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POSSIBLE INFLUENCE OF INTRARENAL GENERATION OF KININS ON PROSTAGLANDIN RELEASE FROM THE RABBIT PERFUSED KIDNEY

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1 The effects of bradykinin and kininogen on renal prostaglandin release were studied in rabbit isolated kidneys perfused with oxygenated Krebs solution.

2 The concentration of prostaglandin-like material in kidney effluent was determined by bioassay after extraction of the samples with organic solvents. In 7 experiments the samples were assayed after separation of prostaglandins E and F by thin layer chromatography.

3 Addition of bradykinin to the perfusing fluid increased the venous and urinary effluxes of prostaglandin E-like substance by sixfold and fivefold, respectively, but efflux of prostaglandin F-like material was unaffected.

4 Addition of kininogen to the perfusing fluid augmented the venous and urinary release of prostaglandin E-like substances by fifteenfold and ninefold respectively and caused a twofold increase in the efflux of prostaglandin F-like material into the venous effluent.

5 Aprotinin, a kallikrein inhibitor, reduced the prostaglandin releasing action of kininogen but not of bradykinin. In contrast, inhibition of prostaglandin synthesis by indomethacin suppressed the release of prostaglandin evoked by either bradykinin or kininogen.

6 This study suggests that augmented release of prostaglandins in response to kininogen is a consequence of renal generation of kinins. Thus, changes in the intrarenal activity of the kallikrein-kinin system may modulate renal prostaglandin release.

Introduction

Kinins and prostaglandins of the E series share the ability to produce renal vasodilatation, diuresis, and natriuresis, and thereby may contribute to the regulation of blood pressure, renal blood flow and salt-water excretion (Adetuyibi & Mills, 1972; Margolius, Geller, de Jong, Pisano & Sjoerdsma, 1972; McGiff, Crowshaw & Itskovitz, 1974; Nasjletti, Colina-Chourio & McGiff, 1975a).

The demonstration that close arterial infusion of bradykinin augments the release of E prostaglandins into renal venous blood (McGiff, Terragno, Malik & Lonigro, 1972) suggested a relationship between blood levels of kinins and renal prostaglandin release. A relationship between kinins generated intrarenally and prostaglandin release depends on the ability of the kinin to reach sites of prostaglandin synthesis. Renal prostaglandin synthesizing enzymes occur predominantly in the medulla (Larsson & Änggard, 1973) while kallikrein is located in the cortex Nustad, 1970). The present experiments were undertaken to investigate the influence of intrarenal generation of kinins on the release of renal prostaglandins.

Methods

Kidney perfusion

Male New Zealand white rabbits (2.5–3.0 kg), maintained on a normal diet, were anaesthetized with sodium pentobarbitone (25 mg/kg i.v.) and injected with heparin (250 units/kg i.v.). After opening the abdomen, the left kidney was isolated and perfused as described by Regoli & Gauthier (1971). Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2.6H_2O$ 2.5, KH_2PO_4 1.2, $MgSO_4\,7H_2O$ 1.17, dextrose 8.4 and $NaHCO_3$ 25.0 was aerated with 95% O_2 and 5% CO_2 and

maintained at 37°C; it was delivered as pulsatile flow at constant rate (10 ml/min) by a roller pump. The ureter and renal vein of the perfused kidney were cannulated with polyethylene tubing and the perfusion fluid was not recirculated. A pressure transducer (Statham P23DB) was connected to a side arm of the arterial cannula and the perfusion pressure was recorded on a polygraph (Hewlett-Packard). Mean renal perfusion pressure, initially high (range 120–160 mmHg), fell rapidly to a steady lower level (87 ± 4 mmHg) within 3–5 min of starting perfusion. The volume of the urinary effluent averaged 0.96 ± 0.08 ml/min during the first 10 min of perfusion indicating that about 10% of the perfusing fluid was excreted. The concentration of sodium, measured by flame photometry, was identical in venous and in urinary effluents. These observations are consistent with the demonstration by Regoli & Gauthier (1971) that the Krebs-perfused rabbit kidney does not reabsorb sodium nor does it concentrate urine. Ten min after starting perfusion the experiments were begun and the kidneys were perfused for a further 60 to 70 minutes. Venous and urinary effluents were collected for three or four different periods of 10 min each separated by 15 min intervals during which neither bradykinin nor kininogen were present in the perfusing fluid. The first period was used as a control while during the second and third periods bradykinin (1–10 ng/ml) or kininogen (0.5–5 mg/ml) were alternatively added to the perfusion medium. In some experiments the third period was used as another control. When the effects of indomethacin (5 μg/ml) or aprotinin (250 KIE/ml) were examined, these agents were added to the perfusion fluid before starting the perfusions and maintained throughout. To investigate time-related changes in prostaglandin effluxes, we perfused kidneys with Krebs solution alone for 70 min and collected samples of venous and urinary effluents at 10 min intervals. Kallikrein activity was measured in these samples to assess the ability of the perfused kidney to release an active kinin forming enzyme.

