Biochem. J. (1981) **198**, 357–363
Printed in Great Britain

Peptide-induced prostaglandin biosynthesis in the renal-vein-constricted kidney

Stuart I. MYERS,* Robert ZIPSER† and Philip NEEDLEMAN*
**Departments of Pharmacology and Surgery, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, U.S.A., and †Department of Medicine, University of Southern California–Los Angeles County Medical Center, Los Angeles, CA 90033, U.S.A.*

(*Received 19 March 1981/Accepted 6 April 1981*)

The ipsilateral kidney was removed from a rabbit 48h after unilateral partial renal-vein-constriction and was perfused with Krebs–Henseleit media at 37°C. Hourly administration of a fixed dose of bradykinin to the renal-vein-constricted kidney demonstrated a marked time-dependent increase in the release of bioassayable prostaglandin E_2 and thromboxane A_2 into the venous effluent as compared with the response of the contralateral control kidney. The renal-vein-constricted kidney produced up to 60 times more prostaglandin E_2 in response to bradykinin after 6h of perfusion as compared with the contralateral kidney; thromboxane A_2 was not demonstratable in the contralateral kidney. Inhibition of protein synthesis *de novo* in the perfused renal-vein-constricted kidney with cycloheximide lessened the hormone-stimulated increase in prostaglandin E_2 by 94% and in thromboxane A_2 by 90% at 6h of perfusion. Covalent acetylation of the renal cyclo-oxygenase by prior oral administration of aspirin to the rabbit inhibited initial bradykinin-stimulated prostaglandin E_2 biosynthesis 71% at 1h of perfusion. However, there was total recovery from aspirin in the renal-vein-constricted kidney by 2h of perfusion after bradykinin stimulation. Total cyclo-oxygenase activity as measured by [^{14}C]arachidonate metabolism to labelled prostaglandins by renal cortical and renal medullary microsomal fractions prepared from 6h-perfused kidneys demonstrated that renal-vein-constricted kidney-cortical cyclo-oxygenase activity was significantly greater than the contralateral-kidney-cortical conversion, whereas medullary arachidonate metabolism was comparable in both the renal-vein-constricted kidney and contralateral kidney. These data suggest that perfusion of a renal-vein-constricted kidney initiates a time-dependent induction of synthesis of prostaglandin-producing enzymes, which appear to be primarily localized in the renal cortex. The presence of the synthetic capacity to generate very potent vasodilator and vasoconstrictor prostaglandins in the renal cortex suggests that these substances could mediate or modulate changes in renal vascular resistance in pathological states.

We have previously demonstrated that constriction of the renal vein (48h) *in vivo* induces exaggerated prostaglandin E_2 and thromboxane A_2 synthesis during subsequent renal perfusion *ex vivo* (Zipser *et al.*, 1980). Prelabelling the renal-vein-constricted kidney with [^{14}C]arachidonate and chromatographing the renal-venous effluent demonstrated prostaglandin E_2 as the major prostaglandin product after bolus injection of the vasoactive substances bradykinin and angiotensin II. The perfused renal-vein-constricted kidney demonstrated an increasing prostaglandin E_2 and thromboxane A_2 production with increasing time of perfusion over 6h. Thromboxane A_2 was identified by both its biological half-life and by the conversion of [^{14}C]-arachidonate into [^{14}C]thromboxane B_2 by microsomal preparations from renal-vein-constricted kidney. Thus the renal-vein-constricted kidney exhibited similarities with the model of 72h ureter obstruction (hydronephrotic kidney), which also demonstrated a time-dependent exaggerated release of prostaglandin E_2 and thromboxane A_2 in response to stimulation with these vasoactive peptides (Nishikawa *et al.*, 1977; Morrison *et al.*, 1977;

0306-3283/81/080357-07$01.50/1

1978*a*). Exaggerated prostaglandin synthesis in the hydronephrotic kidney was shown to be dependent on new protein synthesis and was inhibited by infusion of cycloheximide (Morrison *et al.*, 1978*b*; Needleman *et al.*, 1979). The present study was designed to determine if the time-dependent exaggerated prostaglandin E_2 and thromboxane A_2 production in the renal-vein-constricted kidney is dependent on protein synthesis *de novo* and to attempt to ascertain the primary renal site for the enhanced prostaglandin synthesis.

