Prostaglandin-Like Substances Appearing In Canine Renal Venous Blood During Renal Ischemia

Their Partial Characterization by Pharmacologic and Chromatographic Procedures

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ABSTRACT

Renal prostaglandins (PGs) might mediate an antihypertensive function of the kidney. The blood-superfused organ technique possesses the sensitivity (threshold < 0.4 ng/ml blood) and specificity required for identification of PGs in blood. Induction of unilateral renal ischemia in 14 chloralose-anesthetized dogs reduced renal blood flows from a mean value of 257 to 109 ml/min on the ischemic side and from 250 to 209 ml/min on the contralateral side. Concomitantly, PG-like substances were detected by assay organs in the venous blood of ischemic (13 experiments) and contralateral (11 experiments) kidneys. In one experiment, in a spontaneously hypertensive dog, PGs were not detected during renal ischemia.

Renal venous blood and renal medullary tissue were extracted for acidic lipids and assayed for PG-like substances. Extracts of venous blood collected during renal ischemia and extracts of renal medulla yielded substances with biological activity indistinguishable from PG-like substances or PG standards. Chromatographic characterization of PG-like substances suggests that they are predominantly a mixture of PGE$_2$ and PGF$_2$α.

ADDITIONAL KEY WORDS antihypertensive function of kidney angiotensin II blood-superfused organ technique renal medulla acidic lipids renal prostaglandins renal blood flows renal vascular hypertension

An antihypertensive function of the kidney was suggested by Fasciolo who reported that sustained hypertension in the dog required removal of the kidney contralateral to the kidney with its artery clipped (1). In normotensive animals, bilateral nephrectomy produces hypertension (renoprival) (2) which is allayed by renal transplantation or administration of renal extracts (3-5). Grollman proposed that the antihypertensive or protective action of the kidney resulted from a hormone of renal origin (6). Of those substances having antihypertensive properties which have been found in the kidney (7-9),

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only the PGs have been characterized chemically (10). Prostaglandins E₂ (PGE₂) and A₂ (PGA₂), which are found in renal medulla (11), are potent vasodepressor unsaturated hydroxy-acids having many of the properties of ideal antihypertensive hormones (12). Their relationship to the proposed renal antihypertensive hormone(s) is unknown, primarily because their identification in blood is limited by the sensitivity of available chemical methods (13, 14). An enzymatic analysis which was recently developed to assay PGs (15) is not now applicable to their measurement in blood.

Ferreira and Vane (16) have adapted a bioassay method, the blood-superfused organ technique developed by Gaddum (17), to identify PGs released from an organ into its effluent. We used this method to assay continuously the hormonal content of blood. The streaming of fluid over assay organs is called superfusion. Because this method bypasses conventional procedures for characterization and identification of hormones, selectivity must be achieved by including several different organs in the assay system, i.e., using the technique of parallel pharmacologic assay (18). Each group of assay organs, superfused by blood from a single site, is a bank of organs. By the use of three banks of organs, simultaneous assay of blood from each renal vein and from the aorta permits determination of the generation or disappearance, or both, of blood-borne substances of renal origin having activity on the assay organs. For example, during renal ischemia, the presence of PG-like substances in renal venous blood and their absence in aortic blood suggests their disappearance on passage through the heart or lungs.

To verify the tentative identification by the bioassay system of substances having the properties of PGs and to obtain biochemical characterization of PG-like substances, renal venous blood was removed at the time these substances were detected by the assay organs. At each stage of extraction and biochemical purification, samples were compared to PGE₂ and prostaglandin F₂α (PGF₂α) standards for their effects on assay tissues. Thus, a reference assay of high specificity was used during characterization of PG-like substances to check the reliability of our biochemical procedures for recovery of substances from the blood tentatively identified as PGs by the bioassay system. Finally, we recovered from the renal medulla substances having the same properties as PG-like substances extracted from renal venous blood.

Methods

Fourteen male mongrel dogs weighing 23 to 32 kg were anesthetized with morphine sulfate (2 mg/kg, sc) and chloralose (100 mg/kg, iv). Anesthesia was maintained with chloralose (50 mg/kg, iv). The trachea was cannulated and the lungs were ventilated mechanically. The renal arteries were isolated through a transabdominal incision. Two eight-channel direct writers (Hewlett-Packard, models 7720, 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 flows measured by electromagnetic flowmeters (Statham, model M-4001); and (3) changes in length of assay organs detected by isotonic transducers (Harvard Apparatus, model 356). We have verified the method for recording blood flow and obtaining zero flow (19). The renal artery was constricted by a screw clamp placed distal to the flowmeter sensor. The clamp was tightened without displacing the Hood vessel from its bed or placing traction upon it. Plasma renin activity of thoracic caval Hood was measured by the method of Pickens et al. (20) and is reported in nanograms (ng) of angiotensin II per 100 ml of plasma, generated during a 4-hour incubation (ng/100 ml plasma).