Drugs

The following drugs were used: synthetic bradykinin (BRS 640, Sandoz Pharmaceuticals); indomethacin (Merck, Sharp and Dohme; aprotinin (Trasylol, Bayer) and pentobarbitone sodium (Abbott). Drugs were prepared in Krebs solution immediately before use.

Preparation of kininogen

Canine plasma kininogen was prepared in our laboratory as follows: blood from pentobarbitone anaesthetized dogs (30 mg/kg, i.v.) was collected in plastic bottles containing enough sodium citrate and exadimethrine to establish concentrations of 3.1 mg/ml and 0.4 mg/ml respectively. After centrifugation (1500 *g*, 30 min, 4°C) and addition of disodium ethylenediaminetetraacetate (EDTA) (0.03 M) the mixture was kept at 62°C for 2 h and centrifuged. The supernatant was cooled to 2–4°C and ammonium sulphate was added (1.9 M) followed by continuous stirring (1 h) and centrifugation (1500 *g*, 30 min, 4°C). The precipitate was dissolved in a minimum amount of water and dialyzed against distilled water for 48 h (4 changes of water). Following centrifugation, the supernatant was acidified with 5 N HCl (pH 2.5, 32°C), neutralized (pH 7) 20 min later and freeze-dried. The kininogen content of this preparation was measured with a substrate exhaustion technique (Fasciolo, Espada & Carretero, 1963). One mg of the dry material was dissolved in 4.5 ml of 0.01 M phosphate buffer (pH 7.4) containing 0.15 M NaCl and incubated at 37°C for 15 min with either trypsin (0.5 mg) or dialyzed rabbit urine (0.2 ml). The enzymatic reaction was terminated by heating in boiling water (10 min) and the kinin content of the samples assayed by their vasodilator activity in the canine hind limb preparation (Nasjletti, Colina-Chourio & McGiff, 1975b), using synthetic bradykinin as the reference standard. One mg of the kininogen preparation had an activity equivalent to 140 ng of bradykinin. This amount of kinin precursor was contained in 0.03 ml of the original plasma. Contamination of the kininogen preparation with kinin forming enzymes was not likely since kinins were not generated when the protein material (10 mg) was dissolved in Krebs solution (1 ml) and incubated for 10 min at 37°C. Similar incubations, carried out in the presence of synthetic bradykinin (50 ng) resulted in complete recovery of the peptide, which indicates the absence of kininases. The kininogen preparation was free of renin substrate as indicated by its failure to liberate angiotensin when it was incubated with an excess of rabbit renin in the presence of angiotensinase inhibitors (Nasjletti & Masson, 1969). This was expected since inactivation of renin substrate by heating and acidification has been reported (Braun-Menéndez, Fasciolo, Leloir, Muñoz & Taquini, 1946).

Determination of prostaglandins

The content of prostaglandin-like substances in venous and urinary effluents of perfused kidneys was determined as follows: samples were combined with five volumes of acetone, passed through a millipore filter and evaporated to 5–10 ml under reduced pressure at 35°C. This was followed by acidification (pH 3.5) with 1 N HCl and extraction (three times) with an equal volume of ethyl acetate. The combined ethyl acetate phases were evaporated to dryness and the residue taken up in 0.5–1 ml of saline (0.15 M NaCl) immediately before bioassay. Total

prostaglandin-like activity (Table 1, Figures 1 and 2), representing a mixture of E and F prostaglandins, was bioassayed in terms of prostaglandin E_2 on rat stomach strips superfused with Krebs solution (Vane, 1969). Values are uncorrected for losses of 15% incurred during extraction.

