Biosynthesis of Prostaglandins in Rabbit Kidney Medulla

PROPERTIES OF PROSTAGLANDIN SYNTHASE

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A simple radioactive-substrate assay for prostaglandin synthase (EC 1.14.99.1), which uses t.l.c. to measure simultaneously different prostaglandins synthesized from one precursor substrate, was developed. Rabbit kidney-medulla prostaglandin synthase catalyses the formation of prostaglandin E₂, prostaglandin F_{2α} and prostaglandin D₂ from arachidonic acid. Fractionation of crude homogenates indicated that the microsomal fraction possessed the highest specific activity of prostaglandin synthase, whereas the soluble fraction exhibited little enzyme activity but rather contained a heat-labile inhibitory macromolecular factor(s), which might be attributed to the serum albumin present in this fraction. The microsomal fraction possessed low intrinsic enzyme activity, but the activity could be fully stimulated by the presence of both GSH (reduced glutathione) and a phenolic cofactor. Only cysteine could partially replace GSH, whereas other thiols were inactive and some were even inhibitory. A variety of phenolic compounds, including catecholamines, dopamine (3,4-dihydroxyphenethylamine), 5-hydroxytryptamine and quinol, were active in stimulating prostaglandin synthase. In all cases, the stimulation was reflected in the synthesis of all three prostaglandins with ratios not significantly altered by different phenolic cofactors. The synthesis of each of the different prostaglandins appeared to have similar pH optima. The enzyme system was not inhibited by thiol-group inhibitors or a variety of metal chelators except for cyanide and 8-hydroxyquinoline. Characterization of the kidney-medulla prostaglandin synthase system indicated that it exhibited properties similar to those of the enzyme system present in seminal vesicles.

Prostaglandins are thought to be virtually ubiquitous in mammalian tissues and to exert a wide variety of striking physiological and pharmacological actions (Bergstrom *et al.*, 1968; Ramwell *et al.*, 1968; Horton, 1969). They are derived biosynthetically from certain C₂₀ polyunsaturated fatty acids by reactions catalysed by prostaglandin synthase (EC 1.14.99.1) (Bergstrom *et al.*, 1964; Van Dorp *et al.*, 1964). Properties of this enzyme complex from sheep and bovine seminal vesicles have been described in detail (Samuelsson, 1969; Takeguchi *et al.*, 1971; Flower *et al.*, 1973), but extension of this work to other tissues and organs has been limited. Biosynthesis of prostaglandins in the rabbit kidney medulla has been investigated by Hamberg (1969) and Crowshaw (1971, 1973), who showed that arachidonic acid could be readily converted into prostaglandin E₂ (11 α ,15 α -dihydroxy-9-oxoprostano-5-*cis*,13-*trans*-dienoic acid) and prostaglandin F_{2 α} (9 α ,11 α ,15 α -trihydroxyprosta-5-*cis*,13-*trans*-dienoic acid). However, these studies are still limited to using tissue slices or

crude homogenates. Little information has been accumulated about the properties of prostaglandin synthase from renal tissue or of the factors regulating its activity. Such observations would be of considerable interest in view of the proposition (Lee, 1969) that renal prostaglandins may normally exert a regulatory antihypertensive function.

We have developed a simple radioactive-substrate assay for prostaglandin synthase which uses t.l.c. to separate different prostaglandins formed from the substrate, arachidonic acid. This assay allows the quantitative measurement of the enzyme complex from kidney-medulla tissue and the characterization of its properties.

