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THE EFFECT OF ANGIOTENSIN I CONVERTING ENZYME INHIBITOR (SQ 20881) ON THE RELEASE OF PROSTAGLANDINS BY RABBIT KIDNEY, *in vivo*

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1 Prostaglandin E- and F-like material has been estimated in renal venous blood of the left kidney of anaesthetized rabbits following renal nerve section. Prostaglandins were estimated by bioassay following solvent extraction and column chromatography.

2 Electrical stimulation of the renal nerves of the left kidney to reduce renal blood flow by approximately 15% for 15 min resulted in a significant increase in the concentration of prostaglandin E-like material in the renal venous blood. The peak values were normally seen either in the last 5 min of the stimulation period or in the first 5 min after the end of the stimulation period. The concentration of prostaglandin F-like material was not significantly altered.

3 Similar reduction of renal blood flow of the left kidney by renal artery constriction also resulted in a significant increase in the concentration of prostaglandin E- but not F-like material in renal venous blood. The timing and magnitude of the response was comparable with that observed with renal nerve stimulation.

4 The effect of an angiotensin I converting enzyme inhibitor, SQ 20881, on the response to both renal nerve stimulation and renal artery constriction has been studied. The administration of the drug did not significantly reduce the release of prostaglandins from the denervated kidneys, however, the increase in prostaglandin E-like material, in response to both stimuli, was abolished.

5 The results suggest that the increase in prostaglandin E-like material released from the kidney in response to low frequency stimulation or to modest reductions in renal blood flow is dependent on the release of renin and that the effect is mediated by the formation of angiotensin II and not angiotensin I.

Introduction

The renin-angiotensin, prostaglandin and kallikrein systems of the kidney appear to be closely interrelated; however, a clear picture of these relationships has not yet emerged. Infusion of angiotensin II into the renal artery leads to an increase in the release of prostaglandin-like material from the kidney in the dog (McGiff, Crowshaw, Terragno & Lonigro, 1970; Aiken & Vane, 1973) and from the isolated Krebs-perfused kidney of the rabbit (Needleman, Kauffman, Douglas, Johnson & Marshall, 1973). Stimulation of the renal nerves, which leads to the intrarenal generation of angiotensin II and increase in renin release from the kidney (Vander, 1965; Coote, Johns, Macleod & Singer, 1972; Loeffler, Stockigt & Ganong, 1972) has also been associated with an increase in prostaglandin release in the dog (Dunham & Zimmerman, 1970; Mancia, Romero & Strong, 1974) and the rabbit (Davis & Horton, 1972). Another stimulus which leads to an increase in renin release from the kidney is renal artery constriction (Johns & Singer, 1974). This stimulus has also been reported to induce an increase in the release of prostaglandin from the kidney in the dog (McGiff, Crowshaw, Terragno, Lonigro, Strand, Williamson, Lee & Ng, 1970; Gross, Mujovic, Jubiz & Fisher, 1976). Although these results suggest that the increased formation of angiotensin II within the kidney leads to the local formation of prostaglandin, other evidence indicates that the relationship between these substances is very complex, and that the kallikrein-kinin system may also be involved. Larsson, Weber & Änggård (1974) have shown that administration of arachidonic acid in rabbits led to an increase in plasma renin activity (PRA), while indomethacin, which inhibits prostaglandin synthesis, led to a significant decrease in PRA. The dog also responds to infusion of arachidonic acid with an increase in renin secretion which has been attributed to a direct action of prostaglandin on the juxtaglomerular cell (Bolger, Eisner, Ramwell & Slotkoff, 1976). When indomethacin or meclofenamate were used to block the synthesis of prostaglandin, the rise in

PRA in the rabbit, in response to haemorrhage or furosemide administration, was diminished (Romero, Dunlop & Strong, 1976). In man, PRA was reduced after oral administration of indomethacin (Donker, Arisz, Brentjens, van der Hem & Hollemans, 1976).