Blood-Superfused Organ Technique

Three banks of assay organs were arranged in parallel (Fig. 1). Each bank of organs consisted of three tissues each: rat stomach strip, rat colon, and chick rectum which were continuously superfused in series by renal venous blood (21-23). This combination of assay organs was shown to possess the specificity and sensitivity required for detection of PGs (16) (Fig. 2). PGE₂ and PGF₂α when injected into the extracorporeal circuit (18B of Fig. 1) produced contractions of all three tissues. For the blood-borne substances which are likely to be present in renal venous effluent, only PGs would elicit the responses expected.
Schematic diagram of blood-bathed organ system (one bank). The blood-perfused organ system schematized for one bank of organs (three banks of organs were used). Blood was withdrawn at the rate of 10 to 15 ml/min by a pump. After traversing a constant-temperature circuit, the blood cascaded over three assay organs arranged in series. Changes in the length of the assay organs were transduced and recorded on a multichannel recorder. The blood was collected in a reservoir and returned at a constant rate to the animal. Selective blockade of assay organs is possible by direct intraluminal application of a blocking agent. A major use of this method is shown by the schematized kidney on the right. Thus, a substance may be given into the renal artery (IRA) and its effects on assay organs compared to its direct effects on administration into the extracorporeal circuit (IBB = into the bathing blood) indicated by "injection site" in the diagram of the assay system. Indirect effects of hormones may thereby be determined, e.g., release of intrarenal substances by angiotensin II.

Vasopressin in very high concentrations was reported to produce patterns of response of the assay organs similar to PGs (18). However, vasopressin, 150 to 200 μU/ml blood, was without effect on our assay organs.

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FIGURE 2
Differential patterns of responses of assay organs to vasoactive substances. Patterns of responses of assay organs to substances which may be found in renal venous blood. The activities of three assay organs, rat stomach strip, rat colon, and chick rectum, selected for their sensitivity and specificity to PCs were recorded simultaneously. Infusions of substances were made directly into Krebs solution which superfused the assay organs.

PGE$_2$ and PGF$_2$a were the only substances which produced contraction of all three assay organs. Activities of three assay organs, rat stomach strip, rat colon, and chick rectum, selected for their sensitivity and specificity to PCs were recorded simultaneously. Infusions of substances were made directly into Krebs solution which superfused the assay organs. PGE$_2$ and PGF$_2$a were the only substances which produced contraction of all three assay organs.

Assay organs, loaded with 1 to 3 g, were suspended in polypropylene chambers from autotonic levers (25) and their contractions transduced and recorded. A polyethylene catheter (o.d., 2.0 mm) was inserted into each renal vein and into the aorta through which blood was withdrawn continuously by a peristaltic pump (Harvard Apparatus, model 1203) at the rate of 10 to 15 ml/min per bank of organs. After superfusing the banks of organs, the blood was returned to the external jugular vein of the dog. The superfusate was maintained at 38°C. No glass or polyvinyl chloride connections were used in the system (26, 27). Dextran was given (10 ml/kg, iv) on establishing the extracorporeal circuit to offset any effects its dead space (150 ml) might have. The assay organs were initially superfused with Krebs solution gassed with 95% oxygen and 5% carbon dioxide. Heparin (1500 IU/kg, iv) was given before superfusing the assay organs with blood. Blood-borne substances, which affected the activity of assay organs, were tentatively identified by infusing standards into the extracorporeal circuit (18B of Fig. 1) to reproduce the activity. Most of the observations were made after 1 to 2 hours of superfusion with blood when the sensitivities of the assay organs to PG standards, initially reduced when blood replaced Krebs solution, had returned to their former state. Estimates of the concentration of PG-like substances in extracts (Fig. 3) were made on the same day on assay organs having stable sensitivities as indicated by their response to successive injections of PG standards. When assay organs were used for comparison on different days (Fig. 4), their sensitivities differed by less than 20% as determined by their response to PGE$_2$ and angiotensin II standards.

RAT BLOOD PRESSURE ASSAY
Sprague-Dawley strain male rats (250 to 300 g) were anesthetized with pentobarbital (50 mg/kg, ip). After tracheotomy and bilateral vagotomy, atropine (1 mg/kg) and pentolinium (20 mg/kg) were given intraperitoneally. Arterial blood pressure was measured by a transducer (Statham, model P23Db) and recorded on a direct writer (Hewlett-Packard, model 7712). Catheters were placed in the vena cava and aorta for injections. The activity of samples obtained from PGE, PGF, and PGA zones of thin-layer chromatography plates was estimated by bracket assay against the changes in rat blood pressure produced by corresponding standards of PGE$_2$, PGF$_{2a}$, and PGA$_2$. Blood pressure, made highly stable by vagotomy and pentolinium, was recorded on an expanded scale; changes greater than 5 mm Hg were considered significant. There was a

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Lipid extracts of canine renal medulla were purified by chromatographic procedures. The initial extract and the eluates at each step of purification were tested for activity on the assay organs superfused by Krebs solution. The responses of assay organs to these samples were then compared to those produced by standard solutions of PGE$_2$ and PGF$_2\alpha$. The amount of recovered PG-like substance(s) at each stage of purification is determined by the amount of the standard solution required to reproduce the response of the sample (either PGF$_2\alpha$ or PGE$_2$ equivalents) after correction for dilution of the sample. Thus, the PGE eluate from the first column contained activity equivalent to 33.0 ng PGE$_2$. The PGF eluate, however, was found to contain both PGE- and PGF-like activity, which were separated by a second column chromatography. This yielded a pure PGF eluate, estimated to contain 25.0 ng PGF$_2\alpha$. A minor eluate was estimated to contain an additional 7.5 ng PGE$_2$ (total PGE$_2$ content, 40.5 ng). In addition, a third compound without activity on the assay organs present in PGA eluates from the column was tested for vasodepressor activity in the pentolinium-treated rat (see Fig. 7). This material contained vasodepressor activity equivalent to 21.0 ng PGA$_2$. TLC = thin-layer chromatography; Equiv. = equivalents; RSS = rat stomach strip; RC = rat colon; CR = chick rectum.