Materials

all-*cis*-5,8,11,14-Eicosatetraenoic acid (arachidonic acid), GSH (reduced glutathione), catecholamines, 5-hydroxytryptamine (serotonin), *N*-acetyl-5-methoxytryptamine (melatonin), 5-hydroxyindol-3-ylacetic acid, L-thyroxine, L-tri-iodothyronine, L-ascorbate, histamine, quinol, 8-hydroxyquinoline, *o*-phenanthroline, EGTA [ethanedioxybis(ethylamine)tetra-acetic acid], NaN₃, thioglycolic acid,

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dithiothreitol, 2-mercaptoethanol, sodium mersalyl, *p*-hydroxymercuribenzoate, *N*-ethylmaleimide, 5,5'-dithiobis-(2-nitrobenzoic acid), diethyldithiocarbamate and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. [$1\text{-}^{14}\text{C}$]Arachidonic acid (58 mCi/mmol) was obtained from Applied Science Laboratories, State College, Pa., U.S.A. *p*-Aminophenol was a product of Distillation Products, Eastman Kodak Corp., Rochester, N.Y., U.S.A. Pre-coated silica-gel G plates were obtained from Brinkman Instruments, Westbury, N.Y., U.S.A. Frozen rabbit kidney medulla was purchased from Pel-Freeze Biologicals, Rogers, Ark., U.S.A. Indomethacin [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-ylacetic acid] and meclofenamic acid [*N*-(2,6-dichloro- α,α,α -trifluoro-*m*-tolyl)anthranilic acid] were kindly provided by Merck, Sharp and Dohme Research Laboratories, Rahway, N.J., U.S.A. Prostaglandins E_2 and $\text{F}_{2\alpha}$ were kind gifts from Dr. John Pike of the Upjohn Co., Kalamazoo, Mich., U.S.A. Prostaglandin D_2 (9 α ,15 α -dihydroxy-11-oxoprostano-5-*cis*,13-*trans*-dienoic acid) was prepared as described by Foss *et al.* (1972). Pig kidney 15-hydroxyprostaglandin dehydrogenase (EC 1.1.1.141) was prepared as described by Tai *et al.* (1974).

Methods

Preparation of homogenates and cell fractions

The following operations were carried out at 4°C. Frozen rabbit kidney medulla was thawed and homogenized in a Waring Blendor for 30 s in 2 vol. of 0.1 M-Tris/HCl buffer, pH 7.5. The suspension was further homogenized in a Potter-Elvehjem homogenizer by three up-and-down strokes of the Teflon pestle. The homogenate was centrifuged at 700g for 10 min in a Lourdes refrigerated centrifuge. This precipitate was designated the nuclear pellet. The supernatant was then centrifuged at 8500g for 10 min in the same centrifuge and the precipitate was designated the mitochondrial pellet. The supernatant was further centrifuged at 105000g for 60 min in a Beckman model L-2 centrifuge (rotor no. 40). A floating layer was removed by a pipette, and the supernatant (S_{105}) was then collected. The sedimented material, designated the microsomal pellet, was homogenized in 0.1 M-Tris/HCl buffer, pH 7.5, in a volume equal to one-quarter of the original medulla weight and stored in 1 ml batches at -76°C. Unless otherwise stated, this microsomal fraction was used throughout the study.

Purification of arachidonic acid

Both the commercially available unlabelled and labelled arachidonic acid invariably contained some polar impurities. Removal of these impurities was done by silica-gel G t.l.c. with light petroleum

(b.p. 37.4–49°C)/diethyl ether/acetic acid (70:30:1, by vol.) as the solvent system. The region corresponding to arachidonic acid was scraped off the plate and extracted with ethyl acetate and the extract after evaporation under N_2 atmosphere was stored at -20°C. In some cases the substrate was purified by chromatography on a silica-gel column equilibrated and developed with *n*-hexane containing 1% acetic acid as described by Flower *et al.* (1973).

