Mass spectrometric determination of prostaglandin E_2, $F_{2\alpha}$ and A_2 in the cortex and medulla of the rabbit kidney

CARIN LARSSON, ERIK ÄNGGÅRD*, *Department of Pharmacology, Karolinska Institutet, 104 01 Stockholm 60, Sweden*

Earlier work has demonstrated very active biosynthesis of prostaglandins (PG) in the renal medulla, but not in the cortex (for ref. see McGiff, Crowshaw & Itskovitz, 1974; Zins, 1975). The effects of drugs known to inhibit PG biosynthesis have however indicated that endogenous PGs have effects on cortical blood flow (Aiken & Vane, 1973; Itskovitz, Terragno & McGiff, 1974; Itskovitz & McGiff, 1974; Kirschenbaum, White & others, 1974; Larsson & Änggård 1974; Solez, Fox & others, 1974; Tannenbaum, Splawinski & others, 1975).

An explanation to this apparent contradiction was provided by our earlier work (Änggård, Larsson & Samuelsson, 1971; Larsson & Änggård, 1973) demonstrating high concentrations of 15-hydroxy PG dehydrogenase in the kidney cortex and that ^{3}H-labelled arachidonic acid was converted to 15-ketometabolites, presumably by way of PGE_2 and $PGF_{2\alpha}$. PGs formed in the cortex are thus subject to a more active metabolism than PGs formed in the medulla. One object of the present study was to determine with a direct and specific method—gas chromatography-mass spectrometry—the presence of PGs in the kidney cortex.

Another objective of the present study was to determine whether or not PGA_2 is naturally present in the rabbit kidney. PGA_2 was an important constituent of the 'medullin' discovered by Lee, Crowshaw & others (1967) but might be formed by dehydration from PGE_2 during the isolation procedure. In the present work we have employed a double labelling procedure to determine how much PGA_2 was present originally in the tissue and how much was formed during the work up and analysis.

^{3}H-Labelled PGA_2, ^{14}C-PGE_2, and ^{3}H-$PGF_{2\alpha}$ (all 30 Ci $mmol^{-1}$) were from New England Nuclear Corp., Boston, Mass, U.S.A. The radiopurity was >97%. Tetradeutero PGE_2 and $PGF_{2\alpha}$ were obtained from the Upjohn Company (Kalamazoo, Michigan) through the courtesy of Dr. U. Axen. These compounds gave single spots with R_F values identical with those of unlabelled reference compounds. Tetradeutero-PGA_2 was prepared from tetradeutero-PGE_2 by treatment with concentrated acetic acid at 80° for 16 h and purified by silicic acid chromatography. Unlabelled PGs were supplied through the generosity of Ono Pharmaceutical Co., Osaka, Japan.

Male New Zealand rabbits were anaesthetized with urethane (1·2–2 g kg^{-1}) and the kidneys removed. The cortex and medulla were dissected and a 20% homogenate prepared in a 0·1M potassium phosphate buffer pH 7·4 containing deuterium and radioactively labelled internal standards of PGA_2, PGE_2 and $PGF_{2\alpha}$. PG standards were added in the following amounts: ^{3}H-PGA_2 and ^{3}H-$PGF_{2\alpha}$ 0·2 ng, ^{14}C-PGE_2 50 ng and tetradeutero PGA_2, $-PGE_2$ and $-PGF_{2\alpha}$ 1 μg each, except for medulla when 5 μg of tetradeutero-PGE_2 and $PGF_{2\alpha}$ were added. The possible conversion of PGE_2 to PGA_2 (Schneider, Pike & Kupiecki; 1966; Andersen 1969) during the isolation procedure was followed by determination of ^{14}C-PGE_2 converted to ^{14}C-PGA_2. The homogenate was centrifuged for 1 h at 100 000 *g*, the supernatant decanted off and acidified with formic acid to pH 4 and applied on an Amberlite XAD-2 column (Gréen, 1971). The PGs were eluted with methanol. The extracted PGs were evaporated to dryness and esterified with diazomethane in methanol and the methylester applied on a Sephadex LH-20 column (Änggård & Bergkvist, 1970) for group separation into PGA, E and F. The fractions with radioactive PGA_2, PGE_2 and $PGF_{2\alpha}$ were collected. The amount of ^{14}C recovered in the PGA_2 fraction and PGE_2 fraction respectively was used to calculate the formation of PGA_2 from PGE_2 during the isolation procedure until analysed by mass fragmentography. Before injection of samples into the mass spectrometer, they were silylated according to Nicosia & Galli (1974) with *N*-trimethylsilylimidazole and piperidine. In this derivatization PGE and PGA compounds are converted to the corresponding PGBs. The samples were reduced to dryness, dissolved in 50 μl of benzene and stored at −20°. The instrument was equipped with a LKB 9000 accelerating voltage alternator. The column was a 1%, 4 foot SE-30, temperature was 240° and the energy of the electrons 22·5 eV. In the analysis of the PGB_2-derivative the instrument was focused on the *m/e* values at 321 and 325 corresponding to the protium and deuterium forms respectively. In the analysis of $PGF_{2\alpha}$ the fragments at 307 and 311 were used. PGA_2, PGE_2 and $PGF_{2\alpha}$ present in the sample were calculated from standard curves, obtained from known amounts of the protium and deuterium forms of the respective PGs.

