Release of a Prostaglandin E-Like Substance from Canine Kidney by Bradykinin

COMPARISON WITH ELEDOISIN

By John C. McGiff, Norberto A. Terragno, K. U. Malik, and Andrew J. Lonigro

ABSTRACT

Renal vasodilation produced by two dissimilar vasodepressor polypeptides, bradykinin and eledoisin, was correlated with changes in renal venous concentrations of substances having the properties of prostaglandins of the E and F series in anesthetized dogs. Samples of renal venous blood were extracted for acidic lipids, and the prostaglandin E and prostaglandin F zones of the chromatographed extracts were eluted and assayed in vitro for prostaglandins of the E and F series by a parallel bioassay system (sensitivity 0.015 ng/ml blood). During the first 2 minutes of infusion, bradykinin increased the concentration of a prostaglandin E-like substance in renal venous blood from a mean control level of 0.16 ng/ml to 1.05 ng/ml (P <0.01); this increase occurred simultaneously with the greatest increase in renal blood flow to 432 ml/min from a control value of 282 ml/min. After 12 minutes of bradykinin infusion, the concentration of the prostaglandin E-like substance had decreased to 0.30 ng/ml, and renal blood flow had fallen to 398 ml/min. In contrast, eledoisin infused in equipotential doses did not increase the concentration of the prostaglandin E-like substance. The concentration of prostaglandin F-like substances was not affected by either polypeptide. A transient increase in urine flow occurred during the first 2 minutes of bradykinin infusion only. These results suggest that a prostaglandin E-like substance participates in the renal vasodilator and the diuretic responses to bradykinin.

KEY WORDS - renal vasodilation, renal prostaglandins, bioassay, tissue hormones, renal lipids, vasodilator polypeptides

Vasodilator substances differ not only in their capacity to increase blood flow to an organ but also in their effects on vascular elements within an organ. Thus, the threshold dose of bradykinin which increases renal blood flow is similar to that dose which increases femoral blood flow (1). In contrast, the threshold dose of isoproterenol which increases femoral blood flow is without effect on renal blood flow (1). Furthermore, bradykinin constricts veins (2), but isoproterenol usually dilates them (3). For the renal circulation, vasodilator agents may be further distinguished by their differential effects on the regional redistribution of blood flow within the kidney (4). In this paper we describe an additional differential action of vasodilator agents: their effect on release of prostaglandins from the canine kidney is examined. It has been proposed that prostaglandins function in an intrarenal regulatory system (5). We related the renal activity of two chemically unrelated vasodilator polypeptides, the nonapeptide bradykinin and the endecapeptide eledoisin, to changes in the

From the Department of Pharmacology, Section of Clinical Pharmacology, The Medical College of Wisconsin, Milwaukee, Wisconsin 53233.

This work was supported in part by U. S. Public Health Service Grant HE 13624, American Heart Association Grant 70-775, and by the Missouri Heart Association.

Dr. McGiff is a Burroughs Wellcome Fund Scholar in Clinical Pharmacology.

Received November 8, 1971. Accepted for publication April 24, 1972.

Circulation Research, Vol. XXXI, July 1972
concentration of prostaglandins of the E (PGE) and F (PGF) series in renal venous blood. Bradykinin increased renal blood flow and urine flow; these changes coincided with an increase in the concentration in renal venous blood of a substance which was indistinguishable from a prostaglandin of the E series (PGE-like) by physicochemical and biological criteria. In contrast, equidilator doses of eledoisin did not affect the concentrations of prostaglandins of either the E or the F series in renal venous blood.

**Methods**

Eight mongrel dogs, weighing 22-31 kg, were fasted for 12 hours prior to the experiment but allowed free access to water. They were anesthetized with morphine sulfate (2 mg/kg, sc) and chloralose (100 mg/kg, iv). To maintain anesthesia, chloralose (40 mg/kg, iv) was given every 3-4 hours. The trachea was cannulated, and the lungs were ventilated mechanically. The renal arteries, the ureters, and the right renal vein were isolated through a transabdominal incision. Two eight-channel direct writers (Hewlett-Packard, models 7720 and 7718) recorded: (1) mean aortic blood pressure measured by a Statham transducer (model P23Db) via a catheter inserted in a retrograde direction into a femoral artery, (2) renal blood flow measured by electromagnetic flowmeters (Statham, model M-4001), (3) urine drops counted by a Grass photoelectric transducer (PTTI), and (4) changes in length of assay organs measured by isotonic transducers (Harvard Apparatus, model 358).