McGiff, Itskovitz, Terragno & Wong (1976), in studies based on the administration of kinins, have suggested that kinins increase prostaglandin synthesis by making more substrate available to the prostaglandin synthetase complex. In a study of the isolated Krebs-perfused rabbit kidney, bradykinin added to the perfusion fluid augmented the release of a prostaglandin E-like substance, while aprotonin, a kallikrein inhibitor, reduced it. Addition of a kininogen had the same effect as bradykinin (Nasjletti & Colina-Chourio, 1976). On the other hand Obika & Mills (1976) found that arterial infusion of prostaglandin E in dogs leads to an increase in urinary excretion of kallikrein, and they suggest that the diuresis and natriuresis associated with this treatment may be secondary to the release of kallikrein.

The present study is an attempt to examine the relationship between the release of renin and the release of prostaglandins from the kidney, *in vivo*, in response to moderate stimuli, i.e. stimulation of the renal nerves and renal artery constriction, to reduce renal blood flow by about 15%. These experiments were performed on denervated kidneys of anaesthetized rabbits, and the prostaglandin content of renal venous blood was examined before, during and after the stimulus was applied. The experiments were also performed after the administration of the angiotensin I converting enzyme inhibitor (CEI), SQ 20881, which blocks the conversion of angiotensin I to angiotensin II and which inhibits kininase, thus potentiating the effect of locally generated kinins (Engel, Schaeffer, Gold & Rubin, 1972; Erdös, 1975).

It was hoped that this approach might avoid some of the problems associated with isolated organs, especially when perfused with synthetic media, or with the administration of substances not normally reaching the kidney via the renal artery. As SQ 20881 blocked the formation of angiotensin II while preventing the breakdown of kinins the experiments were expected to give some information about the relative importance of these substances in mediating the release of prostaglandins in response to the stimuli under investigation.

Methods

Preparation of animals

New Zealand Red rabbits of either sex (2.1–3.2 kg) were anaesthetized with intravenous sodium pentobarbitone (marginal ear vein), and the trachea was cannulated as well as a jugular vein and a carotid artery. The left kidney was exposed via a retroperitoneal incision. The renal nerves were sectioned and a flow probe placed on the renal artery (Biotronix, 1.25 mm) and connected to a flow meter (S.E. Laboratories M275). Renal blood flow was recorded on a Grass Polygraph. The lumboadrenal vein was cannulated and the tip of the cannula was manoeuvered into the renal vein. After surgery was completed heparin (1000 u/kg) was administered.

Collection of renal venous blood samples

At least 1 h was allowed to elapse following renal denervation before the first renal venous blood sample was collected. Each renal venous blood sample (3–5 ml) was collected into a cooled 5 ml plastic syringe over a 5 min period. During this period about one half to three quarters of the blood volume removed was replaced with dextran (Lomodex 70, Dextran, Fisons Ltd.) intravenously. The blood was placed in a graduated test tube surrounded by ice and extracted immediately.

Extraction of blood

Immediately after collection, 0.1 ml of ethanol containing approximately 0.01 μCi [n-5, 6, 8, 11, 12, 14, 15-^{3}H]-prostaglandin E_2 (specific activity 120–170 Ci/mmol, Radiochemical Centre, Amersham), was added to the blood and mixed. The blood was lysed with an equal volume of ice-cold deionized water. One half of the diluted blood volume of 0.5 molar sodium phosphate buffer at pH 5.0 containing 10 mg/ml L-ascorbic acid was added. The ascorbic acid acted as an anti-oxidant (Edwards, Strong & Hunt, 1969) and significantly improved extraction recoveries. The lysed blood in phosphate buffer was extracted with 40 ml of 5% methanol in dichloromethane. Following centrifugation at 0°C the organic solvent phase was decanted off and dried down under vacuum on a rotary evaporator (40–45°) and stored under nitrogen. This extraction procedure is a modification of the method of Golub, Zia & Horton (1974).

Column chromatography

The method used is a modification of the method of Caldwell, Burstein, Brock & Speroff (1971). Prostaglandin E- and F-like substances were separated using silicic acid plus celite microcolumns loaded with methanol. The methanol was removed with benzene:ethyl acetate (60:40) and the extracts (or standards) were applied to the column in benzene:ethyl acetate:methanol (60:40:10).