Methods and materials

Preparation of renal-vein constriction and kidney perfusion

Partial renal-vein constriction was performed as previously described (Zipser *et al.*, 1980). At 24–48h after partial constriction of the left renal vein, the animals were anaesthetized (pentobarbitol, 30mg/kg, intravenously) and treated with heparin (250i.u./kg, intravenously). The abdominal cavity was entered and polyethylene catheters (PE 160; Adams, Division of Becton, Dickinson and Co., Parsippany, NJ, U.S.A.) were tied into both renal arteries. Patency of the renal vein was assessed by obstructing the renal vein adjacent to the inferior vena cava and observing blood flow after sectioning of the vessel distally. The constricting suture was removed from the renal-vein-constricted kidney. The kidneys were removed, placed in warming jackets at 37°C and perfused with oxygenated (O_2/CO_2, 19:1) Krebs–Henseleit buffer (Krebs & Henseleit, 1932) at a constant flow rate of 12ml/min.

Superfused organ system

Isolated assay tissues were continuously superfused by the renal-venous effluent to monitor constantly the presence of prostaglandin-like compounds (Ferreira & Vane, 1967). The rat stomach and chick rectum were used to monitor changes in basal prostaglandin E_2-like activity, and the rabbit aorta strip to monitor changes in basal thromboxane A_2-like activity. A mixture of antagonists to catecholamines, 5-hydroxytryptamine (serotonin), acetylcholine and histamine was infused directly over the assay tissues (Gilmore *et al.*, 1968). Indomethacin (10μg/min) was perfused directly over the assay tissues to prevent endogenous prostaglandin production (Eckenfels & Vane, 1972). Bradykinin (Boehringer Mannheim, New York, NY, U.S.A.) was prepared in a 1mg/ml stock solution of saline (0.9% NaCl). Changes in smooth-muscle contraction of the assay organs were measured with a Harvard smooth-muscle transducer (model 386; Harvard Apparatus Co., Millis, MA, U.S.A.).

Quantification of prostaglandin E_2 by bioassay

The renal-venous effluent was collected in 50ml samples before (basal) and immediately after peptide administration, acidified to pH3.5 with 1M-HCl and extracted twice with an equal volume of ethyl acetate. The extract was then vacuum-evaporated to dryness and reconstituted with 7.5ml of chloroform/methanol (2:1, v/v). Immediately before assay, the sample was dried under N_2, reconstituted with 100mM-potassium phosphate buffer, pH8.0, and bioassayed on rat stomach strips to quantify the amount of prostaglandin E_2-like activity present. Known prostaglandin E_2 standards were used to construct standard curves on the rat stomach tissues. Measurement of bradykinin-stimulated prostaglandin E_2 release was calculated as the difference between peptide-stimulated and unstimulated (basal) release measured immediately before bradykinin administration. Percentage recovery of prostaglandin E_2-like activity was calculated by injecting known standards of prostaglandin E_2 over the superfusion system, acidifying, extracting and bioassaying as described above and was 70% in our assay. Bradykinin and cycloheximide were not found to affect the assay organs in the superfusion system or affect the percentage recovery.

Radioimmunoassay

Antisera was raised to thromboxane B_2 by Dr. Aubrey R. Morrison, Department of Pharmacology, Washington University School of Medicine. The displacement curves of this antisera demonstrated cross-reactivity less than (*a*) 0.012% with prostaglandin E_2, (*b*) 0.009% with 6-oxoprostaglandin $F_{1\alpha}$, (*c*) 0.075% with prostaglandin $F_{2\alpha}$, (*d*) 0.002% with 13,14-dihydro-15-oxoprostaglandin E_2 and (*e*) 0.002% with 13,14-dihydro-15-oxoprostaglandin $F_{2\alpha}$. The titre used in this assay was that which bound 30–40% of the radioactive ligand in assay and was 1:200000. The [^{3}H]thromboxane B_2 was available commercially from New England Nuclear (Boston, MA, U.S.A.) and had specific radioactivity 100Ci/mmol.