The linear relationship between the amount of PG injected and changes in blood pressure. No more than three unknowns were tested on each rat. The reproducibility of these determinations was ± 15% when performed on different rats using replicate samples of a single fraction.

**PREPARATION OF RENAL MEDULLARY EXTRACT**

Renal medullas were obtained from 30 dogs, which were blood donors for experimental surgery. The kidneys were removed immediately postmortem. Either the medullas were excised at once or the kidneys were fresh frozen and the
Top: Infusion of vasoactive agents over assay organs superfused by Krebs solution reproduced the responses of assay organs superfused by blood from an ischemic kidney. Bottom: Unilateral renal arterial constriction (RAC) was induced at the first arrow and released at the second arrow. Changes in activity of rat colon and rat stomach strip (superfused by renal effluent) which occurred immediately after induction of RAC and the subsequent increasing contractions of rat stomach strip and chick rectum were matched by angiotensin I and PGE,

standards infused simultaneously over assay organs (superfused by Krebs solution) at 2 cm. The peak activity of assay organs elicited by removal of RAC was reproduced by a single injection of PGE,

standard. The assay organ monitoring arterial blood showed increased activity within 8 minutes after induction of renal ischemia which was related to the generation of an angiotensin-like substance. Renal blood flows: L = left, contralateral; R = right, ischemic; aortic BP is mean aortic blood pressure. Vertical scales: 2 cm for assay organ activity; renal blood flows in min/mL; aortic BP in mm Hg. Other abbreviations as in Figure 3.

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Silicic Acid Chromatography: Elution Procedure Used for Separation of Prostaglandin-Like Substances

<table>
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<tr>
<th>Elution sequence</th>
<th>Solvent composition (v/v)</th>
<th>Elution volume (ml)</th>
<th>Type of activity detected</th>
<th>Rat blood pressure assay</th>
<th>TLC Identification</th>
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<tbody>
<tr>
<td>1</td>
<td>Benzene-ethyl acetate (9:1)</td>
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<td>Carboxylic acids</td>
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<td>PGA compounds</td>
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<td>Vasodepressor</td>
<td>PGF-like compounds</td>
</tr>
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<td>Benzene-ethyl acetate (1:9)</td>
<td>200</td>
<td>PGE-like</td>
<td>Vasodepressor</td>
<td>PGE compounds</td>
</tr>
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<td>Methanol</td>
<td>200</td>
<td>PGE-like</td>
<td>Biphasic</td>
<td>PGF and polar compounds</td>
</tr>
</tbody>
</table>

TLC = thin-layer chromatography.

medullas were dissected out within 24 hours. Each kidney was sectioned in a coronal plane and the medulla separated from the cortex by dissection through the corticomedullary junction (approximately 8 to 15 g of medulla was obtained per kidney). Medullary tissue was stored at —10°C until extracted by a method which we have reported (11). This method in brief required homogenization of medullary tissue in ethanol, vacuum filtration of the homogenate for 24 hours, and evaporation of the filtrate which was diluted with water and extracted with ethyl acetate. A concentrate of the latter was extracted with phosphate buffer, from which we subsequently obtained acidic lipids by extraction with chloroform. The chloroform extract was evaporated to dryness, and the residue reconstituted in 10 ml ethanol.

Silicic acid chromatography was performed by the methods previously reported (10, 11) using the same solvent systems as those described by Samuelsson for the purification of PGs in human seminal fluid (28). To minimize losses of PGs during the procedure, the extract was separated on a 20-g silicic acid column and small volumes of solvents were used in the elution sequence (Table 1). The time during which PGs were adsorbed on the silicic acid was relatively short and the eluates were quickly evaporated. These precautions were necessary to minimize formation of artifacts, e.g., PGA₂ from endogenous PGE₂ (10). By these shortened procedures, fractions containing PGs were eluted with more polar solvent mixtures than previously observed (10, 28). However, by following standard procedures, we consistently obtained the reproducible sequence of column separations shown in Table 1.

The column was developed with increasing concentrations of ethyl acetate in benzene as shown in Table 1. Five eluates were collected at a flow rate of 1.0 to 2.0 ml/min. The fractions were evaporated to dryness and dissolved in 1.0 ml ethanol for assay on the isolated, superfused tissues and on rat blood pressure. All fractions were assayed and those with biological activity were stored in 4°C for further assay and thin-layer chromatographic characterization.