In six experiments, bradykinin (20-100 ng kg⁻¹ min⁻¹) and eledoisin (37.5-150 ng kg⁻¹ min⁻¹) were infused alternately into the renal artery for 20 minutes at a rate which increased renal blood flow by at least 30% without affecting aortic blood pressure. The rate of infusion of eledoisin was limited by its hypotensive effect, which attenuated or abolished its renal vasodilator action. Hypotension occurred invariably when the rate of administration of eledoisin exceeded 150 ng kg⁻¹ min⁻¹. Unlike bradykinin, eledoisin is neither inactivated in blood nor removed by the lungs (6). Changes in renal blood flow and in concentrations of PGE- or PGF-like substances in renal venous blood were unaffected by the sequence of administration of the polypeptides. Intervals of 30 minutes were allowed between infusions of either polypeptide. In two additional experiments, only bradykinin was infused. Bradykinin and eledoisin were infused into the renal artery by a Braun continuous-infusion apparatus (model Unita 1) in appropriate concentrations so that the rate did not exceed 1 ml/min. A sustaining infusion of 0.9% saline was administered intravenously at a rate of 4-6 ml/min. Heparin (1500 IU/kg, iv) was given just prior to superfusing (streaming of fluid over the assay organs) the assay tissues with blood. In five of the eight experiments, rates of excretion of sodium from the experimental kidney were determined for three periods before and two periods, 10 minutes in duration each, during infusion of bradykinin into the renal artery. Urinary sodium concentration was determined by flame photometry.

In nine and six additional experiments PGE₂ (4 ng kg⁻¹ min⁻¹) and PGF₂α (40 ng kg⁻¹ min⁻¹), respectively, were infused into the renal artery for 5-10 minutes, and their effects on renal blood flow and urine flow were related to the concentration of either prostaglandin in renal arterial blood. We had determined in preliminary experiments that PGF₂α infused at the same rate as PGE₂ was without effect on renal blood flow.

We have reported the adaptation of the blood-bathed organ technique of Vane (7) to the continuous assay of renal venous effluent (8). In brief, three assay organs, rat stomach strip, rat colon, and chick rectum, were superfused in series by renal venous blood withdrawn by a pump at 15 ml/min and returned to the dog via the left jugular vein. The in vivo assay system, in addition to continuously assaying changes in the concentration of prostaglandin-like substances in renal venous blood, was used to determine the optimal time for collection of renal venous blood. These samples of blood were then assayed for concentrations of PGE₂ and PGF₂α substances by the same assay organs in vitro after the acidic lipids had been extracted and the extracts chromatographed.

For example, the first collection of blood during bradykinin infusion was prompted by the initial large contraction of the assay organs coincident with the peak increase in renal blood flow (Fig. 1). The unsustained contraction of the assay organs during bradykinin infusion suggested a waning of the concentration of prostaglandin-like substances in renal venous blood. These estimates of changes in concentrations of prostaglandins provided by continuous monitoring of renal venous blood in vivo were confirmed by the results of the in vitro assay: an initial severalfold increase in the concentration of a PGE-like substance was observed, but it was not sustained (Fig. 2). Thus, based on the information provided by the in vivo assay system, venous blood (100 ml) was collected in ethanol before and during infusion of bradykinin into the renal