Determination of total cyclo-oxygenase activity and radiochemical analysis

The renal-vein-constricted and contralateral kidneys were perfused *ex vivo* for 6h. The kidney was then removed and the cortex and medulla were separated, weighed and added to 3vol. (v/w) of 100mM-potassium phosphate buffer, pH8.0, at 4°C and homogenized with a Polytron homogenizer. The samples were then centrifuged at 8000*g* for 15min in a Sorval centrifuge (model RC2-B) at 0–5°C. The supernatant was removed and centrifuged at 100000*g* for 60min in a Beckman L2-75B ultracentrifuge. The microsomal pellet was resuspended

in 100mM-potassium phosphate buffer, pH 8.0, in a volume equivalent to one-fourth of the original wet weight of tissue. Incubations of cortical and medullary microsomal fractions were conducted in a final volume of 1ml. Samples (200–250μl) of cortical and medullary microsomal fractions containing approx. 0.26mg of protein for cortical and 0.33mg for medullary incubations were used, and the remainder of the 1ml incubation volume was made up of 100mM-potassium phosphate buffer, pH 8.0. All incubations were conducted at 37°C in the presence of 1.2mM-L-adrenaline (epinephrine), 1mM-reduced glutathione and [^{14}C]arachidonic acid (1μg, 300000c.p.m.) for various lengths of time. A time course of the cortical and medullary assays was performed. Medullary microsomal conversion of [^{14}C]arachidonate into [^{14}C]prostaglandins was found to be linear with time of incubation between 5 and 20min. Cortical microsomal fraction demonstrated linearity of conversion of [^{14}C]arachidonate into [^{14}C]prostaglandins between 10 and 60min time of incubation. The reaction was stopped by addition of 2M-formic acid to adjust the pH to 3.0–3.5. The samples were extracted twice with 2vol. of ethyl acetate and centrifuged at 1000*g* for 2min. The supernatant was collected, dried under N_2 and reconstituted with chloroform/methanol (2:1, v/v). Authentic unlabelled prostaglandin standards (kindly supplied by the Upjohn Co., Kalamazoo, MI, U.S.A.) and microsomal extracts were applied to the silica-gel thin-layer plate (Brinkman Instruments, Westbury, NY, U.S.A.), and were separated in a solvent system of chloroform/methanol/acetic acid/water (90:8:1:0.8, by vol.). The prostaglandin standards were located by using I_2 vapour and the plates were analysed in a Vangard radioisotope scanner (Packard Instrument Co., La Grange, IL, U.S.A.). The radioactive peaks were scraped off and their radioactivities counted in liquid-scintillation 'cocktail' (4a20; RPI Corp.) with a Beckman radioisotope counter (model LS-3133T). No apparent quenching problems were encountered during the quantification of the prostaglandins present on the thin-layer scrapings, as evidenced in our experimental samples with the automatic external standards.

Materials

Cycloheximide and acetylsalicylic acid were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and [1-^{14}C]arachidonic acid (specific radioactivity 55mCi/mmol; Amersham–Searle, Des Plaines, IL, U.S.A.) was used. Prostaglandin standards were generously given by Dr. John Pike of the Upjohn Co.

Presentation of results

Statistical significance was calculated for the bioassay, radioimmunoassay and radiochemical results by Student's *t* test. All results are expressed as means ± S.E.M.