Extraction and Characterization of Biologically Active Substances Produced by Renal Ischemia

In three experiments (dogs 1, 2, and 3), during renal ischemia, venous blood was removed from both kidneys at 20 to 30 ml/min per kidney by a roller pump (Watson-Marlow, model MHRE) coincident with the appearance of PG-like substances in renal venous blood as detected by the assay organs. Removal of blood was accompanied by intravenous infusion of a corresponding amount of dextran (dogs 1 and 2) and blood from a donor (dog 3). Control samples (100 to 200 ml) of venous blood were collected for estimation of content of the PG-like substances. After induction of unilateral renal ischemia, 100 ml (dog 1) or 500 ml (dog 2) of venous blood were collected from each kidney. In dog 3, venous blood, removed from the ischemic kidney, was collected in two parts consecutively; one extending for 8 minutes up to and including the release of constriction, the other extending 12 minutes into the period of recovery.

Blood samples were immediately centrifuged (International Equipment Co., model HT) for 15 minutes at 8,000 g at 4°C and the plasma was collected and diluted with four parts of ethanol. The precipitated protein and peptides were separated by centrifuging at 2,000 g at room temperature. The combined supernatant fluids were then evaporated in vacuo. This procedure was modified for dog 3 as follows. The blood samples were immediately centrifuged (International Equipment Co., model HT) for 15 minutes at 8,000 g at 4°C and the plasma was collected and diluted with four parts of ethanol. The precipitated protein and peptides were separated by centrifuging at 2,000 g at room temperature. The combined supernatant fluids were then evaporated in vacuo. This procedure was modified for dogs 1 and 2 as follows. Blood samples were immediately centrifuged (International Equipment Co., model HT) for 15 minutes at 8,000 g at 4°C and the plasma was collected and diluted with four parts of ethanol. The precipitated protein and peptides were separated by centrifuging at 2,000 g at room temperature. The combined supernatant fluids were then evaporated in vacuo. This procedure was modified for dog 3 as follows. The blood samples were immediately centrifuged (International Equipment Co., model HT) for 15 minutes at 8,000 g at 4°C and the plasma was collected and diluted with four parts of ethanol. The precipitated protein and peptides were separated by centrifuging at 2,000 g at room temperature. The combined supernatant fluids were then evaporated in vacuo. This procedure was modified for dogs 1 and 2 as follows. Blood samples were immediately centrifuged (International Equipment Co., model HT) for 15 minutes at 8,000 g at 4°C and the plasma was collected and diluted with four parts of ethanol. The precipitated protein and peptides were separated by centrifuging at 2,000 g at room temperature. The combined supernatant fluids were then evaporated in vacuo. This procedure was modified for dog 3 as follows. Blood samples were immediately centrifuged (International Equipment Co., model HT) for 15 minutes at 8,000 g at 4°C and the plasma was collected and diluted with four parts of ethanol. The precipitated protein and peptides were separated by centrifuging at 2,000 g at room temperature. The combined supernatant fluids were then evaporated in vacuo. This procedure was modified for dogs 1 and 2 as follows. Blood samples were immediately centrifuged (International Equipment Co., model HT) for 15 minutes at 8,000 g at 4°C and the plasma was collected and diluted with four parts of ethanol. The precipitated protein and peptides were separated by centrifuging at 2,000 g at room temperature. The combined supernatant fluids were then evaporated in vacuo. This procedure was modified for dog 3 as follows. Blood samples were immediately centrifuged (International Equipment Co., model HT) for 15 minutes at 8,000 g at 4°C and the plasma was collected and diluted with four parts of ethanol. The precipitated protein and peptides were separated by centrifuging at 2,000 g at room temperature.

EXTRACTION AND CHARACTERIZATION OF BIOLOGICALLY ACTIVE SUBSTANCES PRODUCED BY RENAL ISCHEMIA

In three experiments (dogs 1, 2, and 3), during renal ischemia, venous blood was removed from both kidneys at 20 to 30 ml/min per kidney by a roller pump (Watson-Marlow, model MHRE) coincident with the appearance of PG-like substances in renal venous blood as detected by the assay organs. Removal of blood was accompanied by intravenous infusion of a corresponding amount of dextran (dogs 1 and 2) and blood from a donor (dog 3). Control samples (100 to 200 ml) of venous blood were collected for estimation of content of the PG-like substances.
water and then evaporated to dryness. The residue was dissolved in a known volume of ethanol for biological testing and chromatographic purification.

Aliquots of the final ethanolic extract were emulsified in saline using ultrasound (Heat Systems Co., model W Branson Sonifier). The maximum permitted concentration of ethanol was 20%, but in most cases the final concentration was less than 10%. Sonification yielded an emulsion which was suitable for injection into the banks of assay organs and into rats for blood pressure assay. Similar extraction procedures have been reported to recover from 5 to 55% of PGs (29) and to eliminate all other substances known to contract the assay organs leaving only acidic and neutral lipids (30). PGs recovered by the extraction procedure were detected if their concentrations in the final dilution of the extract were greater than 10 ng/ml. Responses of the assay organs bathed in Krebs solution to the unknown solutions and to the PG standards were compared at two or more dosages. A dose-response curve for the most sensitive assay organ was obtained in each experiment. For PGE2, this was rat colon; for PGE2, rat stomach. The concentration of PG-like substances in the sample was verified by bracket assay as well as by relating its effect on the recorded lengths of assay organ to the dose-response curve obtained from PGE2 or PGE2 standards. Control solutions of identical composition (ethanol in 0.9% saline) and volume were without effect on the assay organs.

