtained for the anesthetized dog. Recently, it has become apparent that the effects of indomethacin on renal blood flow are different in conscious and anesthetized dogs; this suggests that prostaglandin synthesis may be enhanced by anesthesia. However, the effects of indomethacin on renal blood flow in the conscious rabbit are not known. The effects of indomethacin on renal vascular resistance in the anesthetized rabbit were not modified after treatment with doses of reserpine which abolished the extensive histochemical evidence of adrenergic innervation and markedly decreased the response to renal nerve stimulation. Reserpine enhanced the response of the renal vascular bed to norepinephrine but was without effect on the response to angiotensin. These data suggest that indomethacin does not increase renal vascular resistance by opposing the effects of the adrenergic nervous system on the kidney.

In summary, results of the present study show that PGE2 inhibits renal vascular responses to nerve stimulation and pressor hormones. Since the reduction in response to nerve stimulation was greater, these data suggest that PGE2 inhibits release of transmitter and depresses the response of vascular smooth muscle to pressor hormones. The effects of PGE2 and reserpine on responses to nerve stimulation were similar whereas effects on responses to pressor hormones were different. These data show that PGE2 in very low concentrations possesses the ability to modulate renal vasoconstrictor responses in situ. However, experiments with indomethacin in doses that decrease renal responses to arachidonic acid, the prostaglandin precursor, suggest that endogenous PGE2 does not modulate responses to pressor hormones or nerve stimulation and that this substance probably does not mediate the

**Figure 5** (A) stretch preparations of rabbit renal interlobar artery showing branching varicose adrenergic vasomotor fibers in control animal. (B) 18 hours after reserpine, 1 mg/kg ip, almost no specific fluorescence associated with adrenergic innervation is present.
Figure 6 Influence of indomethacin 2.5 mg/kg iv, on renal vasodilator responses to bradykinin and nitroglycerin. Responses to the dilators were obtained before and 15-30 minutes after administration of the synthesis inhibitor.

Acknowledgments

We thank the Upjohn Company and Merck for the prostaglandins and indomethacin used in this study. We also thank Elizabeth Thomason for her help in preparing the manuscript.

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nerve stimulation, pressor and depressor hormones.
G D Fink, B M Chapnick, M R Goldberg, P W Paustian and P J Kadowitz

_Circ Res._ 1977;41:172-178
doi: 10.1161/01.RES.41.2.172

_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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