The concentrations of PGA_2, PGE_2 and $PGF_{2\alpha}$

Table 1. *Mass spectrometric determination of prostaglandins in homogenates of the rabbit kidney.*

	Cortex $\mu g\ g^{-1}$	Medulla $\mu g\ g^{-1}$
PGE_2	0·19 ± 0·04	4·36 ± 1·04
$PGF_{2\alpha}$	0·21 ± 0·07	1·64 ± 0·50
PGA_2*	0·015 ± 0·007	0·21 ± 0·14

*Not corrected for the PGE_2 to PGA_2 conversion. All or almost all can be accounted for by this conversion (about 8% of PGE_2).

* Correspondence

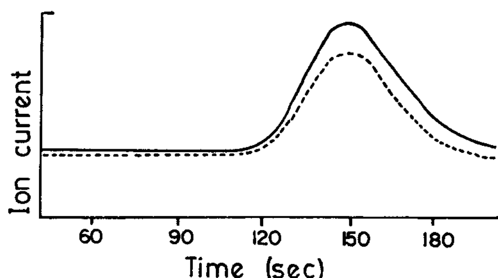

FIG. 1. Mass fragmentographic analysis of PGE_2 in renal cortical itssue. The instrument was focused on 321 (———) and 325 (- - - -) corresponding to the protium and deuterium form respectively.

present in the cortex and medulla of rabbit kidney are shown in Table 1. In general the PGE_2 and $PGF_{2\alpha}$ concentrations were 10–120 fold higher in the medulla than in the cortex. However, compared with concentrations of PGs in other tissues (for refs see Piper, 1973) the amounts in the renal cortex are quite high. A mass fragmentogram of a PGE_2 analysis in cortical tissue is shown in Fig. 1.

The concentrations of PGs found in this study are not those present *in vivo*. If a rabbit kidney is homogenized in organic solvents immediately after removal the concentrations of PGs are not in the low ng g^{-1} range (Änggård, Bohman & others, 1972). The major part of the PGs are thus formed during the work up and therefore represent the biosynthetic capacity of the tissue rather than true tissue levels.

The concentrations of $PGF_{2\alpha}$ were similar to those of PGE_2 in the cortex. In the medulla the $PGF_{2\alpha}$ concentrations were somewhat lower than those of PGE_2 but in the same range. The effects of drugs stimulating and inhibiting PG biosynthesis can be rationalized in terms of formation of PGE_2 (McGiff, & others, 1974). $PGF_{2\alpha}$ on the other hand is relatively inactive on parameters of renal function. Since a 9-keto reductase has recently been described in the kidney (for refs see Stone & Hart, 1975) it is possible that $PGF_{2\alpha}$ could originate from PGE_2 rather than by a reductive cleavage of the PG endoperoxides.

The relative distribution of PGs in the kidney also raises the question of the significance of the endoperoxide precursors PGG_2 and PGH_2 (Hamberg, Svensson & others, 1974). The activity of these PGs as well as their metabolism into other PG related compounds will need to be studied further.

The amounts of PGA_2 were very small. That found in samples from the medulla could be entirely accounted for by the conversion (about 8%) of E_2 into A_2. In some experiments, trace quantities of PGA_2 were found in the cortex. These amounts were less than 1% of the total PGs found in the sample. Our conclusion is, therefore, that in the rabbit kidney PGA_2 is not present in amounts sufficient to elicit any important physiological actions. These results are in agreement with the recent findings of Frölich, Sweetman & others (1975) and of Steffenrud (1975) who, using mass fragmentography, were unable to demonstrate the presence of PGA_2 in the kidneys of dog, rabbit and man and in human peripheral plasma down to 10–25 pg ml^{-1}.