Enzyme assay

Prostaglandin synthase activity was assayed with [$1\text{-}^{14}\text{C}$]arachidonic acid as substrate. The assay mixture contained 0.1 M-Tris/HCl buffer, pH 8.0, 1 mM-GSH, 1 mM-L-adrenaline, 60 nmol of [$1\text{-}^{14}\text{C}$]arachidonic acid (40000 c.p.m.) and microsomal fraction as indicated in the Table and Figure legends in a final volume of 1 ml. Incubations were in air at 37°C for 5 min with shaking and were stopped by the addition of 0.03 ml of 12 M-formic acid (final pH 3.5). The reaction mixture was then extracted with two 3 ml portions of ethyl acetate. The combined extracts were evaporated under N_2 at 40°C in a water bath. The residue was taken up in a small volume of acetone, quantitatively spotted on silica-gel G t.l.c. plates (2 cm × 20 cm) and developed to a height of 12.5 cm with the organic layer of ethyl acetate/acetic acid/iso-octane/water (11:2:5:10, by vol.) as the solvent system. The plates were divided into four bands, prostaglandin $\text{F}_{2\alpha}$ ($R_F = 0.17$), prostaglandin E_2 ($R_F = 0.29$), prostaglandin D_2 ($R_F = 0.43$) and arachidonic acid plus hydroxy fatty acids ($R_F > 0.8$) after co-chromatography with the authentic compounds and detection with I_2 vapour. Each band was transferred to a scintillation vial and the radioactivity was determined in dioxan containing 10% (w/v) naphthalene, 0.4% 2,5-diphenyloxazole and 0.005% 1,4-bis-(5-phenyloxazol-2-yl)benzene with a Beckman liquid-scintillation counter model LS-233.

Reaction with 15-hydroxyprostaglandin dehydrogenase

The reaction products were scraped off the t.l.c. plate and extracted with two 1 ml portions of ethanol. The extract was blown dry with N_2 gas and the residue was dissolved in 1 ml of 0.1 M-potassium phosphate buffer at pH 7.5 containing 1 mM-EDTA and 1 mM- NAD^+ . Pig kidney hydroxyprostaglandin dehydrogenase (2.4 munits) was added and the reaction was carried out at 37°C for 45 min. The reaction mixture was acidified, extracted and chromatographed in the same manner as described under 'Enzyme assay'.

Reduction with NaBH_4

Samples were dissolved in 1 ml of methanol, cooled to 0°C, and 5 mg of NaBH_4 was added.

Reaction was allowed to proceed at room temperature for 60 min. The reaction mixture was diluted with 1 ml of water, acidified to pH 3.5 with 12M-formic acid, and extracted with 4 vol. of ethyl acetate. The organic layer was evaporated to dryness with a stream of N_2 gas.

Protein determination

Protein concentrations were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Results

Subcellular localization of prostaglandin synthase

The microsomal pellet of rabbit kidney-medulla tissue catalysed the formation of 9.87 nmol of prostaglandin/mg of protein in 5 min, which was the highest specific activity of prostaglandin synthase among the subcellular fractions analysed. The nuclear fraction and mitochondrial pellet catalysed the formation of 4.59 and 2.06 nmol of prostaglandin/mg of protein in 5 min respectively, whereas the soluble fraction contained little activity. The following studies were carried out by using microsomal fraction as the source of the enzyme system.

Identification of reaction products

When microsomal fraction was incubated with [$1-^{14}C$]arachidonic acid in the presence of the activators adrenaline and GSH (see below), three prominent radioactive peaks in the polar region of the thin-layer chromatogram were observed, as shown in Fig. 1. The most polar peak ($R_F = 0.17$) was identified as prostaglandin $F_{2\alpha}$, the second polar peak ($R_F = 0.29$) as prostaglandin E_2 , and the third polar peak ($R_F = 0.43$) as prostaglandin D_2 . Identification of these reaction products was achieved by co-chromatography of authentic prostaglandin $F_{2\alpha}$, prostaglandin E_2 and prostaglandin D_2 with these radioactive peaks in three solvent systems, namely, solvent system I (ethyl acetate/acetic acid/iso-octane/water, 11:2:5:10, by vol.), solvent system II (benzene/dioxane/acetic acid, 20:20:1, by vol.) and solvent system III (chloroform/methanol/acetic acid, 18:1:1, by vol.). The reaction products had exactly the same mobility as the authentic prostaglandin $F_{2\alpha}$, prostaglandin E_2 and prostaglandin D_2 respectively in all three solvent systems. Further confirmation of the identities of these reaction products was obtained by isolation from the t.l.c. plate and by treatment with 15-hydroxyprostaglandin dehydrogenase from pig kidney (Tai *et al.*, 1974). The first and second polar products were converted by this enzyme in the presence of NAD^+ into metabolites that had the same mobilities on t.l.c. plates developed in solvent system