*Circulation Research, Vol. XXXI, July 1972*
FIGURE 1

Effects of bradykinin (BK), infused for 20 minutes into the renal artery (IRA), on urine flow, mean aortic blood pressure (BP), renal blood flow, and assay organs (rat stomach strip [RSS]; rat colon [RC], and chick rectum [CR] superfused by venous blood of the same kidney in a chloralose-anesthetized dog. Every tenth drop is marked by the decade counter. Contraction of the assay organs produced by infusion of bradykinin into the renal artery (lower left) was matched by a PGE standard but not by bradykinin infused directly into the extracorporeal circuit (IBB), thereby bypassing the kidney (matching infusions, lower right). The times that renal venous blood was collected for assay are indicated by the black bars. Concentrations of PGE- and PGF-like substances in the purified extracts of renal venous blood were determined by bracket assay as shown (upper right) for an eluate of the PGF zone of the thin-layer plate. TLC = thin-layer chromatography.
Summary of effects (means ± se) of bradykinin and eledoisin, infused for 20 minutes into the renal artery (IRA), on concentrations of prostaglandin E- ("PGE") and F- ("PGF") like substances in renal venous blood, on renal blood flow, and on mean aortic blood pressure (BP). The boundaries of the standard error for all values are indicated by the broken lines. Bradykinin, 2 minutes after the onset of infusion (early) produced a sixfold increase in the concentration of "PGE" in renal venous blood and a 53% increase in renal blood flow. Twelve minutes after the onset of infusion (late), the concentration of "PGE" and renal blood flow had decreased, although they remained significantly greater than control values. Mean aortic blood pressure and the concentration of "PGF" were not affected by infusion of bradykinin. Eledoisin, infused at rates which increased renal blood flow comparably to bradykinin, did not change concentrations of "PGE" or "PGF" in renal venous blood.

alters of their biological activities over a period of 3 months.

Changes in renal blood flow and in the concentrations of PGE- and PGF-like substances were tested for statistical significance by the t-test based on paired observations or by the sign test. A P value of 0.05 or less was considered statistically significant. Statistical analyses were performed according to methods described by Steel and Torrie (11).

Results

Effects of Bradykinin

Bradykinin increased mean renal blood flow initially by 150 ml/min from a control value of 282 ml/min (P < 0.01) without affecting aortic blood pressure (Fig. 2). The concentration of a PGE-like substance was invariably increased severalfold from a control value of 0.16 ± 0.04 (se) ng/ml 2 minutes after starting the infusion of bradykinin. The mean increase was 0.89 ± 0.16 ng/ml and the range was 0.25 ng/ml to 1.02 ng/ml (P < 0.01) (Fig. 2). During bradykinin infusion, the peak concentrations of PGE- and PGF-like substances in renal venous effluent at 2 minutes corresponded in time to the greatest increase in renal blood flow and the maximal activity of the assay tissues superfused directly with renal venous blood. Thus, in Figure 1, the bradykinin-induced increase in renal blood flow of 193 ml/min at 2 minutes coincided with the peak contraction of the assay tissues superfused with renal venous blood. Twelve minutes after starting the bradykinin infusion,
renal blood flow had fallen from its highest value of 523 ml/min to 482 ml/min, and the assay tissues had returned to their control state. These changes in the activity of the assay tissues in vivo produced by bradykinin corresponded to the concentration of a PGE-like substance in renal venous blood determined on samples obtained during infusion of bradykinin: an elevenfold increase above control at 2 minutes fell to levels less than twofold above the control value at 12 minutes. During the first 1-2 minutes of bradykinin infusion, a transient increase in urine flow occurred simultaneously with the phase of the most rapid increase in renal blood flow (Fig. 3). However, this initial transient increase in urine flow in response to bradykinin was not reflected by changes in either urine volume or sodium excretion determined before and during infusion of bradykinin. Thus, the mean control values for urine volume and sodium excretion for the experimental kidney were 0.27 ml/min and 19 µEq/min, respectively, and the corresponding values for the same kidney during infusion of bradykinin were 0.29 ml/min and 23 µEq/min ($P > 0.1$ for changes in urine volume and sodium excretion). Bradykinin has been reported to produce a sustained diuresis (12). Failure to do so under these experimental conditions is presumably related, in part, to the anesthetic agent, chloralose, since changes in urine flow and sodium excretion in response to bradykinin were reported to be variable in dogs anesthetized with chloralose (13).