The active extracts were dissolved in 30 ml of benzene-ethyl acetate (9:1, v/v) and purified by silicic acid column chromatography by the method described previously (14). The extracts were applied to the top of the column and fractions (500 ml) were collected by stepwise elution with increasing concentrations of ethyl acetate in benzene (Table 1) at a flow rate of approximately 1.0 ml/min. Each fraction was evaporated, dissolved in ethanol, and aliquots assayed for effect on rat blood pressure and for activity on the assay organs bathed in Krebs solution. Column chromatography of extracts from dog 3 was omitted and purification was achieved by thin-layer chromatography as described below.

**Thin-Layer Chromatography**

Thin-layer chromatography was performed by the methods described in detail by Green and Samelson (31), modified as described previously (32). AgNO3 was incorporated into the adsorbent (AgNO3/adsorbent ratio 1:30, w/w) for further separation of individual PGs. Placing...
RENAL PROSTAGLANDIN-LIKE SUBSTANCES

**Detection by continuous bioassay of substance(s) appearing in renal venous blood following induction of renal ischemia in a chloralose-anesthetized dog.** Constriction of the right renal artery (RAC) was induced at the first arrow and released at the second arrow. The response of the assay organs superfused by venous blood of the ischemic kidney was reproduced by PGE₂ standard given into the extracorporeal circuit (IBB) 20 minutes after release of RAC. Vertical scales: 2 cm for assay organ activity; renal blood flows in ml/min; aortic BP in mm Hg. Abbreviations as in Figures 3 and 4.

**STATISTICAL METHODS**

Statistical analyses, using the method of matched pairs, of changes in renal blood flow and mean aortic blood pressure during and following renal ischemia were performed as described by Steel and Torrie (33). A P value of 0.05 or less was considered statistically significant.

**DRUGS**

Drugs used were: angiotensin I (iso-leucine), Schwartz/Mann, Orangeburg, N. Y.) and angiotensin II amide (CPA, Summit, N. J.); prostaglandins E₂ (PGE₂), F₂α (PGF₂α) and A₂ (PGA₂) and propranolol (Ayerst Laboratories Inc., New York, N. Y.). Prostaglandins E₂, F₂α and A₂ were crystalline samples and were prepared as 1 mg/ml stock solutions in 95% ethanol and diluted before use with 0.9% saline so that volumes added to the extracorporeal circuit of the banks of organs did not exceed 0.1 ml.

**Results**

**RENAL BLOOD FLOWS AND ARTERIAL BLOOD PRESSURE**

Table 2 shows the mean values for renal blood flow and mean aortic blood pressure obtained during control periods, immediately after induction of renal artery constriction (28 minutes mean duration), immediately before removal of constriction, and during recovery. The control renal blood flow was 4.5 ml/min/g of kidney.

**Response of the Assay Organs to Renal Ischemia**

During unilateral renal ischemia, in 13 of 14 experiments, PG-like substances appeared in...
Left: Detection by continuous bioassay of substance(s) appearing in the venous blood of the contralateral kidney of a chloralose-anesthetized dog in response to unilateral renal arterial constriction (RAC) induced at the first arrow. Assay organs, superfused by the effluent of the contralateral kidney, demonstrated increased activity several minutes after induction of RAC. After release of RAC, infusion of angiotensin II into the artery (IRA) of the same kidney resulted in activity of the assay organs similar to that observed during renal ischemia.

Right: In contrast, angiotensin II, infused directly over assay organs, produced significant activity on only one assay organ, rat colon (RC). PG-like infusion, however, produced effects on all assay organs similar to that seen during renal ischemia or during infusion of angiotensin II, IRA. These assay organs were superfused by Krebs solution. Abbreviations as in Figure 3.

The earliest change in activity of the assay organs resulting from renal artery constriction was demonstrated by the bank of organs in response to renal ischemia. The control mean aortic blood pressure of 165 mm Hg in this dog was the highest recorded in these experiments.
Changes in length of the assay organs could not be related to alterations in tensions of gases or pH of the blood resulting from renal ischemia, since induction of renal ischemia comparable to that found in Table 2 did not alter PCO₂, Po₂ or pH of renal venous blood.

In Figure 4, plasma renin activity increased from a control value of 333 to 4,033 ng angiotensin 11/100 ml coincident with peak contraction of rat colon. In two additional experiments, plasma renin activity of blood obtained within 10 minutes after induction of renal artery constriction was increased by more than tenfold; viz., from control values of 270 and 293 to 2,707 and 3,180 ng angiotensin 11/100 ml, respectively, coincident with the appearance of an angiotensin-like substance in aortic blood and in effluent of the ischemic kidney as detected by changes in activity of the assay organs.

During the period of renal ischemia, the renal venous blood contained, in addition to renin, a substance that stimulated smooth muscle in doses of 10 ng/100 ml or less. This substance was distinct from angiotensin II, since it failed to elevate blood pressure in pentolinium-treated rats. It did, however, contract muscle strips of guinea pig ileum in a dose-dependent manner, in contrast to angiotensin II, which was inactive in this assay system.