Results

Determination of bioassayable prostaglandin E_2-like activity

Basal prostaglandin E_2-like activity from the contralateral kidney remained essentially unchanged over the 6h period of perfusion [26 ± 4ng/50ml at 1h ($n = 4$) versus 29 ± 5ng/50ml at 6h ($n = 9$)]. Basal prostaglandin E_2-like activity from the renal-vein-constricted kidney increased from 17 ± 4ng/50ml at 1h ($n = 4$) to 335 ± 67ng/50ml ($n = 13$) at 6h of perfusion ($P < 0.001$ renal-vein-constricted kidney versus contralateral kidney from 1 to 6h of perfusion). Bradykinin-stimulated prostaglandin E_2-like activity increased from 21 ± 8ng/50ml ($n = 4$) at 1h to 87 ± 50ng/50ml ($n = 9$) at 6h of perfusion in the contralateral kidney, whereas that of the renal-vein-constricted kidney ranged from 147 ± 85ng/50ml ($n = 4$) at 1h of perfusion to 5831 ± 970ng/50ml at 6h ($n = 13$) ($P < 0.001$ for renal-vein-constricted kidney versus contralateral kidney at each hourly interval) (Fig. 1). Constant infusion of cycloheximide decreased prostaglandin E_2-like activity in the renal-vein-constricted kidney after bradykinin stimulation at 6h of perfusion to 360 ± 77ng/50ml (a 94% inhibition of prostaglandin biosynthesis) (Fig. 2). Covalent acetylation of renal cyclo-oxygenase with aspirin (acetylsalicylic acid) inhibited bradykinin-stimulated prostaglandin release by 71% at 1h to a prostaglandin E_2-like activity of 43 ± 16ng/50ml ($n = 7$) versus the control value of 147 ± 85ng/50ml ($n = 4$) ($P < 0.05$). The effectiveness of the aspirin treatment *in vivo* was also validated by the inability of renal microsomal fractions, taken from the aspirin-treated animal, to metabolize [^{14}C]arachidonate (shown below). However, by 2h of perfusion prostaglandin E_2-like activity released in the aspirin-treated renal-vein-constricted kidney was almost identical with that in the control renal-vein-constricted kidney [724 ± 411ng/50ml ($n = 9$) versus 512 ± 160ng/50ml ($n = 13$)].

Determination of thromboxane B_2 production by bioassay and radioimmunoassay

At 2h of perfusion the renal-vein-constricted kidney released a substance that contracted the rabbit aorta after hormonal stimulation. This rabbit-aorta-contracting substance increased steadily with time after hormonal stimulation, as measured by direct bioassay. Direct radioimmunoassay for thromboxane B_2 in the first 50ml of renal-venous effluent of the renal-vein-constricted kidney collected

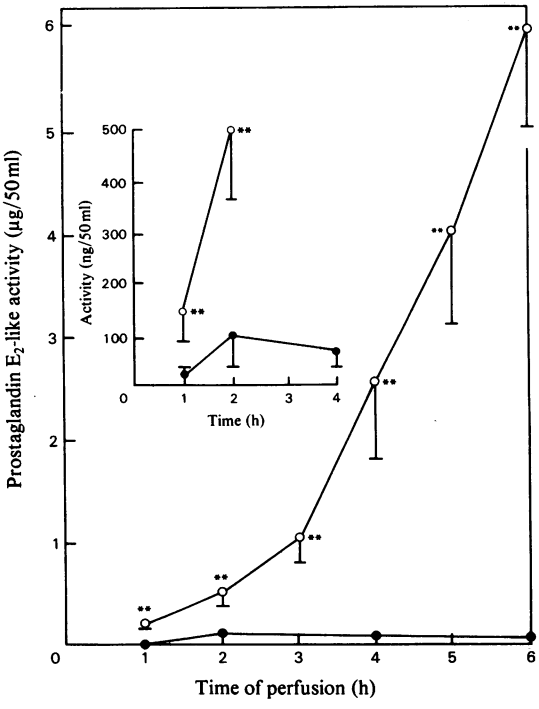

Fig. 1. *Comparison of prostaglandin E_2-like activity released after hourly bradykinin stimulation of the 24–48 h-renal-vein-constricted kidney* (○) *and surgically unaltered contralateral kidney* (●)

Each kidney was stimulated with 300 ng of bradykinin each hour for 6 h. The prostaglandin E_2-like activity was determined from dose–response curves of standard doses of prostaglandin E_2 on the rat stomach strip and expressed as μg of prostaglandin E_2-like activity/50 ml of renal-venous effluent. Values are given means ± S.E.M. ($n = 13$ for renal-vein-constricted kidney, $n = 4$ at 1 h and $n = 9$ at 2–6 h for the contralateral kidney). $^{**}P < 0.001$ compared with control.