Renal blood flow 12 minutes after starting bradykinin infusion had decreased to a mean value of 398 ml/min from the peak value of 432 ml/min, and the simultaneously determined renal venous concentration of the PGE-like substance had decreased to a mean of 0.30 ± 0.06 ng/ml (Fig. 2). The latter, although significantly less ($P < 0.01$) than the mean concentration of 1.05 ng/ml determined at the time of the peak value of renal blood flow, was significantly greater than the control level of 0.16 ng/ml ($P < 0.05$). The concentration of a PGF-like substance in renal venous blood did not change significantly during bradykinin infusion. Within 7 minutes after stopping bradykinin infusion, mean renal blood flow returned to 274 ml/min; this value was not significantly different from the control renal blood flow. In five experiments the concentrations of PGE- and PGF-like substances determined within 30 minutes after stopping bradykinin infusion were similar to control values; for the PGE-like substances control and recovery values were 0.18 ± 0.05 ng/ml and 0.15 ± 0.07 ng/ml, respectively, and for the PGF-like substances the corresponding values were 0.06 ± 0.03 ng/ml and 0.1 ± 0.04 ng/ml.

EFFECTS OF ELEDOSIN

Eledoisin increased mean renal blood flow to 404 ml/min from a control value of 297 ml/min ($P < 0.01$) without significantly affecting systemic blood pressure. However, the range of this increase in renal blood flow was 48 ml/min to 193 ml/min. Eledoisin, unlike bradykinin, produced neither an initial peak in renal blood flow nor an increase in urine flow during the first 2 minutes of its infusion. Concentrations of either a PGE- or a PGF-like substance in renal venous blood did not change during infusion of eledoisin (Fig. 2). Renal blood flow returned to control levels within 5 minutes after stopping eledoisin infusion.

EFFECTS OF PGE$_2$ AND PGF$_{2a}$

In nine experiments PGE$_2$ infused into the renal artery at a rate of 4 ng kg$^{-1}$ min$^{-1}$, resulting in concentrations in renal arterial blood ranging from 0.21 ng/ml to 1.06 ng/ml (mean 0.53 ng/ml), increased renal blood flow by 35% of control and urine drops by two- to threefold and did not affect systemic blood pressure (Table 1). PGF$_{2a}$ infused into the renal artery at a rate of 40 ng kg$^{-1}$ min$^{-1}$, resulting in concentrations in renal arterial blood as high as 10 ng/ml, did not affect renal blood flow, urine flow, or mean aortic blood pressure.

Discussion

Increased concentration of a PGE-like substance in renal venous blood in response to
TABLE 1

Effects of PGE₂ (4 ng kg⁻¹ min⁻¹) and PGF₂α (50 ng kg⁻¹ min⁻¹) Infused into the Renal Artery on Blood Pressure, Renal Blood Flow, and Urine Flow

<table>
<thead>
<tr>
<th></th>
<th>MABP (mm Hg)</th>
<th>RBF (ml/min)</th>
<th>UF (drops/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>105 ± 3</td>
<td>230 ± 14</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>PGE₂</td>
<td>105 ± 3</td>
<td>306 ± 18</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>Probability levels</td>
<td>NS</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Control</td>
<td>111 ± 5</td>
<td>182 ± 12</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>111 ± 5</td>
<td>181 ± 13</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Probability levels</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

The values are means ± se; for renal blood flow and urine flow they refer to the experimental kidney only. MABP = mean aortic blood pressure; RBF = renal blood flow; UF = urine flow. Statistical analyses were made using the paired t-test for experimental (prostaglandin) and control periods. NS indicates no statistically significant difference.