EXTRACTION AND PURIFICATION OF CANINE RENAL MEDULLA

Portions of the initial acidic lipid extract were dissolved in saline and tested for prostaglandin-like activity on the assay organs. The extract appeared to contain a mixture of prostaglandins E and F (Fig. 3). This was confirmed by separating a portion of the acidic lipid extract directly by thin-layer chromatography on silica gel G layers. Two zones from the plate contained smooth muscle stimulating activity. The more polar material had the same chromatographic mobility and biological properties as PGF 2α. The less polar material was found to be indistinguishable from PGE2. Purification of the lipid extract by column chromatography on silicic acid yielded three eluates each having characteristic biological activity (Table 1). Polar material in the methanol eluates had the same chromatographic and biological properties as PGs of the F series. Similarly, column eluates which contained a less polar compound were characterized as a PG of the E series. Finally, a relatively nonpolar PG was present in the benzene-ethyl acetate (2:3, v/v) eluates which did not contract smooth muscle but which was vasodepressor when assayed in the pentolinium-treated rat (Fig. 7). These properties are characteristic of PGs of the A series (13, 34).

None of the previous procedures distinguished PGE2 from PGE1, PGF2α from PGF2β, or PGA2 from PGA1. To separate these compounds which differ only in their degree of unsaturation, the purified extracts containing PG-like material were subjected to further thin-layer chromatography on silver nitrate-impregnated silica gel plates and the distribution of PG-like material was determined by bioassay (Fig. 3). This procedure permitted the tentative identification of PGE2, PGF2α, and PGA2, as the major, if not exclusive, PGs of the E, F, and A series found in canine renal medulla.

Additional biological characterization of the PG fractions was obtained by rat blood pressure assay. The property of PGAs to pass freely across the pulmonary circulation (35), in contrast to PGEs and PGFs (16), was used for its tentative identification. Thus, PGA2 had equal effects on rat blood pressure on either intravenous or intra-arterial injection, whereas PGE2 and PGF2α were without activity on rat blood pressure at these doses on intravenous administration. In contrast, intra-arterial injection of PGE2 and PGF2α which bypassed the lungs allowed demonstration of the vasodepressor action of PGE2 and the pressor action of PGF2α (Fig. 7). We have adapted this property of the rat lung, differential removal of PGs, to obtain additional verification of the presence of a particular PG in purified samples of dog renal medulla. The column eluate which contained PGA-like material was estimated to contain vasodepressor activity equivalent to 21 μg PGA2. Similarly, samples from the PGE column eluates when injected into the rat demonstrated vasodepressor activity which was identical to that of PGE2 standards. This activity was equivalent to 40 μg PGE2. Finally, when the purified PGF eluate was characterized by its pressor effect on rat blood pressure, the activity which was identical to that produced by injections of PGF2α standards (Fig. 7) was equivalent to 18 μg PGF2α. These estimates compare...
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Concentration (ng/ml Blood) of PG-like Substances (Assayed as PGE₂) Extracted from Renal Venous Blood

<table>
<thead>
<tr>
<th>Blood sample</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
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<tr>
<td>Kidney</td>
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<tr>
<td>Ischemic</td>
<td>1.0</td>
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</table>

Indicated concentrations are not corrected for losses incurred by extraction and chromatography. *PGs were not detected (ND) if their concentrations in the final dilution were less than 10 ng/ml of the extract. Volume of blood collected and the method of extraction is described in Methods. In the case of dog 3, the first period refers to blood collected during ischemia and immediately after release (500 ml); collection of blood (550 ml) continued during recovery, is designated 2nd period.

favorably with those estimates obtained by smooth-muscle bioassay; viz., 40.5 μg PGE₂ and 25 μg PGF₂α (Fig. 3).

Characterization of PG-like substances in blood

Renal venous blood removed from ischemic and contralateral kidneys of dogs was extracted to yield crude lipid extracts. Aliquots of these extracts, reconstituted in saline, had biological activity similar to a mixture of PGE₂ and PGF₂α as detected by the response of the assay organs (Table 3). Because of the reported variability of recoveries of PGs from blood (29), we have not corrected the estimated concentrations of PG-like substances for losses incurred during the extraction procedure (Tables 3 and 4).

In an attempt to obtain larger quantities of PG-like substances for further characterization, a total of 1760 ml of renal venous blood was removed terminally from both kidneys in dog 1 during a second period of unilateral renal ischemia with simultaneous dextran replacement. After acidic lipid extraction and silicic acid chromatography using the procedures indicated in Table 1, samples removed

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
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<tbody>
<tr>
<td>TABLE 4</td>
</tr>
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</table>

**TABLE 3**

Concentration (ng/ml Blood) of PG-like Substances (Assayed as PGE₂) Extracted from Renal Venous Blood

<table>
<thead>
<tr>
<th>Blood sample</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic</td>
<td>1.0</td>
<td>0.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Control</td>
<td>ND*</td>
<td>ND*</td>
<td>ND*</td>
</tr>
<tr>
<td>Contralateral</td>
<td>1.0</td>
<td>0.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Indicated concentrations are not corrected for losses incurred by extraction and chromatography. *PGs were not detected (ND) if their concentrations in the final dilution were less than 10 ng/ml of the extract. Volume of blood collected and the method of extraction is described in Methods. In the case of dog 3, the first period refers to blood collected during ischemia and immediately after release (500 ml); collection of blood (550 ml) continued during recovery, is designated 2nd period.