In seven experiments (Figure 3) the ethyl acetate extract was taken up in a minimum amount of chloroform-methanol (4:1, v/v), applied as a band to a thin layer chromatography plate (Silica gel G plates, 0.5 mm thick, Brinkmann Instruments), and chromatographed using the solvent system chloroform: methanol: acetic acid (18:2:1, by volume). Marker plates, prepared by spotting 10 μg of authentic prostaglandins E_2 and $F_{2\alpha}$, were run concurrently with preparative plates. All plates were developed until the solvent had reached 16 cm from the origin. The marker plates were visualized by spraying with 10% phosphomolybdic acid in ethanol, followed by heating to 100°C for 15 minutes. The R_F value of prostaglandins E_2 and $F_{2\alpha}$ were approximately 0.44 and 0.26 respectively. Zones on the preparative plate corresponding to the position of prostaglandins E_2 and $F_{2\alpha}$, the area in between them and the remainder of the plate divided into five 2 cm zones were scraped off and eluted with chloroform-methanol (4:1, v/v). Eluates were dried in N_2, reconstituted in 0.9% w/v NaCl solution (saline), and bioassayed for their content of prostaglandin E- and F-like materials using prostaglandins E_2 and $F_{2\alpha}$ respectively as reference standards. Efflux of prostaglandin-like material (flow rate × concentration) was expressed as nanograms of prostaglandins E_2 and $F_{2\alpha}$ equivalent per min (ng/min). The values were uncorrected for losses incurred on extraction and chromatographic purification. In six experiments, after addition of 50 ng of authentic prostaglandin E_2 to 50 ml of venous effluent obtained from a kidney perfused with Krebs solution containing indomethacin (5 μg/ml), we recovered 65 ± 6% (s.e.) of prostaglandin-like material. The concentration of prostaglandin-like substance in the samples was determined by bracket assay on rat stomach strips superfused with Krebs solution (Vane, 1969; Vane, 1971). To provide evidence in support of the tentative identification of prostaglandins in the samples, a rat colon and a chick rectum were included in the assay system. Thus, agreement between responses evoked by a sample and a prostaglandin standard on the rat stomach strip, rat colon and chick rectum, suggests that the myotropic activity of the sample is due to a prostaglandin (Vane, 1969). All three isolated organs were suspended in glass chambers and superfused in series with Krebs solution (3 ml/min, 37°C) (McGiff *et al.*, 1972).

Contractions of the tissues were measured by an isotonic transducer (model 356 Harvard Apparatus Company, Inc.) and recorded on a polygraph (model 7718, Hewlett-Packard). In the dose range used (0.5–5 ng), prostaglandin E_2 contracted the rat stomach strip and chick rectum, but had little effect on the rat colon; whereas prostaglandin $F_{2\alpha}$ contracted the rat colon and the chick rectum but had only one third the potency of prostaglandin E_2 in contracting the rat stomach strip. The crude ethyl acetate lipid extract contracted the three tissues in a manner similar, but not identical, to prostaglandin E_2. Thus, a dose of the lipid extract equivalent to prostaglandin E_2 in evoking contraction of the stomach strip, was slightly more effective than prostaglandin E_2 in contracting the rat colon. This discrepancy might be explained if prostaglandin E- and F-like material were present in the sample. This possibility was confirmed after thin layer chromatography of the crude lipid extract. Thus, the reconstituted eluate of the plate at zones corresponding to the migration of prostaglandins E_2 and $F_{2\alpha}$ standards contained material which contracted all three assay organs in a manner indistinguishable from prostaglandins E_2 and $F_{2\alpha}$ respectively. The demonstration of agreement between the substances isolated from the ethyl acetate extract and authentic prostaglandins E_2 and $F_{2\alpha}$ in terms of thin layer chromatographic behaviour and myotropic effects on three smooth muscle preparations is compatible with the interpretation that the myotropic activity of the lipid extract is due to prostaglandins. Release of prostaglandins E_2 and $F_{2\alpha}$ by the rabbit kidney has been established unequivocally by Davis & Horton (1972) using mass spectrometric analysis.

All prostaglandins assays were performed in duplicate; the difference between duplicate assays of 45 samples averaged 13.5 ± 2.5% (s.e. mean).

Determination of kallikrein

Kallikrein activity in venous and urinary effluents was determined by the method of Marin-Grez & Carretero (1972), which is based on the determination of the amount of kinins formed when an aliquot of sample is incubated with an excess of kininogen. The lyophilized substrate (500 mg) was dissolved in 10 ml of a solution containing 0.15 M NaCl, 0.001 M 1–10 phenanthroline and 0.003 M EDTA. Each incubation mixture contained an aliquot of either venous (1 ml) or urinary (0.2 ml) effluents, 1 ml of kininogen solution and 0.15 M NaCl to a final volume of 3 ml. After incubation for 20 min at 37°C and pH 7.5, the mixture was acidified (pH 5–5.5) and heated in a boiling water bath for 5 minutes. Following centrifugation, the supernatant was neutralized (pH 7.0) and assayed in the canine hind limb preparation with synthetic bradykinin as reference standard (Nasjletti, *et al.*, 1975b). Kallikrein activity was expressed as nanograms of bradykinin equivalents released per millilitre of sample per min of incubation (ng ml^{-1} min^{-1}). Kallikrein release (flow rate × kallikrein activity), was expressed as nanograms of bradykinin equivalents per min (ng/min). The specificity of the kallikrein assay was assessed by the

demonstration that addition of aprotinin (50 KIE) to mixtures of kininogen and urinary effluents (0.2 ml), blocks the generation of kinins. All the results were expressed as mean ± standard error (s.e.). Statistical significance was determined by Student's *t*-test based on unpaired or paired observation (Steel & Torrie, 1960). A *P* value of 0.05 or less was considered statistically significant.