**BRADYKININ, ELEDOSIN, AND PROSTAGLANDINS**

However, before this proposal merits serious consideration, several questions should be considered. First, can changes in renal blood flow of the magnitude occurring in these experiments be mediated, even partially, by a substance which is synthesized or stored, or both, exclusively in the renal medulla? Second, are the activities of renal prostaglandins restricted to the renal medulla? The assumption on which these two questions are based—the exclusion of prostaglandins from the renal cortex in terms of activity, synthesis, or storage—challenges biochemical and histochemical evidence which assigns the major activity of the enzyme responsible for the degradation of prostaglandins, a 15-hydroxydehydrogenase, to the renal cortex (14, 15). The activity of this enzyme in the kidney surpasses that in the lung, the site of the major biological inactivation of circulating prostaglandins (7). Pursuant to this finding, Ånggård et al. (14) have urged a reconsideration of the evidence that the synthesis and site of activity of prostaglandins is restricted to the renal medulla. Third, is the identification of the substance appearing in renal venous blood dependent on relatively nonspecific methods? This question overlooks our procedures and criteria for characterization of the substance appearing in the venous effluent in response to bradykinin. Changes in the concentration of prostaglandin-like substances as determined by the in vivo assay system corresponded to those values obtained in vitro on samples subjected to preliminary extraction and biochemical purification. Extraction eliminated unwanted substances, primarily amines and polypeptides, which might have interfered with the detection of prostaglandins in the in vivo assay system. Thin-layer chromatography resulted in the exclusion of all acidic lipids except those having the mobility characteristics of prostaglandins of either the E or F series, and the substances recovered from the E and F zones affected the assay tissues in the same way as did bradykinin in vivo. The application of this highly sensitive and specific bioassay system to the detection of prostaglandins in venous effluents has received...
abundant verification by Vane and his associates (7, 16). Similarly, Horton (17) has observed that identification of prostaglandins on the basis of their biological effects and chromatographic behavior, although tentative, is highly suggestive. Inasmuch as we were dealing with small quantities of these substances, the use of procedures which depended on rigorous chemical identification was precluded.

We suggest that the PGE-like substance is probably PGE$_2$, the major renal prostaglandin, since we have previously characterized the PGE-like substance released during renal ischemia as PGE$_2$ (8). Furthermore, infusion of authentic PGE$_2$, at a rate which produced concentrations in renal blood comparable to those of the PGE-like substance occurring in response to bradykinin, produced similar increments in renal blood flow (Table 1) (10, 18). The failure of bradykinin to increase the concentration of a PGF-like substance is consistent with our proposal that the release of PGE$_2$, a vasodilator, by bradykinin is related to the renovascular action of the kinin; PGF$_2\alpha$, which is present in the renal medulla in concentrations only slightly less than those of PGE$_2$ (19), possesses pressor properties (20) and is without effect on renal blood flow (Table 1). Heretofore, only ischemic or pressor stimuli have been shown to release renal prostaglandins (5, 8, 21). The failure of another polypeptide, eledoisin, given in equidilator doses to increase the concentration of either PGE- or PGF-like substances suggests the specificity of this relationship for bradykinin and challenges the hypothesis that prostaglandins subserve a general mechanism as local mediators of blood flow for vasoactive agents. Collier et al. (22, 23) have suggested that the vascular activity of bradykinin appears to be dependent, at least in part, on a mediator(s), since aspirin reduces the duration of the vasodepressor action of bradykinin. The recent demonstration by Ferreira et al. (16) that aspirin inhibits the synthesis of prostaglandins suggests that the putative mediator of the vasodepressor activity of bradykinin is a prostaglandin. Some of the seemingly paradoxical actions of bradykinin such as vasoconstriction in vitro and vasodilation and venoconstriction in vivo might be comprehensible, if they resulted from a mixture of direct effects and secondary effects related to release of an intermediary (24). The latter is not without precedent, since bradykinin releases catecholamines and vasopressin (25, 26). Although the renal vasodilator action may be partially mediated by a prostaglandin of the E series or a substance very similar to it, we cannot exclude a complementary or even antagonistic direct effect of bradykinin.

Acknowledgment

We thank Mrs. S. A. Kraemer for her help. We are grateful to the Department of Biostatistics (Dr. Alfred A. Rimm, Director) for assistance. We thank Dr. J. E. Pike of Upjohn for prostaglandins and Dr. J. H. Trapold of Sandoz Pharmaceuticals for bradykinin and eledoisin. We thank the Upjohn Company for its generosity in partially supporting this investigation.

References

8. MCGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A., LONIGRO, A.J., STRAND, J.C., WILLIAMSON,
BRADYKININ, ELEDOISIN, AND PROSTAGLANDINS


Release of a Prostaglandin E-Like Substance from Canine Kidney by Bradykinin:
COMPARISON WITH ELEDOISIN
John C. McGiff, Norberto A. Terragno, K. U. Malik and Andrew J. Lonigro

Circ Res. 1972;31:36-43
doi: 10.1161/01.RES.31.1.36
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1972 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/31/1/36

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/