Distribution of Prostaglandins in Rabbit Kidney

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Three prostaglandins (PGE_2 , $\text{PGF}_{2\alpha}$ and PGA_2) are present in rabbit kidney medulla. An acidic lipid extract (0.165g) obtained from 2kg of frozen rabbit kidney cortex was separated by silicic acid chromatography to yield eluates containing fatty acids, possible non-polar prostaglandin metabolites, PGA , PGE and PGF compounds. Ultraviolet spectra of the eluates before and after treatment with sodium hydroxide did not yield chromophores typical of any known prostaglandins or related metabolites. By using more sensitive bioassay procedures (contraction of rabbit duodenum) weak activity equivalent to $60\mu\text{g}$ of PGE_2 and $10\mu\text{g}$ of $\text{PGF}_{2\alpha}$ was detected in the PGE and PGF eluates respectively. Extraction and bioassay of fresh kidney cortex revealed no prostaglandin-like activity. Attempts to biosynthesize prostaglandins in fresh homogenates of rabbit kidney cortex from endogenous precursors and from added arachidonic acid were unsuccessful. When freshly prepared homogenates of rabbit kidney cortex were incubated with added PGE_1 no evidence of enzymic breakdown was obtained. It is concluded that rabbit kidney prostaglandins are present predominantly in the medulla and there are no cortical mechanisms for their biosynthesis or inactivation under normal conditions.

Lee, Crowshaw, Takman, Attrep & Gougoutas (1967) reported the isolation and purification of three prostaglandins from rabbit kidney medulla and identified them as PGE_2 , $\text{PGF}_{2\alpha}$ and PGA_2 .† PGE_2 was found to be the most abundant prostaglandin present and it was demonstrated that at least part of the isolated PGA_2 could be derived as an artifact from this PGE_2 . In earlier experiments with medullary homogenates (Lee, Hickler, Savaris & Thorn, 1963), all of the vasodepressor activity of kidney was localized in the medulla and thus we decided to use medulla only in the large-scale extraction procedures. However, the actual prosta-

glandin content of rabbit kidney cortex has never been determined. Therefore large quantities of rabbit kidney cortex were extracted and separated by silicic acid chromatography. Each fraction was analysed for smooth-muscle-stimulating activity and for u.v.-absorption properties characteristic of PGA and PGE compounds and some of their known metabolites.

A preliminary report of part of this work has been published (Crowshaw, 1969).

MATERIALS AND METHODS

Fresh rabbit jejunal strips were obtained from rabbits of the New Zealand Flemish strain. Fresh rabbit kidney cortex was separated from medulla by scissor dissection as described by Lee *et al.* (1967). For large quantities (2-3kg) this procedure was performed by Pel-Freeze Biologicals Inc., Rogers, Ark., U.S.A. The fresh cortex was immediately frozen in solid CO_2 for shipment and stored at -20°C before use. Solvents were Spectro grade and redistilled before use (E. J. Curtis Co., North Wilmington, Mass., U.S.A.). Silicic acid (Bio-Sil Ha, -324 mesh) was obtained from Bio-Rad Laboratories, Richmond, Calif., U.S.A. T.l.c. was done on silica gel G or GF (E. Merck A.-G., Darmstadt, West Germany). Authentic PGE_1 , PGE_2 , $\text{PGF}_{1\alpha}$ and $\text{PGF}_{2\alpha}$ were given by Dr D. A. van Dorp, Unilever Laboratories, Vlaardingen, The Netherlands, and by Dr J. Pike, Upjohn Co., Kalamazoo, Mich., U.S.A. Frozen rabbit kidney cortex (2kg) was partially thawed and homogenized in a Waring Blender

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† Trivial names and abbreviations are based on the systematic nomenclature proposed by Bergström & Samuelsson (1967) [see Nugteren, van Dorp, Bergström, Hamberg & Samuelsson (1966) for certain revisions]: PGE_1 , prostaglandin E_1 (11,15-dihydroxy-9-oxoprost-13-enoic acid); PGE_2 , prostaglandin E_2 (11,15-dihydroxy-9-oxoprost-5,13-dienoic acid); $\text{PGF}_{1\alpha}$, 9,11,15-trihydroxyprost-13-enoic acid; $\text{PGF}_{2\alpha}$, 9,11,15-trihydroxyprost-5,13-dienoic acid; PGA_1 (formerly PGE_1 -217), 15-hydroxy-9-oxoprost-10,13-dienoic acid; PGA_2 (formerly PGE_2 -217), 15-hydroxy-9-oxoprost-5,10,13-trienoic acid; PGB_1 (formerly PGE_1 -278), 15-hydroxy-9-oxoprost-8(12),13-dienoic acid; PGB_2 (formerly PGE_2 -278), 15-hydroxy-9-oxoprost-5,8(12),13-trienoic acid.