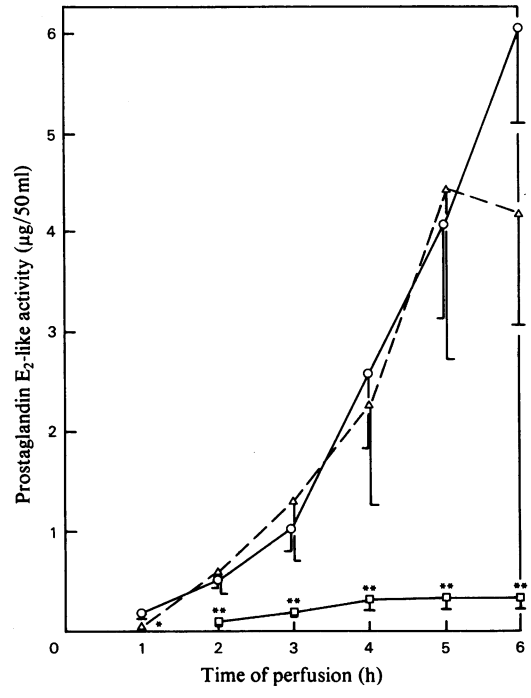

Fig. 2. *Comparison of prostaglandin E_2-like activity after hourly bradykinin stimulation of the 24–48 h-renal-vein-constricted kidney* (○), *the renal-vein-constricted kidney from animals pretreated (orally) with aspirin (2 g) 2 h before the start of perfusion* (△) *and the perfused renal-vein-constricted kidney continuously infused with cycloheximidine (50 μg/min)* (□)

For experimental details see the Methods and materials section. Each kidney was stimulated with 300 ng of bradykinin each hour for 6 h. The prostaglandin E_2-like activity is expressed as μg of prostaglandin E_2-like activity/50 ml of renal-venous effluent. Values are given as means ± S.E.M. ($n = 4$ at 1 h and $n = 13$ at 2–6 h for renal-vein-constricted kidney; $n = 7$ at 1 h and $n = 9$ for aspirin-pretreated renal-vein-constricted kidney; $n = 9$ for cycloheximide-treated renal-vein-constricted kidney). $^{*}P < 0.05$ compared with control; $^{**}P < 0.001$ compared with control.

after bradykinin stimulation demonstrated 13 ng of thromboxane B_2/50 ml at 1 h of perfusion, which increased to 51 ng/50 ml at 6 h of perfusion. Infusion of cycloheximide decreased bradykinin-stimulated thromboxane B_2 production to less than 0.1 ng/50 ml of renal-venous effluent at 1 h of perfusion, which increased only to 5 ng of thromboxane B_2/50 ml after bradykinin stimulation at 6 h of perfusion ($P < 0.01$ at 1, 3 and 6 h for renal-vein-constricted control kidney versus renal-vein-constricted kidney plus cycloheximide; see Table 1). Pretreatment of the renal-vein-constricted rabbits with oral aspirin decreased bradykinin-stimulated thromboxane B_2 production by 60% at 1 h of perfusion to 5 ng/50 ml ($P < 0.05$). However, by 3 h of perfusion there was no difference in the amount of thromboxane B_2 released by the control renal-vein-constricted kidney and the renal-vein-constricted kidney group pretreated with aspirin, showing synthesis of new cyclo-oxygenase and thromboxane synthetase activity (see Table 1).

Radiochemical analysis of total cyclo-oxygenase activity

Microsomal fractions prepared from the 6 h-perfused renal-vein-constricted kidney cortex and medulla were incubated with [^{14}C]arachidonate in the presence of cofactors. The renal-vein-constricted kidney and contralateral kidney medullas demon-