Results

In nine isolated kidneys perfused for 70 min with Krebs solution only, the mean release of prostaglandin-like substances into the venous and urinary effluents at the start of perfusion was 1.9 ± 0.8 and 2.0 ± 0.6 ng of prostaglandin E_2 equivalents per min respectively and did not differ significantly from the values obtained in three subsequent observation periods (Table 1). Release of kallikrein into the urinary effluent was nearly constant throughout the perfusion but release into the venous effluent was detected in only two out of eight experiments (Table 1).

Addition of bradykinin to the perfusing fluid to establish concentrations of 1 ng/ml and 10 ng/ml, increased the venous efflux of prostaglandin-like substances from 1.7 ± 0.6 ng/min and 1.3 ± 0.3 ng/min to 3.6 ± 0.7 ng/min ($P < 0.02$) and 9.9 ± 2.0 ng/min ($P < 0.02$) respectively (Figures 1 and 2). Concomitantly, the urinary efflux of prostaglandin-like substances increased from 1.6 ± 0.4 ng/min and 1.3 ± 0.5 ng/min to 3.7 ± 1.2 ng/min ($P < 0.05$) and 10.4 ± 3.4 ng/min ($P < 0.025$) in response to 1 and 10 ng/ml of bradykinin respectively (Figures 1 and 2). Fifteen minutes after termination of bradykinin infusion (1 ng/ml), effluxes of prostaglandins into

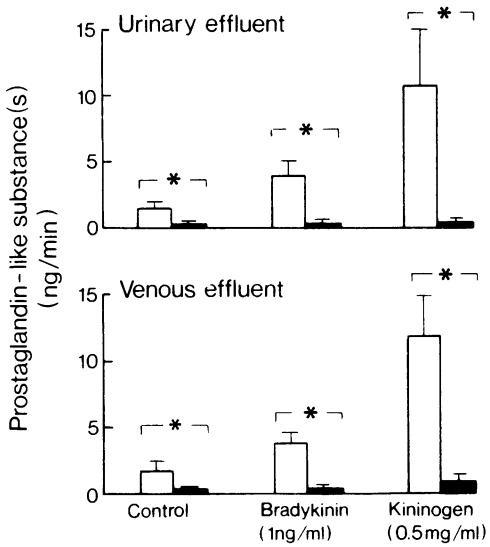

Figure 1 The effect of bradykinin and kininogen on prostaglandin release in the presence (closed columns, $n = 6$) and in the absence (open columns, $n = 11$) of indomethacin (5 μg/ml). Vertical lines indicate the s.e. mean. Probability level (non-paired Student's *t*-test): $^*P < 0.01$.

venous (1.1 ± 0.1 ng/min) and urinary (1.8 ± 0.6 ng/min) effluents were not different from control (1.0 ± 0.2 ng/min, $P > 0.6$ and 1.9 ± 0.4 ng/min, $P > 0.8$, respectively; 4 experiments). Additions of kininogen to the perfusing fluid to establish concentrations of 0.5 mg/ml and 5 mg/ml, increased the

Table 1 Time course of prostaglandin and kallikrein release from rabbit perfused kidneys

Perfusion time (min)	*0–10*	*20–30*	*40–50*	*60–70*
Prostaglandin release into the venous effluent (ng/min) ($n = 9$)	1.9 ± 0.8	2.1 ± 0.4*	2.1 ± 0.4*	1.9 ± 0.4*
Prostaglandin release into the urinary effluent (ng/min) ($n = 9$)	2.0 ± 0.6	1.6 ± 0.3*	1.3 ± 0.3*	1.7 ± 0.3*
Kallikrein release into the venous effluent (ng/min) ($n = 8$)	1.9 ± 1.4	0.3 ± 0.3*	1.2 ± 0.9*	7.4 ± 7.4*
Kallikrein release into the urinary effluent (ng/min) ($n = 8$)	82.5 ± 16.0	74.7 ± 10.6*	81.5 ± 11.8*	90.1 ± 11.1*

Results are means ± s.e. mean; n = number of experiments.
Significance of difference from controls (paired Student's *t*-test): $^*P > 0.1$.