**TABLE 4**

Thin-Layer Chromatographic Purification and Estimation of Prostaglandin-like Substances (Assayed as PGE₂ or PGF₂α) in Extracts from Blood of Dog 3

<table>
<thead>
<tr>
<th>Sample with same mobility as antigenic substance</th>
<th>Total PG-like substances estimated in blood extracts (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Ischemic kidney</td>
</tr>
<tr>
<td>Initial extract*</td>
<td>0.995 PGE₂</td>
</tr>
<tr>
<td>1st TLC: PGE₂ and PGE₂</td>
<td>0.35 PGE₂</td>
</tr>
<tr>
<td>2nd TLC: PGE₂ and PGE₂</td>
<td>0.47 PGE₂</td>
</tr>
<tr>
<td>PGE₂ and PGF₂α</td>
<td>PGE₂ activity present</td>
</tr>
<tr>
<td>AgNO₃ silica gel TLC:</td>
<td>0.53 PGE₂</td>
</tr>
<tr>
<td>PGE₂</td>
<td>ND</td>
</tr>
<tr>
<td>PGE₂α</td>
<td>0.4 PGE₂α</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>0.4 PGE₂α</td>
</tr>
</tbody>
</table>

TLC = thin-layer chromatography. ND = not detected.

*Control blood (200 ml) contained no detectable PG-like activity. See Table 3 for explanation of collection of blood from ischemic and contralateral kidneys. *Extracts were separated on silica gel C plates in solvent system A1 (see Methods). *Extracts of ischemic kidney blood from the 1st period were separated in solvent system A1, due to insufficient separation between PGE₂ and PGF₂α, the PGF material could not be fully characterized. Extracts from the blood of contralateral kidney were separated in system C (see Methods).
Renal prostaglandin-like substances

FIGURE 7

Differential effect on rat blood pressure of three PG standards (PGA₂, PGE₂, PGF₂α) and eluates from argentan thin-layer chromatography (AgNO₃-TLC). Compounds having the chromatographic characteristics of PGA₂, PGE₂, and PGF₂α were tested for their effect on rat blood pressure by intravenous (IV) and intra-ortic (IA) injections. In each case the response was matched by injections of standard solutions of PGs administered in the indicated dose at the arrow. Vertical scales: mean aortic blood pressure in mm Hg.

from the column eluates demonstrated weak but unambiguous PGE₂- and PGF₂-like activity. However, this procedure resulted in considerable loss of biological activity and when acidic lipid extracts obtained from smaller quantities of renal venous blood in dog 2 were separated by silicic acid chromatography, the procedure resulted in complete loss of PG-like activity.

Because of demonstrated low concentrations of PG-like substances in the renal venous blood of dogs 1 and 2 and their poor recovery after column chromatography, the latter procedure was bypassed and lipid extracts of renal venous blood obtained just before release of renal artery constriction in Figure 5 were purified directly by thin-layer chromatography (dog 3). Table 4 shows the results obtained from the successive stages of purification of the extracted renal venous blood obtained during renal ischemia when bioassayed as equivalents of PGE₂ or PGF₂α. Each thin-layer chromatography was performed using one quarter or less of the total extract available. Thus, under the best conditions, we were separating less than 0.25 μg PGE₂-like material present in large quantities of other
PG-like substances isolated from venous blood of the ischemic and contralateral kidneys of dog 3 were purified by argentous thin-layer chromatography (AgNO₃-TLC). The smooth muscle stimulating activity of typical samples of isolated PGE₂-like and PGF₂α-like material are compared with standard solutions of PGE₂ and PGF₂α, respectively. The chromatographic and biological properties of the extracted and purified PG-like substances are indistinguishable from PGE₂ and PGF₂α standards. Abbreviations as in Figure 3.

Discussion
Substances having properties of PGs were released into venous blood of ischemic and contralateral kidneys in response to unilateral renal ischemia. The only exception occurred in a spontaneously hypertensive dog. The proposal that canine kidney releases PGs into venous blood in response to renal ischemia was strengthened by the demonstration that substances having the biological and chromatographic properties of PGs were present in renal medullary extracts. The release of PG-like substances from either kidney may be partially mediated by angiotensin II since its infusion into the renal artery resulted in the appearance of these substances in renal venous blood (Fig. 6). We have reported 

Since complete conversion of angiotensin I to angiotensin II is assumed to occur in the lungs (36), angiotensin I, if it participates in release of PG-like substances, presumably does so only in the ischemic kidney. This suggestion requires experimental confirmation.
RENAL PROSTAGLANDIN-LIKE SUBSTANCES