Table 1. *Biological activity of eluates (100ml) from silicic acid chromatography*

Fraction no.	Solvent composition	Active fractions	Biological activity	
			Rat blood pressure	Rabbit duodenum
1-5	Benzene-ethyl acetate (9:1, v/v)	—	None	None (<1 μ g of PGE ₂ equiv./fraction)
6-15	Benzene-ethyl acetate (4:1, v/v)	—	None	None (<1 μ g of PGE ₂ equiv./fraction)
16-22	Benzene-ethyl acetate (7:3, v/v)	—	None	None (<1 μ g of PGE ₂ equiv./fraction)
23-28	Benzene-ethyl acetate (2:3, v/v)	23, 24, 25	Depressor	Contraction (60 μ g of PGE ₂ equiv.)
29-33	Ethyl acetate	29, 30, 31	None	Contraction (10 μ g of PGF _{2α} equiv.)

for 3 min at room temperature with an equal volume of water (200ml). The homogenate was stirred for 1 h at room temperature with an additional 3 vol. of acetone (600ml) and then stored overnight at 4°C. After filtration, the filtrate was concentrated to approx. 50ml by vacuum distillation at 35°C and diluted with a further 100ml of distilled water. The aqueous extract was partitioned three times against equal volumes of light petroleum (b.p. 35–60°C) and the organic phases were discarded. The aqueous phase was adjusted to pH 2, extracted three times with chloroform and the aqueous phase discarded. The combined chloroform phases were then concentrated and extracted with 0.1M-NaHPO₄, pH 8, to extract all the acidic lipids. Material remaining in the chloroform phases (0.66g) had no smooth-muscle-stimulating activity and was discarded. The combined phosphate buffer phases were adjusted to pH 2 with 2M-HCl, re-extracted three times with chloroform and the washed chloroform extracts were evaporated to dryness *in vacuo* to yield an oil (0.165g). This oil was dissolved in benzene-ethyl acetate (9:1, v/v) and separated on a 20g silicic acid column as described by Lee *et al.* (1967). Fractions (100ml) were collected by stepwise elution with increasing concentrations of ethyl acetate in benzene (Table 1) at an elution rate of approx. 1 ml/min. Two 10ml samples were taken from each fraction and blown down to dryness under N₂. The first sample was reconstituted in 2.5ml of ethanol-0.9% NaCl (1:9, v/v) for biological testing on isolated segments of rabbit duodenum. The second sample was dissolved in ethanol (5ml) and the u.v. spectrum of each fraction was recorded on a Zeiss PMQII spectrophotometer. For further characterization, 3.0ml of each ethanol sample was treated with 3ml of M-NaOH at 55°C for 30 min and the new u.v. spectrum was recorded. Chromophores absorbing at 500 and 237nm are indicative of PGE₂ metabolites 15-oxo-PGE₂ (Änggård & Samuelsson, 1966) and dihydro-15-oxo-PGE₂ (Bergström, Ryhage, Samuelsson & Sjövall, 1963) (Scheme 1) respectively; characteristic u.v. absorption at 278nm develops after alkali treatment of PGA- and PGE-type but not PGF-type prostaglandins (Bergström *et al.* (1963).

Biological assay. Isolated segments of rabbit duodenum were washed with warm Tyrode solution and then suspended in 10ml of oxygenated Tyrode solution at

37°C. All tissues were allowed to equilibrate with the media for 1 h before use; isotonic contractions were recorded at 1g resting tension and eightfold magnification. Contractions obtained in response to various doses of PGE₁, PGE₂ and PGF_{2 α} solutions (10 μ g/ml of 0.9% NaCl) were then compared with the responses obtained with those samples prepared from column eluates or tissue extracts.

For assays of vasodepressor activity Sprague-Dawley strain male rats weighing 250–300g, anaesthetized with pentobarbital (50ng/kg intraperitoneally), were used. After bilateral vagotomy and tracheotomy, atropine (1mg/kg) and pentolinium (20mg/kg) were given intraperitoneally. Arterial blood pressure was measured by a transducer (model P23Db, Statham) via a catheter inserted into the left carotid or femoral artery and recorded on a direct writer (Hewlett-Packard, model 7712). Polyethylene catheters were inserted into a femoral vein for injection of samples and prostaglandin standards.

Biosynthesis and metabolism. A number of incubation experiments were carried out as follows: (1) Fresh cortex (8.8g) was homogenized in 40ml of 0.1M-potassium phosphate buffer, pH 7.8. PGE₁ (50 μ g) dissolved in 0.5ml of buffer was added to the homogenate. (2) Fresh cortex (18.2g) was homogenized in 40ml of Bucher medium (Änggård & Samuelsson, 1966). PGE₁ (500 μ g) dissolved in 2ml of buffer was added to the homogenate and another sample of PGE₁ (500 μ g) was added to a second volume of buffer (40ml). The samples were incubated at 37.5°C for 60 min and the incubations were terminated by the addition of 4 vol. of ethanol. After filtration and evaporation of the ethanol, the residue was taken up in water, acidified to pH 3 and extracted three times with diethyl ether. The combined extracts were evaporated to dryness and portions tested for biological activity as described above.