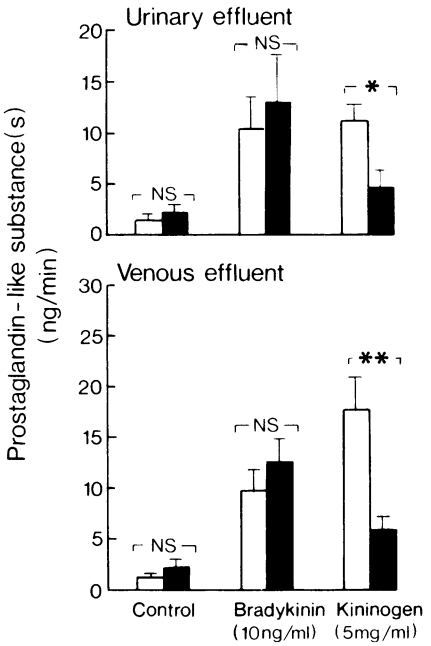

Figure 2 The effect of bradykinin and kininogen on prostaglandin release in the presence (closed columns, $n=8$) and in the absence (open columns, $n=6$) of aprotinin (250 KIE/ml). Vertical lines indicate the s.e. mean. Probability level (non-paired Student's t-test): NS = $P>0.05$; *$P<0.02$; **$P<0.001$.

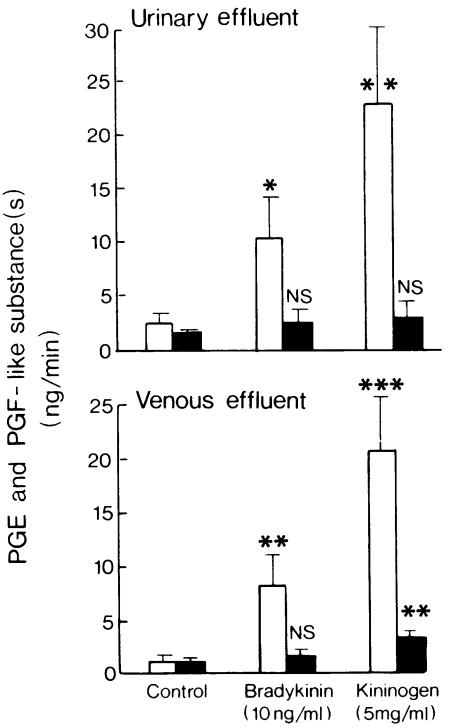

Figure 3 The effect of bradykinin and kininogen on the release of prostaglandin E (PGE, open columns) and prostaglandin F (PGF, closed columns)-like substances from the perfused rabbit kidney ($n=7$). Vertical lines indicate the s.e. mean. Significance of difference from controls (paired Student's t-test): NS = $P>0.05$; *$P<0.05$; **$P<0.025$; ***$P<0.01$.

venous efflux of prostaglandin-like substances from 1.7 ± 0.6 ng/min and 1.3 ± 0.3 ng/min to 10.5 ± 3.0 ng/min ($P<0.01$) and 17.8 ± 2.8 ng/min ($P<0.005$) respectively (Figures 1 and 2). Concomitantly, the urinary efflux of prostaglandin-like material increased from 1.6 ± 0.4 ng/min and 1.3 ± 0.5 ng/min to 10.3 ± 4.4 ng/min ($P<0.05$) and 11.6 ± 1.7 ng/min ($P<0.001$) in response to the low (0.5 mg/ml) and high (5 mg/ml) concentration of kininogen respectively (Figures 1 and 2). Fifteen minutes after termination of the kininogen infusion (0.5 mg/ml), effluxes of prostaglandin into venous (1.7 ± 0.4 ng/min) and urinary (2.2 ± 1.1 ng/min) effluents were not different from control (1.5 ± 0.3 ng/min, $P>0.7$ and 1.7 ± 0.4 ng/min, $P>0.3$ respectively; 4 experiments).

Addition of indomethacin (5 μg/ml) to the perfusion fluid reduced the basal release of prostaglandin into venous and urinary effluents by 92% ($P<0.01$) and 87% ($P<0.01$) respectively and prevented the release evoked by bradykinin (1 ng/ml) and kininogen (0.5 ng/ml) (Figure 1). The kallikrein inhibitor aprotinin (250 KIE/ml) did not alter the basal or the bradykinin-induced efflux of prostaglandins but reduced the effect of kininogen (5 mg/ml) on venous and urinary release of prostaglandin-like substances by 79% ($P<0.001$) and 77% ($P<0.02$) respectively (Figure 2).