Prostaglandins E and F were eluted from the column by increasing the proportion of methanol in the eluting solvent. Sixteen column fractions were collected, each of 6 ml, the solvent being forced through the column under pressure to produce a flow

rate of 1 ml/minute. Column fractions 1–3 were eluted with benzene:ethyl acetate (60:40). These column fractions were discarded since they contained prostaglandins A and B and other unwanted components. Column fractions 4–12 were eluted with benzene:ethyl acetate:methanol (60:40:5). Prostaglandin E characteristically appeared over a well-defined peak, occurring in column fractions 4–7. For routine experiments these were the only column fractions kept, the rest were discarded. Column fractions 12–16 were eluted with benzene:ethyl acetate:methanol (60:40:20). This was the prostaglandin F zone.

Corrections for losses were made by counting 1 ml aliquots of column fractions 4–7 (prostaglandin E) and 12–16 (prostaglandin F) for each sample. The pooled total counts found in the four prostaglandin E fractions (about 30%) were used to determine the losses which occurred during the extractions and purification procedures. The estimate of loss found in the prostaglandin E zone was also applied to the prostaglandin F values as it had been confirmed by bioassay that the losses of both prostaglandins in any one extract were usually similar. The prostaglandin F values were also corrected for a small contamination with prostaglandin E (assessed by radioactivity and usually less than 5%), taking into account the relative potencies of prostaglandins E and F in the bioassay.

Bioassay

For bioassay the column fractions for each prostaglandin (5 ml of each) were pooled and the solvent evaporated with a jet of nitrogen and the fractions stored in the deep freeze. The extract was taken up in 1 ml Tyrode solution and assayed against either prostaglandin E_2 or $F_{2\alpha}$ on the rat isolated stomach strip (Vane, 1957). Indomethacin, 1–10 μg/ml, was added to the Tyrode solution to inhibit spontaneous activity and increase the sensitivity of the tissue. To eliminate effects due to other substances such as acetylcholine, histamine, 5-hydroxytryptamine and the catecholamines, the following blocking agents were routinely added to give final bath concentrations/ml as follows: atropine 0.1 μg; diphenydramine 0.1 μg; bromo-lysergic acid diethylamide 0.2 μg; phenoxybenzamine 1.0 μg (Sirois & Gagnon, 1974).

Renal nerve stimulation

The distal cut ends of the renal nerves were placed over silver wire stimulating electrodes for direct electrical stimulation. A 15-min period of renal nerve stimulation was employed, with the intensity adjusted to maintain a 15% reduction in renal blood flow. Square wave stimuli were delivered from a Grass S8 stimulator. Stimulation parameters were 15 V, 0.2 ms duration and, usually, 1 to 1.5 hertz.

Renal artery constriction

A cotton thread was passed around the renal artery and through a length of polythene tubing attached to a screw device which could be raised or lowered to adjust the degree of constriction applied. Blood flow was reduced by 15% for a period of 15 minutes.

SQ 20881 (Pyr – Trp – Pro – Arg – Pro – Glu – Ile – Pro – Pro)

This was made up as required as a 3 mg/ml solution in 0.9% w/v NaCl solution (saline). Doses of 1.5 mg were administered every 15 min in the first few experiments, and every 10 min in the rest in an attempt to maintain a reasonable degree of converting enzyme inhibition. As data were not available on the rabbit the dose level and timing were chosen on the basis of published data for the rat (Engel *et al.*, 1972).

Experimental design

The maximum number of renal vein blood samples removed from any one animal was six. Two basic experimental protocols were used in the experiments. (1) Six samples were taken during a single experiment, 2 before stimulation, 2 during the last 10 min of a 15 min period of stimulation (either renal nerve stimulation or renal artery constriction), and 2 during consecutive 5-min periods immediately after the end of stimulation. It was found that the prostaglandin content of 6 renal venous blood samples, taken in this manner, but without the stimulus, were almost identical. (2) Six samples were taken during 2 experiments; one experiment performed before and one during the administration of SQ 20881. Three samples were removed during each experiment; one before stimulation, one during the last 5 min of a 15-min period of stimulation and one during the first 5 min immediately after the end of the stimulation. It was found that, in the absence of the SQ 20881, the response in the second experiment was similar to the first, with both stimuli.

Statistical analysis was performed using Student's *t* test.