strated a linear conversion of [^{14}C]arachidonate into labelled prostaglandins between 5 and 20min of incubation. The renal-vein-constricted kidney medulla rapidly converted 54% of [^{14}C]arachidonate into labelled prostaglandin products at 5min incubation, and this rose to 88% at 20min of incubation. The contralateral kidney medulla converted 42% of [^{14}C]arachidonate into labelled prostaglandin products at 5min incubation, and this rose to 88% at 20min of incubation. There was no statistical difference between the renal-vein-constricted kidney and contralateral kidney cyclo-oxygenase activities in the medulla (see Table 2). The distribution of label from [^{14}C]arachidonate to labelled prostaglandin products in the renal-vein-constricted kidney medulla at 20min was as follows: prostaglandin E_2, $58 \pm 7\%$ (mean ± S.E.M.); prostaglandin $F_{2\alpha}$, $15 \pm 2\%$; thromboxane A_2, $6 \pm 1.5\%$, prostaglandin D, $3 \pm 0.3\%$; prostaglandin A_2, $2 \pm 0.4\%$. The contralateral kidney and renal-vein-constricted cortices demonstrated a linear conversion of [^{14}C]-arachidonate into labelled prostaglandin products between 10 and 60min of incubation. The contralateral kidney cortex converted 4% of [^{14}C]-arachidonate into labelled prostaglandin products at 10min of incubation, and this increased to 23% at 60min of incubation. The renal-vein-constricted kidney cortex was considerably more active on a protein basis, converting 18% of [^{14}C]arachidonate into labelled prostaglandin products at 10min incubation, this rising to 55% at 60min of incubation time. Cyclo-oxygenase activity in the renal-vein-constricted kidney cortex was significantly greater than in the contralateral kidney cortex at 10, 30 and 60min of incubation ($P < 0.001$ at each time; see Table 2). Conversion of [^{14}C]arachidonate

Table 1. *Radioimmunoassay of thromboxane B_2 after bradykinin stimulation of renal-vein-constricted kidney*

The results show a comparison of thromboxane B_2 activity in the renal-vein-constricted control kidney and renal-vein-constricted kidneys treated with either a constant infusion of cycloheximide (final concentration 50μg/ml) or oral aspirin (2g, 2h before cannulation). The first 50ml of renal-venous effluent was collected after bradykinin stimulation and assayed for thromboxane B_2 activity by radioimmunoassay. Results are expressed as means ± S.E.M. ($n = 4$, all groups).

	Thromboxane B_2 activity (ng/50ml)		
	1h	3h	6h
Renal-vein-contricted kidney, control	13 ± 7	25 ± 14	51 ± 15
Renal-vein-constricted kidney, +cycloheximide	<0.05**	1.5 ± 0.8**	5 ± 3**
Renal-vein-constricted kidney, +oral aspirin	5 ± 2*	35 ± 15	59 ± 16

* $P < 0.05$ compared with control.
** $P < 0.001$ compared with control.

Table 2. *Renal conversion of [^{14}C]arachidonate into [^{14}C]prostaglandins*

Cortical and medullary microsomal fractions prepared from the contralateral and renal-vein-constricted kidneys were incubated with [^{14}C]arachidonic acid (1μg, 300000c.p.m.) in the presence of 1.2mM-L-adrenaline and 1mM-reduced glutathione for various times. Extracts were chromatographed as described in the Methods and materials section. Results are expressed as means ± S.E.M. of the percentage of total labelled arachidonate converted into total labelled prostaglandin peaks (sum of all prostaglandin peaks), calculated as nmol of prostaglandin formed/mg of protein. Abbreviation: N.S., not significant.

	Conversion of arachidonate into prostaglandins (%)			
Incubation time (min)	Renal-vein-constricted kidney cortex	Contralateral kidney cortex	*P*	*n*
10	18 ± 3	4 ± 2	<0.001	8
30	35 ± 6	12 ± 3	<0.001	8
60	55 ± 7	23 ± 5	<0.001	8
Incubation time (min)	Renal-vein-constricted kidney medulla	Contralateral kidney medulla	*P*	*n*
5	54 ± 8	42 ± 3	N.S.	4
10	73 ± 8	67 ± 4	N.S.	4
20	88 ± 4	88 ± 2	N.S.	4

Table 3. *Renal conversion of* [^{14}C]*arachidonate into* [^{14}C]*prostaglandins*

Cortical and medullary microsomal fractions were prepared from the renal-vein-constricted kidney and the renal-vein-constricted kidney from animals pretreated with aspirin after 1 h of perfusion. The microsomal fractions were then incubated with [^{14}C]arachidonic acid (1 μg, 300000 c.p.m.) in the presence of 1 mM-L-adrenaline and 1 mM-reduced glutathione for 30 minutes. Extracts were chromatographed as described in the Methods and materials section. Results were calculated as nmol of prostaglandin formed/mg of protein and expressed as means ± S.E.M. (numbers of observations in parentheses) of the percentage of total labelled arachidonate converted into labelled prostaglandin peaks or into labelled prostaglandin E_2.