Figure 3 illustrates the effects of bradykinin (10 ng/ml) and kininogen (5 mg/ml) on renal release of prostaglandin E- and F-like materials. Bradykinin did not affect the release of F prostaglandins but increased the venous and urinary efflux of prostaglandin E-like substances from 1.3 ± 0.5 and 2.6 ± 0.9 ng/min to 8.3 ± 2.7 ng/min ($P<0.025$) and 10.3 ± 3.8 ng of prostaglandin E_2 equivalents per min ($P<0.05$) respectively. Similarly, addition of kininogen to the perfusion fluid increased the release of prostaglandin E into venous and urinary effluents from 1.3 ± 0.5 ng/min and 2.6 ± 0.9 ng/min to 20.6 ± 5.4 ng/min ($P<0.01$) and 23.1 ± 7.5 ng/min ($P<0.025$) respectively. This was associated with a small increase in the release of prostaglandin F into the venous effluent from 1.2 ± 0.3 to 3.3 ± 0.6 ng of prostaglandin $F_{2\alpha}$ equivalents per min ($P<0.025$).

In all experiments, neither bradykinin nor kininogen affected the renal perfusion pressure (87 ± 4 mmHg) which remained constant until the experiments were terminated.

Discussion

Renal prostaglandins are synthesized predominantly in the renal medulla (Larsson & Ånggard, 1973) and released into renal venous blood (McGiff, Crowshaw, Terragno & Lonigro, 1970) and urine (Frolich, Wilson, Sweetman, Smigel, Niew, Carr, Watson & Oates, 1975). Our experiments show that addition of bradykinin to the perfusion fluid does not affect renal perfusion pressure but increases the renal effluxes of prostaglandins by selectively increasing the release of prostaglandin E compounds. These results support the findings of McGiff *et al.* (1972) who showed that intrarenal arterial infusions of bradykinin, but not of eledoisin, augment the concentration of prostaglandin E-like substance in the renal venous blood of dogs. We cannot explain the failure of bradykinin to produce vasodilatation in Krebs perfused kidneys. In a similar preparation, Needleman, Kauffman, Douglas, Johnson & Marshall (1973) observed slight vasodilatation in response to bolus injections (100 ng) of bradykinin. The discrepancy may be due to the differences in dosage and in the administration of the peptide.

Our demonstration that blockade of prostaglandin synthesis with indomethacin (Vane, 1971) causes an immediate reduction of prostaglandin efflux is consistent with the view that there is no appreciable storage of prostaglandins in the kidney (Ånggard, Bohman, Griffin, Larsson & Maunsbach, 1972). Therefore, the increased effluxes of renal prostaglandins in response to bradykinin may be accounted for by enhanced release of newly synthesized prostaglandins. In the present experiments a crude kininogen preparation, devoid of renin substrate and of kinin forming enzymes, was shown to share with bradykinin the ability to increase renal prostaglandin efflux by selectively enhancing the release of E prostaglandins. This observation and the finding of a kinin forming enzyme in the urinary effluent of perfused kidneys (Roblero, Croxatto, Corthorn, Garcia & De Vito, 1973) raise the possibility that the prostaglandin releasing action of kininogen is mediated by kinins liberated by kallikrein from the protein substrate. The demonstration that aprotinin, an inhibitor of renal and of urinary kallikrein (Nustad, 1970) reduced the effect of kininogen on prostaglandin release supports this view. Alternative explanations, such as direct effects of either kininogen or other plasma proteins contained in the substrate preparation on the synthesis, release and inactivation of renal prostaglandins are improbable since such effects are not likely to be impaired by aprotinin. Moreover, a direct impairment by the kallikrein inhibitor of the synthesis, release and inactivation of prostaglandins is unlikely since in our experiments aprotinin affected neither the basal effluxes of prostaglandins nor the augmented release evoked by bradykinin. Finally, the possibility that plasmin, an aprotinin-inhibited kininogenase whose precursor appears to originate in the kidney (Vogel & Werle, 1968; Highsmith & Kline, 1973) liberated kinins in our experiments is unlikely since this enzyme is devoid of kininogenase activity when acting upon heated kininogen (Vogt, 1964). Kinins generated intrarenally may also affect the release of renal prostaglandins in intact animals. Thus, kallikreins synthesized in the kidney cortex and presumably secreted in urine at the level of the distal tubules, may act on kininogen to produce kallidin (lysylbradykinin) which in turn could reach the sites of prostaglandin synthesis in the renal medulla via the collecting ducts (Nustad, Kirsten Vaaje & Pierce, 1975; Scicli, Carretero, Hampton & Oza, 1975). This possibility is endorsed by the demonstration in urine of free kinins which originate intrarenally (Miwa, Erdos & Seki, 1968; Nasjletti *et al.*, 1975a) and by the occurrence of kininogen in the kidney (Sardesai, 1968; Werle & Zach, 1970). Moreover, we have observed that rats receiving daily injections of deoxycorticosterone (5 mg) for two weeks have an augmented urinary excretion of kallikrein and of prostaglandin E-like substances and that these effects are prevented by simultaneous treatment with aprotinin (100,000 KIU) for four days (Colina-Chourio, McGiff & Nasjletti, unpublished observation).