Results

Renal nerve stimulation

Five experiments were performed in which 6 renal venous blood samples were taken before, during and after a single stimulation period of 15 min (Table 1). The mean prostaglandin E-like material rose from 1.94 ± 0.43 (s.e. mean) ng/ml and 0.93 ± 0.39 during the control periods to 5.6 ± 1.6 ($P < 0.025$) in the immediate post-stimulation period. In the fifth to tenth minute of post-stimulation period the value had

returned to 0.98 ± 0.41 ng/ml. In the individual experiments, the peak values occurred in the immediate post-stimulation period in 4 experiments and the 5th–10th min of stimulation period in one experiment. Collections were not made in the first 5 min of the stimulation period as preliminary studies showed that there was no change in prostaglandin levels in this period. Prostaglandin F was not determined in the group of animals.

During the administration of SQ 20881

Five experiments were performed, as above, except that the converting enzyme inhibitor was administered every 15 min (3 experiments) and later every 10 min (2 experiments) until the last sample was removed. The first blood collection period was started immediately after the first injection of the drug. The concentrations of prostagland E- and F-like substances were estimated (Table 1). Prostaglandin E values in the control period were 1.73 ± 0.45 and 1.08 ± 0.18 ng/ml. All of the values after this were within this range, or lower. The prostaglandin E concentration during the last 5 min of stimulation was actually significantly lower than the first control sample, 0.64 ± 0.10 ($P < 0.025$). It was not, however, significantly lower than the second control sample ($P > 0.05$). The concentrations of prostaglandin F-like material were 1.10 ± 0.49 and 0.66 ± 0.40 ng/ml in the 2 control samples, and the subsequent four values fell within this range.

Experiments performed before and after the administration of SQ 20881. Four experiments were performed in which the nerve was stimulated before and after the administration of the drug. In a fifth animal two experiments were performed, but the drug was not administered during the second experiment. These experiments are included in the group which did not receive SQ 20881 (Table 3). The timing of the blood samples was based on the fact that most of the peaks occurred in the immediate post-stimulation blood samples in the previous experiments (first paragraph of Results section). In the experiment performed before the administration of the drug the mean prostaglandin E concentration in the control blood was 2.27 ± 0.70 ng/ml. The mean peak value, found either at the end of the stimulation period, or immediately after this, was 7.32 ± 1.6 ng/ml ($P < 0.01$, $n = 6$). The peak values were found in the second blood sample in 3 experiments and the third blood sample in 3 experiments. The mean prostaglandin F value in the control blood was 1.00 ± 0.47 ng/ml. The peak value associated with the renal nerve stimulation was 1.53 ± 0.91 ($P > 0.15$, $n = 4$).

After the administration of SQ 20881, the prostaglandin E concentration was 1.03 ± 0.31 ng/ml before stimulation and was not significantly different in the two subsequent blood samples. The prostaglandin F concentration was 0.77 ± 0.30 before stimulation and was not significantly different in the next two samples.

Pooled data. (Figure 1) In the 11 experiments in which the renal nerve was stimulated in the absence of the blocker, the mean prostaglandin E concentration in the renal venous blood before stimulation was 2.12 ± 0.41 ng/ml. The mean peak value was 6.62 ± 1.12 ($P < 0.025$).

After the administration of SQ 20881 the prostaglandin E concentration before stimulation was 1.42 ± 0.30 ng/ml and the peak value was 1.51 ± 0.37 ($n = 9$, $P > 0.4$). The values for prostaglandin F were 0.96 ± 0.29 before stimulation and the peak value was 1.48 ± 0.42 ($P > 0.1$, $n = 9$).

Table 1 Effect of renal nerve stimulation on prostaglandin-like material in renal venous blood, in the absence and presence of angiotensin I converting enzyme inhibitor (SQ 20881)

Time (min)	*Control* 0–5	*Control* 10–15	*Stim*† 25–30	*Stim*†† 30–35	*Post* 35–40	*Post* 40–45
PGE conc. ng/ml ± s.e.mean $n = 5$ Admin. of SQ 20881.	1.94 ± 0.43	0.93 ± 0.39	1.80 ± 0.91	2.19 ± 0.80	5.60 ± 1.60*	0.98 ± 0.41
PGE conc. ng/ml ± s.e. mean $n = 5$ Admin. of SQ 20881.	1.73 ± 0.45	1.08 ± 0.18	0.90 ± 0.39	0.64 ± 0.10*	1.38 ± 0.59	0.88 ± 0.44
PGF conc. ng/ml ± s.e. mean $n = 5$	1.10 ± 0.49	0.66 ± 0.40	0.92 ± 0.48	0.92 ± 0.60	0.66 ± 0.50	0.72 ± 0.72

† 5th to 10th min of stimulation; †† 10th to 15th min of stimulation.
* $P < 0.025$ when compared with first control value.

