Contribution of Intrarenal Generation of Prostaglandin to Autoregulation of Renal Blood Flow in the Dog

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ABSTRACT

The mechanism of autoregulation of renal blood flow was investigated in anesthetized dogs, using either constant-flow or constant-pressure perfusion of the kidney. Prostaglandinlike material in the renal venous blood was assayed by the blood-bathed organ technique. Compensatory renal vasodilation, induced either by lowering the blood flow or the perfusion pressure, was accompanied by increased output of prostaglandinlike material into the renal venous blood. The prostaglandin synthetase inhibitor, indomethacin, abolished this output, and, at the same time, autoregulation of renal blood flow was abolished. A converting enzyme inhibitor, however, had no effect on renal autoregulation. The results support the hypothesis that autoregulation in the kidney is mediated by release of a vasodilator, prostaglandin, rather than by formation of a vasoconstrictor substance such as angiotensin II.

KEY WORDS
renal prostaglandin release, indomethacin, renal blood flow autoregulation, converting enzyme inhibition, prostaglandin synthetase inhibition, renin-angiotensin system

Several stimuli to the kidney, including ischemia (1), reduction of perfusion pressure (2), infusion of norepinephrine (3), angiotensin (4–6), or bradykinin (7), and sympathetic nerve stimulation (8, 9) cause an increase in prostaglandinlike substances in renal venous blood. McGiff et al. (1) identified the material released from dog kidney by ischemia as predominantly prostaglandin E₂, and Davis and Horton (9) found increased concentrations of prostaglandins E₂ and A₂ in extracts of rabbit renal venous blood after renal sympathetic nerve stimulation. Both prostaglandin E₂ and A₂ are potent dilators of the renal vascular bed and both induce natriuresis (10).

Indomethacin is a specific inhibitor of prostaglandin biosynthesis in all tissues so far studied including the kidney (5, 6, 11–13). The abolition by indomethacin of the release of prostaglandinlike material from an organ helps not only to identify the material as a prostaglandin but also to define the functions of prostaglandins in the organ from which the release occurred. For instance, Aiken and Vane (5, 6) showed that, when release of prostaglandinlike material from the kidney was abolished by indomethacin, the vasoconstrictor effects of angiotensin II on the renal vascular bed were greatly augmented. Lonigro et al. (14) found that renal blood flow in dogs was proportional to the basal output of prostaglandin E₂ and that indomethacin reduced renal blood flow in proportion to its effect on the prostaglandin output.

These results suggest that local prostaglandin release contributes to regulation of renal blood flow. We have, therefore, looked at the output of prostaglandinlike substances from the kidney during autoregulation of blood flow and the effects of indomethacin on autoregulation and prostaglandin output.

Methods

Twenty-nine mongrel dogs of either sex weighing 13.5–28 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv); anesthesia was maintained with additional intravenous injections of 3 mg/kg as required.

Mean arterial blood pressure was recorded from a femoral or a brachial artery with a Statham or an S.E. Laboratory pressure transducer. A jugular vein was cannulated for intravenous infusion. Positive-pressure ventilation was maintained with a Starling respiration pump attached to an endotracheal tube. The rectal temperature of the dog was kept at 37°C.

The left kidney was exposed by a midline incision, and the renal artery and vein were isolated from the surrounding tissues, taking care to avoid manipulation of the kidney and damage to the nerve
supply. Both ureters were cannulated in the midabdo-
men, and urine output was measured with drop
counters.

An intravenous infusion (30 ml/kg) of 0.9% (w/v) sodium chloride was given at the beginning of each
experiment, and then an automatic syringe was used to
replace each milliliter of urine excreted with an
equivalent amount of intravenously administered saline.
Heparin (1000 IU/kg) was also given intravenously.

REbNAL PBRFUSION

A femoral artery was cannulated and attached by
silicone rubber tubing to the inflow of a Sigma
tomotor 18S peristaltic pump. The left renal artery was transected,
cannulated, and attached to the outflow of the pump by
similar tubing. Water at 38°C was circulated through a
jacket surrounding the input and the output tubing
from the pump. Renal arterial pressure was measured
by a pressure transducer through fine polyethylene
tubing inserted into the renal artery via a side arm of
the cannula.