I as those of 15-oxoprostaglandin $F_{2\alpha}$ and 15-oxoprostaglandin E_2 respectively. However, the third product was poorly oxidized by the same enzyme. Since borohydride reduction of prostaglandin D_1 ($9\alpha,15\alpha$ -dihydroxy-11-oxoprost-13-*trans*-enoic acid) afforded predominantly prostaglandin $F_{1\alpha}$ (Foss *et al.*, 1972), the same treatment of the third polar product yielded a major component which had a mobility identical with that of the prostaglandin $F_{2\alpha}$ t.l.c. plate developed in solvent system I as shown in Fig. 2.

A minor non-polar peak, located next to that of the substrate arachidonic acid, was probably hydroxy fatty acid. However, its structure was not further identified.

Assay conditions for prostaglandin synthase

Fig. 3 shows the time-course of the prostaglandin synthase reaction. The rate of formation of each of the prostaglandins was not precisely linear with time at any given period of time, but was nearly linear during the first 5 min of incubation in most experiments; this time-range was therefore chosen. Fig. 4 presents the effect of increasing the amount of microsomal protein on the extent of prostaglandin synthesis. A non-linear relationship between microsomal protein and the amount of prostaglandin formed was

Fig. 1. Thin-layer radiochromatogram of lipids formed on incubation of arachidonic acid with microsomal fraction from rabbit kidney medulla

The microsomal fraction (0.6 mg of protein) was incubated in 1.0 ml of 0.1 M-Tris/HCl buffer, pH 8.0, with [$1-^{14}C$]arachidonic acid (40000 c.p.m., 60 nmol) in the presence of 1 mM each of L-adrenaline and GSH at 37°C for 5 min. Extraction of lipids and t.l.c. were essentially as described in the Methods section. Strips (0.5 cm) of the chromatogram were scraped off the plate and the radioactivity was determined by liquid-scintillation counting. PG, Prostaglandin.

Fig. 2. Thin-layer radiochromatogram of one of the reaction products, prostaglandin D₂, before and after reduction with NaBH₄.

The microsomal fraction was incubated with substrate and activators under the same conditions as described in Fig. 1 except that the incubation was extended to 15 min. Prostaglandin D₂ regions from the t.l.c. plates from four such incubations were pooled, extracted and dried as described in the Methods section. The extract was divided into two equal portions, of which one was treated with NaBH₄. Incubation, extraction and chromatography were as described in the Methods section. (a) Before NaBH₄ reduction; (b) after NaBH₄ reduction. PG, Prostaglandin.

Fig. 3. Time-course of the prostaglandin synthase reaction

Incubation and assay conditions were as stated in the Methods section; 0.6 mg of microsomal protein was used for assay. PG, Prostaglandin.

observed. To obtain a significant amount of prostaglandin formation, an arbitrary 0.5–1 mg of microsomal protein (depending on the activity of the enzyme preparation) was chosen for most of the assays.

Fig. 4. Rate of synthesis of different prostaglandins as a function of microsomal protein

Incubation and assay conditions were as described in the Methods section, except that the amount of the microsomal fraction was varied as indicated. PG, Prostaglandin.

Endogenous effectors in the soluble fraction (S₁₀₅)

Like the microsomal fraction of sheep and bovine seminal vesicles, the microsomal fraction alone of kidney-medulla tissue exhibited low prostaglandin synthase activity. However, addition of heat-treated soluble fraction enhanced the enzyme activity more than did addition of untreated soluble fraction, indicating the presence of both activator(s) and inhibitor(s) in the soluble fraction, as shown in Table 1. When microsomal enzyme activity was assayed in the presence of a saturating concentration of external activators (see below), addition of the soluble fraction to the reaction mixture produced only inhibition. The inhibitory effect was not abolished by prior dialysis of the soluble fraction but was relieved by boiling. These observations suggest that the inhibitor(s) is a heat-labile macromolecule.