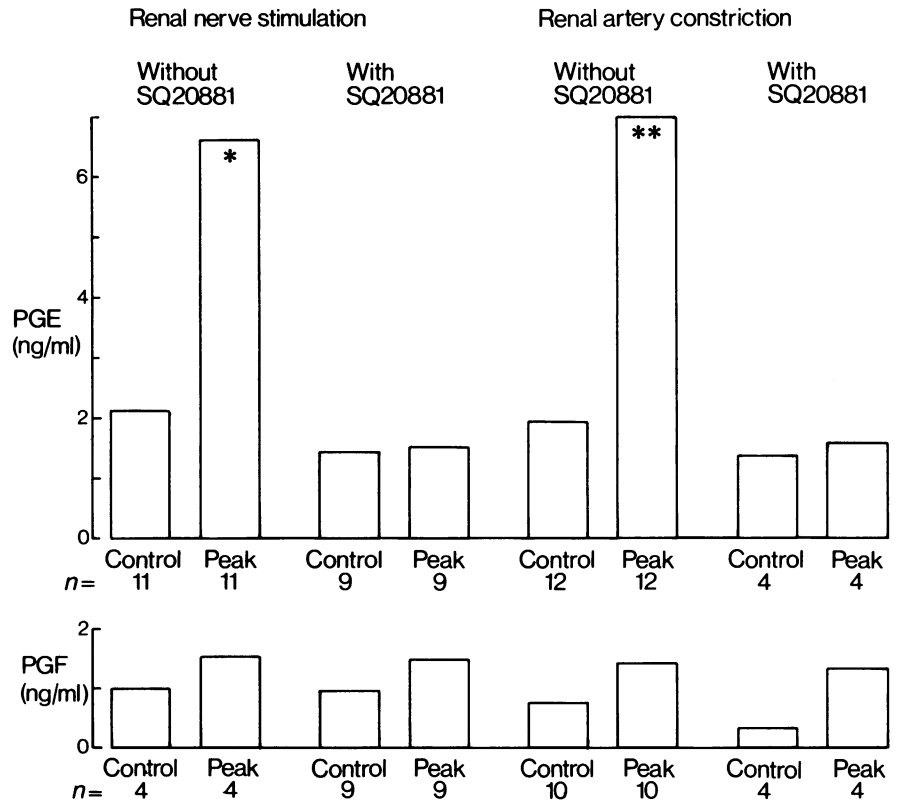

Figure 1 Summary of data from Tables 1 to 3. When two control values were available the first value was used. The peak value refers to the highest value obtained during or immediately after the application of the stimulus.
*$P < 0.025$, **$P < 0.0025$.

Renal artery constriction

Six experiments were performed in which 6 renal venous blood samples were taken before, during and after a single 15-min period of renal artery constriction which lowered renal blood flow by 15% (Table 2). The mean prostaglandin E-like material was 1.60 ± 0.85 and 1.60 ± 0.50 ng/ml in the control periods, and it rose to a peak of 7.7 ± 2.65 ($P < 0.025$) during the last 5 min of the constriction period. It was still significantly increased in the first post-constriction period (3.90 ± 0.87, $P < 0.025$) but fell to slightly under the control values in the second post-constriction period, 1.09 ± 0.39. In the individual

Table 2 Effect of renal artery constriction on the concentration of prostaglandin-like material in rabbit renal venous blood

Time (min)	*Control* 0–5	*Control* 10–15	*Constr*† 25–30	*Constr*†† 30–35	*Post* 35–40	*Post* 40–45
PGE conc. ng/ml ± s.e. mean $n=6$	1.60 ± 0.85	1.60 ± 0.50	3.10 ± 1.27	7.70 ± 2.65*	3.90 ± 0.87*	1.09 ± 0.39
PGF conc. ng/ml ± s.e.mean $n=6$	1.12 ± 0.31	0.55 ± 0.24	1.24 ± 0.54	0.80 ± 0.22	0.88 ± 0.46	0.50 ± 0.32