Additional incubation experiments were performed to study the biosynthesis of PGE₂ and PGF_{2 α} in rabbit kidney cortex. Segments of cortex were cut off the surface of freshly obtained rabbit kidneys, care being taken not to include any portion of the outer medulla. The combined cortex slices (15.0g) were homogenized in 50ml of 0.1M-potassium phosphate buffer, pH 7.8.

Scheme 1. Naturally occurring metabolites of PGE₂ and products formed after treatment with NaOH.

Arachidonic acid (15mg in 75μl of ethanol) was added dropwise to the shaking homogenate and the mixture incubated for 1h at 37.5°C. An identical homogenate of cortex was prepared without added arachidonic acid and incubated. The incubations were terminated by the addition of ethanol and extracted as above. A third homogenate containing added arachidonic acid (15mg) was extracted without any period of incubation.

RESULTS AND DISCUSSION

None of the column samples produced a chromophore at 278nm after alkali treatment, indicating that there were no detectable PGA or PGE compounds present. Further, recently isolated metabolites of the general type shown in Scheme 1 were not detected in the column fractions (no 230nm-absorbing material was present in the eluates, and no 237, 360 or 500nm chromophores were produced by alkali). The more sensitive bioassay procedures indicated the presence of smooth-muscle-stimulating activity in fractions 23, 24 and 25 and in fractions 29, 30 and 31. The elution characteristics of prostaglandins during silicic acid chromatography have been well documented by Samuelsson (1963), and the biologically active compounds in fractions 23-25 and 29-31 have the same chromatographic properties as prostaglandins of the PGE and PGF types respectively. By comparing the biological activity of these fractions with solutions of pure PGE₂ and PGF_{2α}, it was estimated that 60μg of PGE₂-like material and 10μg of PGF_{2α}-like material were recovered from 2kg of rabbit kidney cortex.

As reported by Lee *et al.* (1967), extraction and purification of prostaglandins from 10kg of rabbit kidney medulla yielded 10mg of PGE₂ (1μg/g) and

3.2mg of PGF_{2α} (0.32μg/g). Daniels, Hinman, Leach & Muirhead (1967) reportedly isolated 62mg of PGE₂ from approx. 15kg of medulla (4.1μg/g). Thus the prostaglandin-like activity detected in the cortex is approx. 1% of that found in the medulla, and indicates that rabbit kidney is remarkable among tissues studied to date in having extremely high concentrations of prostaglandins localized within a specific area of the tissue, i.e. in the medulla. The cortex used in this work was obtained frozen from commercial sources, and it is possible that small quantities of medulla may be present in the cortex segments, this medulla being the source of the prostaglandins detected. We have extracted fresh rabbit kidney cortex and incubated homogenates of fresh cortex with and without added arachidonic acid (the known precursor of PGE₂ and PGF_{2α} in ram seminal vesicles) by using previously published procedures (van Dorp, Beerthuis, Nugteren & Vonkeman, 1964). At no time have we recovered prostaglandin-like activity after these procedures, thus confirming that rabbit renal cortex does not contain appreciable quantities of prostaglandins and is also incapable of biosynthesizing them.

A second possibility is that the cortex contains enzymes that efficiently metabolize both PGE₂ and PGF_{2α} to biologically inactive products. We were unable to detect in cortex any of the known PGE₂ metabolites shown in Scheme 1 that are capable of producing characteristic chromophores after treatment with sodium hydroxide. Moreover, when fresh homogenates of cortex were incubated for 1h with PGE₁ (500 and 50μg), prostaglandin-like activity was extracted from the incubation mixtures (500 and 37.5μg respectively). T.l.c. of the crude extracts and bioassay of eluates from the

plates indicated that all the recovered activity was present in one zone with the R_f characteristics of PGE_1 . A control incubation of PGE_1 ($500\text{ }\mu\text{g}$) gave results comparable with the experimental incubation.

These results indicate that there are no efficient mechanisms in rabbit kidney cortex for the metabolism of PGE compounds to biologically inactive metabolites, and this observation is confirmed by the failure to detect known PGE_2 metabolites in cortex. Further, the absence of appreciable quantities of prostaglandins in the cortex suggests that these hormones, located predominantly in the medulla, may act locally as medullary regulators of blood flow with related effects on Na^+ and water excretion (Johnson, Herzog & Lauler, 1967; Vander, 1968).

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