Release of renal prostaglandins in intact animals is continuous and may influence renal blood flow (Lonigro, Itskovitz, Crowshaw & McGiff, 1973), sodium excretion (Lee & Attallah, 1974), renal vascular reactivity (Aiken & Vane, 1973), and ultimately blood pressure (Colina-Chourio, McGiff & Nasjletti, 1975). Similarly, kinins generated intrarenally appear to be involved in the regulation of renal blood flow and salt-water excretion (Nasjletti *et al.*, 1975a; Adetuyibi & Mills, 1972; Marin-Grez, Cottone & Carretero, 1972). Contribution of prostaglandins to some of the renal actions of kinins has recently been suggested. Thus, in studies using canine isolated blood perfused kidneys, the effects of bradykinin on renal blood flow and free-water clearance were reduced by an inhibitor of prostaglandin synthesis (McGiff, Itskovitz & Terragno, 1975). These observations and the present study, suggesting a relationship between intrarenal generation of kinins and renal prostaglandin release, support the proposal that a functional coupling of kinins and prostaglandins within the kidney may be an important element in the regulation of renal blood flow and salt-water excretion. However, this conclusion should be considered provisional until studies in intact animals are available.

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References

ADETUYIBI, A. & MILLS, I.H. (1972). Relation between urinary kallikrein and renal function, hypertension, and excretion of sodium and water in man. *Lancet*, **ii**, 203–207.

AIKEN, J.W. & VANE, J.R. (1973). Intrarenal prostaglandin release attenuates the renal vasoconstrictor activity of angiotensin. *J. Pharmac. exp. Ther.*, **184**, 678–687.

ÄNGGÅRD, E., BOHMAN, S.O., GRIFFIN, J.E., LARSSON, C. & MAUNSBACH, A.B. (1972). Subcellular localization of the prostaglandin system in the rabbit renal papilla. *Acta physiol. scand.*, **84**, 231–246.

BRAUN-MENÉNDEZ, E., FASCIOLO, J.C., LELOIR, L.F., MUÑOZ, J.S. & TAQUINI, A.C. (1946). In *Renal Hypertension* p. 132. Charles C. Thomas: Springfield, Illinois.

COLINA-CHOURIO, J., McGIFF, J.C. & NASJLETTI, A. (1975). Development of high blood pressure following inhibition of prostaglandin synthesis. *Fedn Proc.*, **34**, 368 (abstract).

DAVIS, H.A. & HORTON, E.W. (1972). Output of prostaglandins from the rabbit kidney, its increase on renal nerve stimulation and its inhibition by indomethacin. *Br. J. Pharmac.*, **46**, 658–675.

FASCIOLO, J.C., ESPADA, J. & CARRETERO, O.A. (1963). The estimation of bradykininogen content of plasma. *Acta physiol. lat. amer.*, **13**, 215–220.

FROLICH, J.C., WILSON, T.W., SWEETMAN, B.J., SMIGEL, M., NIES, A.S., CARR, K., WATSON, J.T. & OATES, J.A. (1975). Urinary prostaglandins: Identification and origin. *J. clin. Invest.*, **55**, 763–770.

HIGHSMITH, R.F. & KLINE, D.L. (1973). Plasminogen restoration by the kidney mediated by a plasma factor. *Am. J. Physiol.*, **225**, 1032–1037.

LARSSON, C. & ÄNGGÅRD, E. (1973). Regional differences in the formation and metabolism of prostaglandins in the rabbit kidney. *Eur. J. Pharmac.*, **21**, 30–36.

LEE, J.B. & ATTALLAH, A.A. (1974). Response of intrarenal PGA_2 to salt loading in the rabbit. *Circulation Res.*, **34–35** (suppl. I), 75–82.

LONIGRO, A.M., ITSKOVITZ, H.D., CROWSHAW, K. & McGIFF, J.C. (1973). Dependency of renal blood flow on prostaglandin synthesis in the dog. *Circulation Res.*, **32**, 712–717.

MARGOLIUS, H.S., GELLER, R.G., DE JONG, W., PISANO, J.J. & SJOERDSMA, A. (1972). Urinary kallikrein excretion in hypertension. *Circulation Res.*, **30–31** (suppl. II) 125–131.