†5th to 10th min of renal artery constriction; ††10th to 15th min of renal artery constriction.
* $P < 0.025$ when compared with first control value.

experiments, the peak occurred in the last 5 min of the artery constriction in 4 experiments, and in the immediate post-stimulation period in two. The mean prostaglandin F-like material in the blood taken during the control periods was 1.12 ± 0.31 and 0.55 ± 0.24 ng/ml and it was not significantly different in the subsequent 4 blood samples.

Experiments performed before and after the administration of SQ 20881. Four experiments were performed in which the renal artery was constricted both before and after the administration of SQ 20881. The drug was administered every 10 minutes. In a fifth animal 2 experiments were performed in which the drug was not administered during the second experiment. These experiments are included in the group which did not receive the SQ 20881 (Table 3).

In the experiments performed without inhibitor, the concentration of prostaglandin E-like material was 2.27 ± 0.79 ng/ml in the control period. The mean peak value obtained during or immediately after the constriction was 5.5 ± 1.34 ($P < 0.05$). Before the administration of SQ 20881, the mean concentration of prostaglandin F-like material was 0.75 ± 0.48 ng/ml and it was not significantly altered by the stimulus.

After the administration of the converting enzyme inhibitor, the concentration of prostaglandin E-like material in the renal venous blood was 1.40 ± 0.56 ng/ml in the control period. The mean peak value was 1.60 ± 1.01 ($P > 0.4$). The concentration of prostaglandin F-like material in the equivalent periods was 0.35 ± 0.22 in the control period and 1.35 ± 0.78 for the peak value ($P > 0.1$).

Pooled data. (Figure 1) a total of 12 experiments was performed in which the renal artery was constricted in the absence of the blocker. In the control period before constriction the concentration of prostaglandin E-like material in the blood was 1.96 ± 0.56 ng/ml. The peak value during or immediately after constriction was 7.02 ± 1.34 ($P < 0.0025$). The comparable values for prostaglandin F-like material (10 experiments) was 0.76 ± 0.25 and the peak value during or immediately after constriction was 1.43 ± 0.42 ($P > 0.05$).

Effect of SQ 20881 on basal levels of prostaglandins E and F in renal venous blood

Using the combined data from all the experiments (the first control value when two were obtained) the mean concentration of prostaglandin E-like material in control blood samples was 2.02 ± 0.35 ng/ml ($n = 23$). After the administration of the SQ 20881 the mean concentration of prostaglandin E-like material was 1.55 ± 0.24 ($n = 13$, $P > 0.15$). For prostaglandin F, the values were 0.83 ± 0.21 ($n = 14$) before the administration of the blocker and 0.77 ± 0.22 (n = 13) after the administration of the drug ($P > 0.4$).

Discussion

The present experiments indicate that it is possible to detect small amounts of prostaglandin E- and F-like material in the renal venous blood of denervated kidneys of anaesthetized rabbits. Stimulation of the renal nerves for 15 min, to reduce renal blood flow by about 15%, resulted in a significant increase in the concentration of prostaglandin E-like material in the renal venous blood. We believe this reflects an increase in the intrarenal generation of prostaglandin E-like material. The actual secretion rate would be closely related to these changes in concentration, however, the values would require correction for the small decrease in renal blood flow during the stimulation period and

Table 3 Effect of renal nerve stimulation or renal artery constriction on prostaglandin release from the kidney before and after the administration of angiotensin I converting enzyme inhibitor in the same animal