For constant-pressure experiments, the kidney was
perfused at a set flow rate; changes in the perfusion
pressure indicated changes in renal vascular resistance.
For constant-pressure experiments, the amplified output
of the renal arterial pressure transducer was used to
control the speed of the Servomex motor of the
perfusion pump in such a way that renal arterial
perfusion pressure was maintained constant (15).
Variations in renal blood flow (recorded as a voltage
output from the speed control of the pump) indicated
changes in vascular resistance.

PROSTAGLANDINLIKE ACTIVITY IN RENAL VENOUS BLOOD

A polyethylene catheter was inserted through the
femoral vein and pushed up the vena cava until its bent
tip could be manipulated into the left renal vein. The
diameter of the catheter was much less than that of the
renal vein so that it did not impede renal venous
outflow. Any venous tributaries not carrying renal blood
were ligated. Blood was continuously withdrawn
trough the catheter by a roller pump at a rate of 10
ml/min and superfused over isolated assay tissues
before being returned to a jugular vein (16). The blood
in the extracorporeal circuit was maintained at 38°C by
a water jacket. The assay tissues, a rat stomach strip
(17), a rat colon (18), and a chick rectum (19); were
superfused with Krebs solution bubbled with 95% O2,5%
CO2 and containing a mixture of indomethacin (2
µg/ml) and phenoxybenzamine (2 µg/ml) for at least
1 hour before superfusion with blood was begun.
Treatment with these antagonists made the assay
organs more sensitive and selective for prostaglandin-
like substances.

The movements of the assay tissues were detected
with Harvard smooth muscle transducers. Increases in
prostaglandinlike activity in renal venous blood were
recorded as contractions and quantified by comparison
with the effects of infusions of prostaglandin E2 directly
into the blood superfusing the assay tissues. This
combination of tissues is particularly suitable for
assaying prostaglandinlike activity (16). Sometimes the
assay tissues were alternately superfused with renal
venous blood and femoral venous blood so that the
prostaglandinlike activity in each stream could be
compared.

In one experiment, renal blood flow was recorded
using an electromagnetic flow probe placed around the
noncannulated renal artery. Graded reductions in renal
arterial pressure were induced by varying the degree of
filling of a balloon cuff placed distal to the probe. Renal
arterial pressure was recorded through a fine
hypodermic needle pushed into the arterial lumen.

Mean arterial blood pressure, renal arterial pressure,
renal blood flow, urine output, and changes in tone of
the isolated assay tissues were continuously recorded on
a Beckmann SII dynograph.

Results

BASEL PROSTAGLANDINLIKE ACTIVITY
OF THE RENAL VENOUS BLOOD

In eight dogs (noncannulated renal artery) the
assay tissues were superfused first with femoral
venous blood and then with renal venous blood. In
five experiments superfusion with renal venous
blood led to a contraction of the assay tissues,
which could be repeated several times in each
experiment. These experiments thus suggest that
the output of prostaglandinlike activity in renal
venous blood was higher than that in femoral
blood. Intravenous injection of indomethacin (1–2
mg/kg) did not change the tone of the assay tissues
when they were bathed in femoral venous blood,
showing that the femoral vascular bed was not
releasing detectable amounts of prostaglandin. The
prostaglandinlike activity of renal venous blood
varied in the experiments from 0.2 to 2 ng/ml
(assayed as prostaglandin E2). The relative con-
tractions of the assay tissues induced by infusions of
prostaglandin E2 did not exactly match the
contractions induced by superfusion with renal
venous blood, suggesting that another prostaglan-
din was also present. The contractions of the assay
tissues on changing from femoral to renal venous
blood were abolished by indomethacin (1 mg/kg in
four experiments, 2 mg/kg in one experiment). This
result shows that the contractions induced by renal
venous blood were not due to differences in oxygen
tension between renal and femoral venous blood.

REbNAL PBRFUSION

Constant-Flow Experiments (10 Dogs).—After re-
nal perfusion was started the flow rate was set so
that the perfusion pressure was similar to the mean
arterial blood pressure. Flow rate was changed
within the range which kept perfusion pressure
between 60 and 190 mm Hg.

Reduction in renal blood flow, induced by
lowering the pump speed, was accompanied by an
immediate decrease in renal perfusion pressure,
which then gradually decreased further, reflecting autoregulatory dilation of the renal vessels (Fig. 1). These changes were also accompanied by contractions of the blood-bathed assay tissues, showing an increased concentration of prostaglandinlike material in renal venous blood.