	Conversion of arachidonate into prostaglandins (%)			
	Renal-vein-constricted kidney		Renal-vein-constricted kidney, aspirin-pretreated (*in vivo*)	
	Total prostaglandins	Prostaglandin E_2	Total prostaglandins	Prostaglandin E_2
Cortex	14 ± 3 (11)	6.7 ± 2.8 (11)	2.2 ± 0.5 (6)	1.8 ± 0.9 (6)
Medulla	72 ± 8 (11)	50 ± 6 (11)	2.4 ± 0.5 (6)	1.2 ± 0.2 (6)

into ^{14}C-labelled prostaglandins was then compared for microsomal fractions from untreated renal-vein-constricted kidneys versus microsomal fractions prepared from renal-vein-constricted kidneys removed from aspirin-treated animals and perfused for 1 h. The microsomal fractions were incubated at 37°C for 30 min in the presence of adrenaline and reduced glutathione. The renal-vein-constricted kidney cortex and medulla converted 14 ± 3% and 72 ± 8% ($n = 11$) of [^{14}C]arachidonate into ^{14}C-labelled prostaglandins respectively. This was significantly greater than the conversion into ^{14}C-labelled prostaglandins in the cortex and medulla (2.2 ± 0.5% and 2.4 ± 0.5%, $n = 6$) from the renal-vein-constricted kidney after pretreatment with aspirin ($P < 0.001$). The conversion into prostaglandin E_2 (the major peak) mirrored changes in total cyclo-oxygenase activity (see Table 3).

Discussion

The renal-vein-constricted kidney has been shown previously (Zipser *et al.*, 1980) to exhibit enhanced prostaglandin E_2 and thromboxane biosynthesis after hormonal stimulation, this effect being markedly increased with time of perfusion (1–6 h). Cycloheximide is a potent inhibitor of new protein synthesis (Pestka, 1971). Cycloheximide has also been used as a tool for demonstrating the role of protein synthesis *de novo* of prostaglandin synthase enzyme in several systems, including the isolated Graafian follicle, rat preovulatory follicles, methylcholanthrene-transformed mouse BALB/3T3 fibroblasts and 72 h-ureter-obstructed kidney (Clark *et al.*, 1976; Zor *et al.*, 1977; Pong *et al.*, 1977; Morrison *et al.*, 1978*a*,*b*). In the present study, infusion of cycloheximide inhibited the time-dependent increase in prostaglandin production by 94% at 6 h of perfusion in the renal-vein-constricted kidney. Thus the increase in prostaglandin E_2 biosynthesis is dependent on protein synthesis *de novo*. Aspirin irreversibly covalently acetylates cyclo-oxygenase (Roth & Majerus, 1975), and pretreatment with aspirin initially inhibited prostaglandin biosynthesis by renal-vein-constricted kidney by 71% at 1 h of perfusion. However, prostaglandin biosynthesis had completely recovered by 2 h of perfusion, suggesting that cyclo-oxygenase was among the proteins synthesized *de novo*. Bradykinin-stimulated thromboxane B_2 production by the renal-vein-constricted kidney was decreased by 90% at 6 h of perfusion by infusion of cycloheximide (as measured by radioimmunoassay). Oral aspirin pretreatment inhibited bradykinin-stimulated thromboxane B_2 production by 60% at 1 h of perfusion. However, by 3 h of perfusion there was no statistical difference between thromboxane B_2 production in the aspirin-pretreated renal-vein-constricted kidneys as compared with the control renal-vein-constricted kidneys. These findings suggest that the enhanced thromboxane formation after hormonal stimulation is also dependent on protein synthesis *de novo*. This important difference in cyclo-oxygenase activity between the renal-vein-constricted kidney and the renal-vein-constricted kidney after pretreatment with aspirin was further reinforced by the use of exogenous [^{14}C]arachidonate with cortical and medullary microsomal fractions, as seen in Table 3.