		Renal nerve stimulation						
		Before administration of SQ 20881				*After administration of SQ 20881*		
	n	*Control*	*Peak*	P *value*	n	*Control*	*Peak*	P *value*
PGE conc. ng/ml ± s.e. mean	6	2.27 ± 0.70	7.32 ± 1.60	<0.01	6	1.03 ± 0.31	1.55 ± 0.59	>0.4
PGF conc. ng/ml ± s.e.mean	4	1.00 ± 0.47	1.53 ± 0.91	>0.15	4	0.77 ± 0.30	1.63 ± 0.55	>0.1
		Renal artery constriction						
PGE conc. ng/ml ± s.e. mean	6	2.27 ± 0.79	5.50 ± 1.34	<0.05	4	1.40 ± 0.56	1.60 ± 1.01	>0.4
PGF conc. ng/ml ± s.e. mean	4	0.75 ± 0.48	0.70 ± 0.42	>0.45	4	0.35 ± 0.22	1.35 ± 0.78	>0.1

the small increase in renal blood flow, above the control level, in the immediate post-stimulation period. As the peak of the response was found at the end of the stimulation period in about half of the experiments, and in the immediate post-stimulation period in the other half, the mean increases in concentration recorded in this study closely approximate changes in secretion rate. The present studies support earlier work on the effect of renal nerve stimulation, under slightly different experimental conditions (Dunham & Zimmerman, 1970; Davis & Horton, 1972; Mancia *et al.*, 1974).

Renal artery constriction, to reduce the renal blood flow to the same extent as with neural stimulation, also resulted in a significant increase in the release of prostaglandin E-like material which was similar in magnitude and in timing to that which occurred with neural stimulation. This is in general agreement with the reports of McGiff *et al.* (1970), who constricted the renal artery to reduce renal blood flow by 50%, and of Gross *et al.* (1976), who reduced renal blood flow by 30%, as compared with 15% in the present study.

We chose both of the stimuli used in the present study because we had previously observed, in the cat, that these manoeuvres induced the release of renin from the kidney. The mechanisms by which these stimuli produce their effect on renin release are different, as it is possible to block the effect of neural stimulation with propranolol, but not the effect of renal artery constriction (Johns & Singer, 1974). The present results with the angiotensin I converting enzyme inhibitor suggest that the generation of angiotensin II is necessary in order to demonstrate the increase in the release of prostaglandin E-like material from the kidney, regardless of the stimulus. They support the results of *in vivo* studies by Satoh & Zimmerman (1975) who reported that the effect of prostaglandin synthetase inhibitors on renal blood flow was greater when renin release was high, e.g. during partial renal artery occlusion, than under basal conditions, and that pretreatment with an angiotensin antagonist (1-sar-8-ala-angiotensin II) led to a marked inhibition of the effect of the prostaglandin synthetase inhibitors. The importance of the renin/angiotensin system in mediating the production of prostaglandin following neural stimulation might be queried because this stimulation leads to the release of noradrenaline, and it has been reported by McGiff, Crowshaw, Terragno, Malik & Lonigro (1972) that intrarenal infusion of noradrenaline leads to the release of prostaglandin from the kidney. However, as the angiotensin I converting enzyme inhibitor blocked the response, it would appear that the noradrenaline released in response to renal nerve stimulation in the present experiments was probably having its effect on prostaglandin through its renin-releasing effect. Thus, not only does blockade of prostaglandin production *in vivo* prevent the release of renin induced by a physiological stimulus such as haemorrhage (Romero *et al.*, 1976) but also the blockade of angiotensin II generation in response to the two physiological stimuli of the present study prevent the increase in prostaglandin release. How this apparent see-saw mechanism works is not clear.

Although there is much evidence that administration of kinins or kinogens leads to increased prostaglandin E release from the kidney, the present results do not support the idea that the generation of prostaglandin in the kidney is dependent on the local generation of kinins. The blocker used in these experiments, SQ 20881, is a more potent blocker of kininase than of angiotensin I converting enzyme (Engel *et al.*, 1972). Thus it is likely that if the stimuli which lead to the release of prostaglandin did so by increasing the formation of kinins which then mediated the prostaglandin response, the levels achieved, upon stimulation should have been increased. As this did not occur, it seems more likely that the increased generation of prostaglandin in response to these two stimuli is not directly dependent on the local concentration of kinins. However, it is possible that kinins may have a permissive effect on the prostaglandin response and that preventing their breakdown does not further affect their influence. The effect of aprotonin, which would block the formation of kinins, might resolve this point.