The contraction of the assay tissues continued to increase during the phase of renal vasodilation. In three of the experiments, however, an increased output of prostaglandinlike material occurred with the reduction of renal flow even though there was no autoregulatory dilation of the renal vessels. In the experiments in which successive reductions in flow were induced, the concentration of prostaglandinlike substances increased with each subsequent reduction in flow. The increased concentration of prostaglandinlike material in renal venous blood varied from less than 1 ng/ml up to 5 ng/ml (assayed as prostaglandin E₂).

An imposed increase in flow rate was associated with a relaxation of isolated organs, showing a decreased concentration of prostaglandinlike substances in renal venous blood. When flow rate was maintained constant for 30 minutes or more, there were spontaneous variations in perfusion pressure; reductions in pressure were accompanied by increases in concentration of prostaglandinlike material. Thus, under constant-flow conditions, there were variations in prostaglandin output accompanied by changes in vascular resistance.

After indomethacin administration (1–5 mg/kg, iv) there was a long-lasting rise in perfusion pressure. The vasoconstriction developed quickly but varied in intensity between dogs. There was also a gradual relaxation of the assay tissues so that their tone reached a new stable lower level after 10–20 minutes. The assay tissues were then usually more sensitive to prostaglandin E₂ (Fig. 1). After indomethacin administration, reduction in renal blood flow was not followed by a phase of vasodilation or by an increased concentration of prostaglandinlike material in the renal venous blood (Fig. 1).

Infusions into the renal artery of prostaglandin E₂ to give concentrations of 0.5–1 ng/ml blood caused vasodilation so that the perfusion pressure fell to preindomethacin levels (three experiments).

**Constant-Pressure Experiments (8 Dogs).**—Perfusion pressure was initially set to be the same as mean arterial blood pressure. This pressure gave a flow rate of 160–180 ml/min (10–12.5 ml/kg min⁻¹). Perfusion pressure was then varied within the range of 65 to 190 mm Hg.

A reduction in perfusion pressure was accompanied by a sudden fall in renal blood flow which then gradually recovered (Fig. 2), showing vasodilation of the renal vascular bed. The efficiency of this autoregulation varied from dog to dog and with the extent of the reduction in perfusion pressure. Indomethacin (1–2 mg/kg, iv) induced a fall in renal blood flow (i.e., vasoconstriction) which exactly nullified the autoregulatory vasodilation (Fig. 2).

The recovery of renal venous blood flow at reduced perfusion pressure was accompanied by contraction of the blood-bathed organs, showing increased output of prostaglandinlike material into renal venous blood (Fig. 3). Further reductions in perfusion pressure led to further increases in output.
PROSTAGLANDINS AND RENAL AUTOREGULATION

The left kidney of a 22-kg male dog was perfused with a constant-pressure pump. Reduction in renal perfusion pressure (RPP) led to reductions in renal blood flow (RBF) which were not maintained due to autoregulation. Note that some autoregulation was still present at 65 mm Hg. When indomethacin (2 mg/kg, iv) was given, the autoregulatory recovery of renal blood flow was nullified. BP = blood pressure.

The concentration of prostaglandinlike material in renal venous blood sometimes increased from < 1 ng/ml to 5 ng/ml (assayed as E2) at a time when blood flow remained at 50–70% of the control value. Furthermore, the increased concentrations of prostaglandinlike material were maintained when blood flow had returned or almost returned to previous levels through autoregulation. Restoration of the perfusion pressure to previous values was accompanied by a reversal of the increased output of prostaglandinlike material. These stepwise changes could be reproduced several times (Fig. 3).

Indomethacin (1–2 mg/kg, iv, six experiments) reduced renal blood flow, and, at the same time, the assay tissues relaxed. Reduction in perfusion pressure now no longer led to an increased output of prostaglandinlike material into renal venous blood. At the same time, the autoregulatory renal vasodilation was abolished (Fig. 4).

In any one experiment, it was not possible to obtain sufficient points to plot reliable pressure-flow curves before and after indomethacin treatment. For this reason the results of these experiments were combined (Table 1) by calculating the initial renal vascular resistance and then expressing changes in resistance induced by changes in perfusion pressure as a percent of the initial value.

Resistance was calculated as mean renal arterial pressure divided by mean renal flow, making the approximation that renal venous pressure was negligible. In six of the constant-flow experiments, injection of indomethacin led to such a great increase in perfusion pressure (> 300 mm Hg) that the experiment was abandoned. Figure 5 shows the changes in resistance plotted against the changes in perfusion pressure (also expressed as a percent of initial pressure). Before indomethacin administration, there was a clear reduction in resistance as the pressure was reduced. After indomethacin administration (1 mg/kg, iv), the resistance increased to 150–250% of the initial value and there was no longer a change in resistance with changes in pressure. After administration of a larger dose of indomethacin (2 mg/kg, iv), there was an even greater increase in resistance, and, again, no overall change in resistance could be induced by a change in perfusion pressure.