MARIN-GREZ, M., COTTONE, J. & CARRETERO, O.A. (1972). A method for measurement of urinary kallikrein. *J. appl. Physiol.*, **32**, 428–431.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A. & LONIGRO, A.J. (1970). Renal prostaglandins: Possible regulators of the renal actions of pressor hormones. *Nature, Lond.*, **227**, 1255–1257.

McGIFF, J.C., TERRAGNO, N.A., MALIK, K.U. & LONIGRO, A.J. (1972). Release of a prostaglandin E-like substance from canine kidney by bradykinin. *Circulation Res.*, **31**, 36–43.

McGIFF, J.C., CROWSHAW, K. & ITSKOVITZ, H.D. (1974). Prostaglandins and renal function. *Fedn Proc.*, **33**, 39–47.

McGIFF, J.C., ITSKOVITZ, H.D. & TERRAGNO, N.A. (1975). The actions of bradykinin and eledoisin in the canine isolated kidney: Relationships to prostaglandins. *Clin. Sci. & Mol. Med.*, **49**, 125–131.

MIWA, I., ERDOS, E.G. & SEKI, T. (1968). Presence of three peptides in urinary kinin (substance Z) preparations. *Life Sci.*, **7**, 1339–1343.

NASJLETTI, A., COLINA-CHOURIO, J. & McGIFF, J.C. (1975a). Disappearance of bradykinin in the renal circulation of dogs: Effects of kininase inhibition. *Circulation Res.*, **37**, 59–65.

NASJLETTI, A., COLINA-CHOURIO, J. & McGIFF, J.C. (1975b). Assay of kinins by their effects on canine femoral blood flow. *Proc. Soc. exp. Biol. Med.*, **150**, 493–497.

NASJLETTI, A. & MASSON, G.M.C. (1969). Effects of corticosteroids on plasma angiotensinogen and renin activity. *Am. J. Physiol.*, **217**, 1396–1400.

NEEDLEMAN, P., KAUFFMAN, A.H., DOUGLAS, J.R., JOHNSON, E.M. & MARSHALL, G.R. (1973). Specific stimulation and inhibition of renal prostaglandin release by angiotensin analogs. *Am. J. Physiol.*, **224**, 1415–1419.

NUSTAD, K. (1970). The relationship between kidney and urinary kininogenase. *Br. J. Pharmac.*, **39**, 73–86.

NUSTAD, K., KIRSTEN VAAJE & PIERCE, J.V. (1975). Synthesis of kallikreins by rat kidney slices. *Br. J. Pharmac.*, **53**, 229–234.

REGOLI, D. & GAUTHIER, R. (1971). Site of action of angiotensin and other vasoconstrictors on the kidney. *Can. J. Physiol., Pharmac.*, **49**, 608–612.

ROBLERO, J.S., CROXATTO, H.R., CORTHORN, J.H., GARCIA, R.L. & DE VITO, E. (1973). Kininogenase activity in urine and perfusion fluid of isolated kidney. *Acta physiol. lat. amer.*, **23**, 566–568.

SARDESAI, V.M. (1968). Determination of bradykinin in blood and bradykininogen in tissues. *Can. J. Physiol. Pharmac.*, **46**, 77–79.

SCICLI, A.G., CARRETERO, O.A., HAMPTON, A. & OZA, N.B. (1975). Kallikrein localization in the nephron by the stop-flow technique. *Fedn Proc.*, **34**, 378 (abstract).

STEEL, R.G.D. & TORRIE, J.H. (1960). *Principles and procedures of statistics*. New York, Toronto, London: McGraw-Hill Book Co., Inc.

VANE, J.R. (1969). Release and fate of vasoactive hormones in the circulation. *Br. J. Pharmac.*, **35**, 209–242.

VANE, J.R. (1971). Inhibition of Prostaglandin synthesis as a

mechanism of action for aspirin-like drugs. *Nature, Lond.*, **231**, 232–235.

VOGEL, R. & WERLE, E. (1968). Kallikrein inhibitors. In *Handbook of Experimental Pharmacology*, Vol. 25, ed. Erdos, E.G., pp. 213–249. Berlin and Heidelberg: Springer-Verlag.

VOGT, W. (1964). Kinin formation by plasmin, an indirect process mediated by activation of kallikrein. *J. Physiol., Lond.*, **170**, 153–166.

WERLE, E. & ZACH, P. (1970). Verteilung von kininogen in serum and geweben bei ratten und andergen saugetieren. *Z. Klin. Chem. Klin. Biochem.*, **8**, 186–189.

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