Several recent studies have indicated that renal artery constriction leads to a fall in urinary kallikrein in the dog (Keiser, Andrews, Guyton, Margolius & Pisano, 1976; Mills, Macfarlane, Ward & Obika, 1976). Keiser *et al.* (1976) report a correlation between urinary kallikrein and renal blood flow while Mills *et al.* (1976) stress the relation between renal artery pressure and urinary kallikrein. They suggest that kallikrein release is dependent on a transmural pressure-sensitive system sited beyond the afferent arteriole. The fall in transmural pressure which occurs during renal artery constriction would then lead to a decrease in the generation of kinins. If this interpretation is correct, kinin production is falling in a situation in which renin and prostaglandin are rising. As renal nerve stimulation should also lead to a fall in transmural pressure beyond the afferent arteriole, a fall in the generation of kinins should also occur under these conditions. Evidence on the effect of renal nerve stimulation on urinary kallikrein excretion is lacking. However, if kinin generation is dependent on a transmural pressure sensitive system as postulated by Mills *et al.* (1976) it is highly unlikely that kinins are involved in mediating the release of prostaglandin E during either renal artery constriction or renal nerve stimulation. The present results, indicating that there is no increase in prostaglandin release in response to the stimuli in the presence of a potent kininase, would support this view. It has recently been reported that plasma bradykinin and plasma renin activity in man are both decreased by saline infusion whereas they are

both increased by a postural stimulus, which does not appear to agree with the above suggestion. However, the relation between plasma bradykinin and urinary kallikrein is not clear, and it is possible that the changes in each of these may reflect different phenomena (Wong, Talamo, Williams & Colman, 1975).

The present experiments suggest that the basal level of prostaglandin E-like material released from the denervated kidney is not dependent on the generation of angiotensin II as the concentration was not significantly reduced following the administration of SQ 20881. The present results also indicate that the concentration of prostaglandin F-like substances in renal venous blood are not affected by the generation of angiotensin II either in the basal state or after stimulation of the nerves or after reduction of renal blood flow. Others have found a lack of responsiveness of prostaglandin F in the kidney of the dog (Dunham & Zimmerman, 1970; McGiff *et al.*, 1970). Davis & Horton (1972) did find an increase in prostaglandin F release during renal nerve stimulation in the rabbit, however, they used a much greater intensity of stimulation than was used in the present experiments, i.e. a frequency of 10 Hz as compared with 1 to 1.5 Hz in our studies.

It is apparent that the interrelationship between the renin/angiotensin system and the prostaglandin system is far from clear. In particular, it is not known whether angiotensin influences the synthesis of prostaglandins through its effect on renal haemodynamics or whether it has a direct effect on the prostaglandin producing cells. A recent *in vitro* study, using rat renal papilla, has indicated that angiotensin II will stimulate prostaglandin synthesis by regulating the availability of free precursor (Danon, Chang, Sweetman, Nies & Oates, 1975).

The present results do not, however, confirm several earlier studies in which the isolated Krebs-perfused rabbit kidney was used. In one of these studies, Needleman *et al.* (1973) reported that when angiotensin I was infused into the kidney it caused the release of prostaglandins from the kidney whether it was pretreated with CEI or not. In another study, these workers (Needleman, Douglas, Jakschik, Stoecklein & Johnson, 1974) reported that renal nerve stimulation resulted in prostaglandin release and that this effect was blocked by phenoxybenzamine. Several studies have indicated that renal nerve stimulation of renin release is a β-adrenoceptor mediated effect and that α-adrenoceptor blockade is ineffective (Passo, Assaykeen, Goldfein & Ganong, 1971; Loeffler *et al.*, 1972). This work of Needleman *et al.* (1973; 1974) therefore suggests that renal nerve stimulation on prostaglandin release is not mediated by renin. It also suggests that when renin is involved, the angiotensin I thus formed need not be converted to angiotensin II to be effective. We believe the differences between these results and some of the *in vivo* work is probably related to the use of the unnatural perfusion medium. Such kidneys do not function normally as it has been demonstrated that the isolated Krebs-perfused kidney can neither reabsorb sodium nor concentrate urine (Regoli & Gauthier, 1971). Also, injection of angiotensin I may not produce the same effects as the small quantities of locally generated angiotensin I or II produced in response to a modest stimulus. A repetition of some of the perfusion studies with homologous blood might yield different results.

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