Effects of Converting Enzyme Inhibition.—In two experiments the pentapeptide, pyrrolidonecarboxylic acid-Lys-Trp-Ala-Pro (20-23), which inhibits converting enzyme in the kidney (24, 25), was infused into the perfused kidney at a concentration
TABLE 1

Quantitative Data for Renal Perfusion Pressure and Renal Blood Flow

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Experiments 1-4 were constant-flow experiments and experiments 5-10 were constant-pressure experiments. Six constant-flow experiments and two constant-pressure experiments were not included in the table, because indomethacin caused such an intense vasoconstriction that no further results were obtained. RPP = renal perfusion pressure, RBF = renal blood flow, and R = renal resistance. The percent values are related to the initial value as 100%.

Circulation Research, Vol. XXXIII, October 1973
The left kidney of a 14-kg male dog was perfused with a constant-pressure pump. Reduction in renal perfusion pressure (RPP) led to reduction in renal blood flow (RBF), which then tended to recover due to compensatory vasodilation. After indomethacin administration (1 mg/kg, iv) which caused a reduction in renal blood flow, autoregulation was abolished. BP = blood pressure.

(1 μg/ml blood) which strongly inhibits the effects of angiotensin I on renal blood flow (25). The inhibitor had no effect on either the resting blood flow or the autoregulatory compensation of blood flow induced by reduction in perfusion pressure.

**EFFECTS OF INDOMETHACIN ON BLOOD PRESSURE**

In many of the renal perfusion experiments intravenous injection of indomethacin was followed by a small (5–20 mm Hg), long-lasting rise in mean arterial blood pressure (Figs. 1, 2, and 4).

**NONCANNULATED KIDNEY**

In one experiment, the renal blood flow was measured by an electromagnetic flowmeter and reductions in renal perfusion pressure were induced by an arterial cuff. Results similar to those for experiments in perfused kidneys were obtained; there was good autoregulation of blood flow which was abolished by indomethacin (10 mg/kg, iv) (Fig. 6).

**FAILURE OF AUTOREGULATION**

In two greyhounds, the perfused kidney failed to show autoregulation or prostaglandin release into renal venous blood. Both of these dogs had exceptionally high blood pressure (200/180 mm Hg and 210/180 mm Hg).

**Discussion**

Many organs of the body exhibit autoregulation, and numerous theories about the mechanism underlying this phenomenon have been propounded. The one most generally accepted is that a local vasodilator metabolite is responsible for matching blood flow to the metabolic activity of the tissue. In the kidney, this theory is less well founded both because the blood flow is greatly in excess of the metabolic needs of the organ and because a fall in blood flow is usually accompanied by a fall in the renal requirement for oxygen. One current theory suggests that autoregulation of renal blood flow is not dependent on a vasodilator metabolite but on the effects of locally generated angiotensin II, a vasoconstrictor (26–28).
Our results support the view that in the kidney, as in other tissues, autoregulation is brought about by a vasodilator substance and furthermore demonstrate that this substance may be a prostaglandin released in increasing quantities as the arterial blood pressure decreases. We showed that there was prostaglandin-like material in the renal venous blood and that its concentration increased under conditions which induced autoregulatory vasodilation of the kidney.

The increase in concentration of prostaglandin-like material in renal venous blood when blood flow rate or perfusion pressure was decreased was not simply a consequence of a constant output of prostaglandin coupled with a decrease in blood flow, for the concentration increased far more than could be accounted for by the reduction in blood flow and the increased concentration was maintained even when the blood flow had returned or almost returned to previous levels through autoregulation. In the constant-flow experiments, fluctuations in vascular resistance were accompanied by fluctuations in prostaglandin-like output.

Strong support for the hypothesis that release of a vasodilator prostaglandin is responsible for renal autoregulation comes from our results showing that both autoregulation and the output of prostaglandin-like material from the kidney were abolished by indomethacin administration. As far as is known, indomethacin is a specific inhibitor of prostaglandin synthetase (11-13); therefore, we can conclude that its effects on blood flow and autoregulation are due to abolition of prostaglandin biosynthesis. Since tissues do not store prostaglandins, release must be accompanied by synthesis (29), and inhibition of synthesis would quickly lead to abolition of release.