Exogenous [^{14}C]arachidonate was used to examine cyclo-oxygenase activity in the renal-vein-constricted and contralateral kidney microsomal fractions. There was no apparent quantitative difference in total arachidonate metabolism by the

contralateral medulla or the renal-vein-constricted medulla microsomal fractions (Table 2). On the other hand, contralateral cortical cyclo-oxygenase activity was considerably lower than that of the renal-vein-constricted kidney-cortical microsomal fraction, which suggests that the increase in new enzyme activity is localized in the renal-vein-constricted kidney cortex.

The time-dependent hormone-responsive increase in prostaglandin biosynthesis seen in the renal-vein-constricted kidney closely parallels that seen in the model of 72h ureter obstruction (hydronephrosis). The renal-vein-constricted and hydronephrosis models both demonstrate a dependence on new protein biosynthesis to express the marked time-dependent prostaglandin and thromboxane A_2 biosynthesis and release as demonstrated after hormonal stimulation. Benabe *et al.* (1980) have demonstrated a model of glycerol-induced acute tubular necrosis in the rabbit that also demonstrates a time-dependent increase in prostaglandin and thromboxane A_2 production after hormonal stimulation. Thus three different renal models of pathology in the rabbit all demonstrate a marked induction of prostaglandin and thromboxane A_2 synthesis, suggesting a capacity for a common intrinsic renal mechanism for coping with pathological situations. The renal-vein-constricted-kidney model appears to localize this induced prostaglandin production in the cortex. Since 90% of the total renal blood flow is located in the renal cortex, the enhanced prostaglandin formation would readily influence renal vascular resistance. The biosynthesis of a vasodilator or vasoconstrictor prostaglandin, although so far demonstrated only *in vitro*, could play a role in alterations in renal vascular resistance in pathological states.

We acknowledge the excellent technical assistance of Ms. Sandra Holmberg and Ms. Karen Waters. This research was supported by funding from National Institutes of Health Grants 5T01GM0037118 (to S. I. M.), HL-14397 and HL-20787 (to P. N.) and AM-26487 (to R. Z.).

References

Benabe, J. E., Klahr, S., Hoffman, M. K. & Morrison, A. R. (1980) *Prostaglandins* **19**, 333–347

Clark, M. R., Marsh, J. M. & LeMarie, W. J. (1976) *Prostaglandins* **12**, 209–216

Eckenfels, A. & Vane, J. R. (1972) *Br. J. Pharmacol.* **45**, 451–462

Ferreira, S. H. & Vane, J. R. (1967) *Nature (London)* **216**, 868–873

Gilmore, N., Vane, J. R. & Wylie, J. H. (1968) *Nature (London)* **218**, 113–114

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66

Morrison, A. R., Nishikawa, K. & Needleman, P. (1977) *Nature (London)* **267**, 259–260

Morrison, A. R., Nishikawa, K. & Needleman, P. (1978*a*) *J. Pharmacol. Exp. Ther.* **205**, 1–8

Morrison, A. R., Moritz, H. & Needleman, P. (1978*b*) *J. Biol. Chem.* **253**, 8210–8212

Needleman, P., Wyche, A., Bronson, S. D., Holmberg, S. & Morrison, A. R. (1979) *J. Biol. Chem.* **254**, 9772–9777

Nishikawa, K., Morrison, A. R. & Needleman, P. (1977) *J. Clin. Invest.* **59**, 1143–1150

Pestka, S. (1971) *Annu. Rev. Biochem.* **40**, 697–710

Pong, S., Hong, S. L. & Levine, L. (1977) *J. Biol. Chem.* **252**, 1408–1413

Roth, G. J. & Majerus, P. W. (1975) *J. Clin. Invest.* **56**, 624–632

Zipser, R., Myers, S. I. & Needleman, P. (1980) *Circ. Res.* **47**, 231–240

Zor, U., Strulovici, B. & Linder, H. R. (1977) *Biochem. Biophys. Res. Commun.* **76**, 1086–1091