that arterial blood concentrations of angioten-
sin II of less than 0.1 ng/ml blood released PG-
like substances into renal venous blood (32).
The proposal that angiotensin II might release
renal PG-like substances was also supported by
the observation that their release into the venous
effluent of the contralateral kidney was always
associated with the appearance of angiotensin-
like activity in aortic blood. Furthermore,
increases in plasma renin activity (greater
than tenfold of control) occurred at the time
these substances appeared in the venous blood
of the contralateral kidney.
The blood-bathed organ method used in
this study has the following limitations. (1)
The appearance of several hormones simul-
taneously in response to a stimulus may
obscure the response of the assay organs to
PGs which might be present in renal effluent.
The experiments reported, activity of the
assay organs, not entirely related to the release
of PG-like substances, was accounted for by
the simultaneous appearance of angioten-
sin(s) or catecholamines, or both, with PG-
like substances in renal venous blood. The
most important demonstration that these
responses were due to a mixture of PGs and
angiotensin(s) was provided by infusing
angiotensin I and PGE$_2$ standards (Fig. 4)
directly over the assay organs superfused by
Krebs solution at infusion rates which repro-
duced the original responses. Under these
conditions, other hormones could be excluded
from affecting the responses. Thus, the re-
sponse during renal ischemia could be ac-
counted for entirely by summation of the
effect of angiotensin(s) and PG-like sub-
stances, and occasionally norepinephrine. In
most experiments, the effects of these sub-
stances on the assay organs were reproduced
by PGE$_2$ standards alone (Figs. 5 and 6),
suggesting little modification of the responses
to the PG-like substances by other substances
present in renal venous effluent. (2) Changes
in concentrations of circulating hormones
were appreciated only above those present at
the start of the experiments, i.e., background
levels of hormones could not be detected
(18). Thus, the failure to observe release of
these substances in a hypertensive dog might
represent either deficiency of the substance or
preexisting high basal levels. (3) These assay
organs are insensitive to some substances such
as PGs of the A series (Fig. 2). (4) The
possibility cannot be excluded that unknown
substances could produce activity of assay
organs similar to PGs.
To counter some of the objections cited
above, we obtained additional evidence that
the changes in length of assay organs due to
PG-like substances produced during renal
ischemia behaved as a mixture of PGs as
determined by extraction and chromatograph-
ic characterization. In three experiments renal
venous blood, obtained during renal ischemia
(Fig. 5) coincident with the appearance of
PG-like substances as detected by the assay
organs, was extracted and purified to charac-
terize these substances (Fig. 8). Both extracts
and purified materials obtained by thin-layer
chromatographic methods elicited activity of
the assay organs similar, if not identical, to
that produced by standard solutions of PGE$_2$
and PGF$_2\alpha$ (Fig. 8). In addition, substances
having biological and chromatographic prop-
erties similar to the PG-like substances were
isolated from canine renal medulla and
characterized as a mixture of PGE$_2$, PGF$_2\alpha$, and PGA$_\alpha$.
Whether these observations made in acute
experiments relate to the proposed antihyper-
tensive function of the kidney cannot be
stated. Thus, the apparent release of a mixture
of PGs, which includes one with pressor
properties (PGF$_2\alpha$), as an immediate re-
sponse to acute renal ischemia appears to be
inconsistent with the proposal that a PG is the
renal antihypertensive principle. Although, a
vasodepressor PGE$_2$-like compound was found
in larger quantities in renal venous blood than
a PGF$_2\alpha$-like substance and the latter had only
a slight pressor effects in the dog (37), we are
still faced with the problem of accounting for
their fate on passage across the lung. In a
recent clinical study, Edwards et al. identified a substance having the properties of PGE2 in venous blood of ischemic kidneys (38). In the present study, we have tentatively identified PGE2 as the major constituent of PG-like substances extracted from renal venous blood and the renal medulla.

PGA2 would seem to be the more effective antihypertensive substance of renal origin since it has a vasodepressor potency similar to PGE2 (39) and passes through the lungs without loss of activity. However, assigning an antihypertensive action to PGA2 is not now possible since: (1) the presence of PGA2 in renal medulla may at least in part result from dehydration of PGE2 during purification of the renal medullary extract (10); (2) we cannot determine the presence of PGA2 in renal effluent because of limitations of the bioassay procedures. In addition, there are factors, other than PGs, which might be of greater importance in modifying the evolution of renal vascular hypertension; viz., the antihypertensive neutral lipid identified by Muirhead (7) and the antirenin phospholipid which prevents the generation of angiotensin I (8).

An integrated construction of the release, fate, and effect of vasoactive substances, evoked by renal ischemia under the present experimental conditions, suggests the following sequence. PGs (predominantly PGE2) and renin are released into the venous blood of the ischemic kidney as an early response to renal ischemia. Most of the PGs released from the ischemic kidney are removed on passage across the lung. If any PGA2 is released, however, it will escape destruction, as well from 5 to 10% of the PGE2 or PGA2 (35). Increased release of renin from the ischemic kidney accelerates formation of angiotensin I, which on passage across the lung (and perhaps within the ischemic kidney as well) is converted to angiotensin II. The latter may mediate the release of PGs from the contralateral kidney (35) (Fig. 6). The development of hypertension then depends in part upon angiotensin-PG interactions, i.e., the amount of vaso depressor PG released from kidneys presumably is related to the increased arterial concentration of angiotensin II evoked by renal ischemia. Other factors, such as the state of sodium balance (40), rate of secretion and metabolism of aldosterone (41), level of activity of the adrenergic nervous system (42), and activation or inactivation of an antirenin phospholipid (8) may modify either the vascular response to, or the rate of formation of, the principle interactants (angiotensin-PGs). The above must be considered a provisional scheme which includes some of the suggested major humoral determinants of renal vascular hypertension.

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