We did not attempt to identify the prostaglandin-like material involved, but the parallel bioassay by the three blood-bathed assay tissues used is specific for prostaglandins of the E and F series. Furthermore, the fact that both basal and stimulated output were abolished by indomethacin administration reinforces the conclusion that the spasmogen was either a prostaglandin or a mixture of prostaglandins. McGiff et al. (1) also used the blood-bathed organ technique to demonstrate release of prostaglandin-like substances into renal venous blood during reductions in renal blood flow in the dog. After extraction and thin-layer chromatography, they characterized the material as a mixture of predominantly prostaglandin E₂ and F₂α. Our results are compatible with this conclusion.

If rapid biosynthesis and release of a prostaglandin accounts for renal autoregulation, what are the mechanisms involved? About 90% of renal blood flow is through the cortical region, and autoregulation takes place primarily in the preglomerular resistance vessels (26). However, most, if not all, of the prostaglandin-synthesizing enzyme is located in the renal medulla (30-33). Thus, unless the small amount of prostaglandin synthetase which has been found in the cortex (34) can account for renal autoregulation, some system must exist which transports prostaglandins generated in the medulla to the cortex, where they can reduce the preglomerular resistance. In developing the hypothesis that a prostaglandin may be the natriuretic hormone which controls sodium balance, Lee (35) suggested that the prostaglandin may be transported intrarenally from medulla to cortex via the vasa recta. Presumably, the prostaglandin would enter and leave the blood in the vasa recta by diffusion. In this context, it is interesting that the venous limbs of the vasa recta penetrate deep into the cortex (A. C. Barger, personal communication) and that in another organ, the uterus, a counter-current transport of prostaglandins from vein to artery has been demonstrated (36).

Another transport system might be the urine. Prostaglandin generated in the medulla may travel to the cortex via the ascending limb of the loop of Henle from whence it could reach the afferent arteriole. There is, as yet, little evidence for such a mechanism except that prostaglandins have been detected in urine (37). Certainly, the hypothesis that the rapid biosynthesis and release of one substance (a prostaglandin) can regulate sodium excretion (35) and renal blood flow is an attractive one, for it fits with the conclusion (26) that renal autoregulation is primarily a phenomenon to keep the sodium load constant. Prostaglandin synthetase is contained in cells lining the collecting tubules (32), so the enzyme is well situated to sense the amount of sodium leaving the body.

The intimate mechanism by which prostaglandin synthesis in the kidney is controlled is unknown. In other tissues, distortion of the cell membrane leads to prostaglandin generation; such distortion can be induced by chemical, pathological, mechanical, or even physiological stimuli (29). Slight damage induced by touch or pressure produces prostaglandin synthesis and release (29), and it could be that
PROSTAGLANDINS AND RENAL AUTOREGULATION

the enzyme is also sensitive to changes in the cell membrane induced by osmotic forces.

If it is accepted that prostaglandin generation contributes to autoregulation of renal blood flow, it follows that other mechanisms which have been proposed, such as formation of angiotensin II, have little or nothing to do with autoregulation over the pressure ranges studied, unless prostaglandin release is one link in the chain of events which leads to renin release. Vander (38) was unable to demonstrate renin release by prostaglandin E1 or E2. Our results, in which a converting enzyme inhibitor failed to modify renal autoregulation, also mitigate against angiotensin II being involved in autoregulation. The characteristics of the renin-angiotensin system including the long half-life (> 20 minutes) of renin in the circulation (39) and the activation of angiotensin I in the lungs (38, 40) all suggest that angiotensin II is a circulating rather than a local hormone. On the other hand, the characteristics of generation of the E type of prostaglandins (29, 41) suggest that these substances are local hormones which affect nearby cells and are inactivated by the lungs (42) before reaching the arterial circulation.

There is one other point worthy of comment. McGiff et al. (1) noted that they could not demonstrate prostaglandin release during renal ischemia in one hypertensive dog. In our series of experiments, there were two dogs which failed to autoregulate blood flow and also to release prostaglandins; both of these dogs were also hypertensive. These exceptions add force to the idea that a lack of renal prostaglandins may be involved in the genesis of hypertension (35).

Acknowledgment

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References


Contribution of Intrarenal Generation of Prostaglandin to Autoregulation of Renal Blood Flow in the Dog
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