The most important positive finding of the present study was the unequivocal demonstration of significant amounts of PGs in the renal cortex. This provides a biochemical basis for a direct interaction of PGs with cortical structures such as arterioles, regulating the renal bood flow, and the juxtaglomerular apparatus, regulating release of renin.

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The effect of co-trimoxazole on thymidine uptake by transforming human lymphocytes *in vitro*

H. J. ROGERS*, P. S. LIETMAN, *Division of Clinical Pharmacology, Department of Pharmacology and Experiment a Therapeutics, Johns Hopkins Medical School, Baltimore, Maryland 21205, U.S.A.*

Gaylarde & Sarkany (1972) have reported that co-trimoxazole (trimethoprim, TMP and sulphamethoxazole, SMX) decreases the uptake of labelled thymidine by human lymphocytes cultured in the presence of phytohaemagglutinin *in vitro*. This phenomenon was pronounced (mean suppression 84%) but only observed in 60% of a small group of subjects, prompting these authors to suggest that this could represent some pharmacogenetic difference between these two populations of subjects. During the course of a systematic study of the effects of TMP upon human lymphocytes stimulated to undergo lymphoblastic transformation with concanavalin A (con A) *in vitro* we have re-investigated these findings in this system.

Our population consisted of healthy Caucasian medical students and staff who were taking no drugs (except for one taking an oral contraceptive pill) and who had abstained from coffee or tea for 12 h before venepuncture. Most were non-smokers. Only one subject had been previously exposed to co-trimoxazole: results from this subject were not different from the others. Blood was drawn at approximately the same time each morning to obviate diurnal rhythmicity in lymphocyte responsiveness (Tavadia, Fleming & others, (1975). Heparinized venous blood from each subject was sedimented by gravity for 2 h, the leucocyte-rich plasma being periodically withdrawn into a sterile tube. After twice washing the cells with Medium TC 199 they were resuspended in TC 199 containing 10% foetal calf serum to give a concentration of 10^6 lymphocytes ml^{-1} as determined by visual cell counting. 1 ml cultures were set up in triplicate containing 40 $\mu g\ ml^{-1}$ con A and appropriate additions of drugs made. Both TMP and SMX were dissolved in 50% ethanol and added in a total volume of 10 μl. Preliminary experiments using this vehicle showed that this concentration of ethanol did not significantly alter thymidine uptake. Cultures were incubated at 37° in 5% carbon dioxide/air mixture for 68 h when 1 μCi thymidine [methyl-^3H] (6·7 Ci mM^{-1}: New England Nuclear Corporation, Boston, Mass.) was added. After a further 3 h culture the incubation was terminated by two 3 ml washes with ice-cold 5% trichloroacetic acid and a 3 ml methanol wash. The acid-insoluble residue was dissolved in 0·5 ml 1M Hyamine-10-X in methanol at 70° for 15 min and washed into counting vials with ethanol. A PPO-POPOP scintillant was used to count tritium to an efficiency of 15%. The results are shown in Table 1.

Using drug concentrations similar to those employed by Gaylarde & Sarkany (SMX 2×10^{-5}M; TMP 5×10^{-6}M) we could detect no significant inhibitory effect. At a higher concentration of SMX (4×10^{-4}M which is ~normal therapeutic plasma concn) there was a small but statistically insignificant ($t = 1·3$) inhibitory effect whilst at 5×10^{-5}M, TMP (~ normal plasma concn 10^{-5}M) clearly inhibits thymidine incorporation (Rogers & Lietman, 1975). Combining these drugs at the higher concentrations produced a slightly greater inhibition but this was not significant statistically ($t = 2·35$). Cellular viability as assessed by the trypan blue exclusion method (Ling, 1968) was unaffected by drug treatment.

Since the incubation medium contained 10% foetal

Table 1. *Effects of TMP and SMX alone and in combination of [^3H]thymidine uptake by con A-stimulated lymphocytes*

No. of subjects	Mean age (range)	% incorporation [^{3}H]thymidine *vs* control (with s.d.)		
		SMX 2×10^{-5} M	TMP 5×10^{-6} M	SMX 2×10^{-5} M TMP 5×10^{-6} M
13 (9 male)	25 (20–32)	99·3 s.d. 16·0	102·7 s.d. 12·7	103·7 s.d. 10·4
		SMX 4×10^{-4} M	TMP 5×10^{-5} M	SMX 4×10^{-4} M TMP 5×10^{-5} M
10 (8 male)	26 (21–32)	90·8 s.d. 22·4	69·6 s.d. 13·2	56·4 s.d. 16·2

*Correspondence: Depts. of Pharmacology and Clinical Pharmacology, Guy's Hospital Medical School, London, SE1.