Effect of serum albumin on prostaglandin synthase activity

Serum albumins are known to bind fatty acids, including arachidonic acid. It is possible that these proteins bind arachidonic acid, making this substrate inaccessible to the prostaglandin synthase system. Table 2 shows that increasing amounts of bovine serum albumin inhibited prostaglandin synthesis in both the absence and the presence of boiled soluble fraction.

Table 1. *Effect of soluble fraction (S₁₀₅) on prostaglandin synthesis*

Fraction S₁₀₅, which contained 17.2 mg of protein/ml, was dialysed overnight at 4°C against 100 vol. of the buffer used for preparing homogenates to obtain dialysed fraction S₁₀₅. Boiled fraction S₁₀₅ was prepared by boiling fraction S₁₀₅ for 2 min, the precipitate being removed by centrifugation; 0.5 ml of fraction S₁₀₅ or dialysed fraction S₁₀₅ or boiled fraction S₁₀₅ was used where indicated. The concentrations of L-adrenaline and GSH used for assay were 1 mM each where indicated; 3.2 mg of microsomal protein was used for each assay. The result of a representative experiment is shown.

Additions	Total prostaglandins (nmol)
None	3.11
Fraction S ₁₀₅	4.11
Boiled fraction S ₁₀₅	5.59
L-Adrenaline+GSH	11.73
L-Adrenaline+GSH+fraction S ₁₀₅	7.29
L-Adrenaline+GSH+dialysed fraction S ₁₀₅	7.16
L-Adrenaline+GSH+boiled fraction S ₁₀₅	11.54

Table 2. *Effect of bovine serum albumin on prostaglandin synthesis*

The assays were carried out in the presence of 1 mM each of L-adrenaline and GSH. Boiled fraction S₁₀₅ was prepared as described in Table 1; 0.6 ml was used where indicated. Other conditions were as indicated in the Methods section; 1.5 mg of microsomal protein was used for each assay. The result of a representative experiment is shown.

Additions	Total prostaglandins (nmol)
None	5.90
Bovine serum albumin (1 mg)	4.10
Bovine serum albumin (3 mg)	1.22
Boiled fraction S ₁₀₅	5.08
Boiled fraction S ₁₀₅ +bovine serum albumin (1 mg)	3.88
Boiled fraction S ₁₀₅ +bovine serum albumin (3 mg)	1.81

Effect of various phenolic and thiol compounds on prostaglandin synthase activity

Although the soluble fraction appeared to contain heat-stable factors which stimulated prostaglandin synthase activity, we did not attempt to isolate and identify these factors. Instead, we found that a variety of naturally occurring compounds could not only replace these heat-stable factors but could further enhance stimulation of prostaglandin synthase activity. Table 3 shows that the microsomal enzyme activity was greatly increased by adrenaline

Table 3. *Effect of L-adrenaline and GSH on prostaglandin synthesis*

Incubation and assay were as described in the Methods section, except that the addition of GSH or L-adrenaline was varied as indicated; 0.6 mg of microsomal protein was used for each assay. The result of a representative experiment is shown.

Additions	Prostaglandin synthesis (nmol)			
	F _{2α}	E ₂	D ₂	Total
None	0.43	0.60	0.32	1.35
GSH	0.94	1.01	0.54	2.49
L-Adrenaline	1.73	3.26	0.87	5.86
GSH+L-adrenaline	3.36	4.22	2.24	9.82

Table 4. *Effect of some phenolic compounds on prostaglandin synthesis*

The assay conditions were as described in Table 3, except that GSH was omitted and various compounds were added as indicated.

Additions	Prostaglandin synthesis (nmol)			
	F _{2α}	E ₂	D ₂	Total
None	0.43	0.60	0.32	1.35
L-Adrenaline	1.73	3.26	0.87	5.86
L-Noradrenaline	1.76	3.33	0.95	6.04
L-Isoproterenol	1.68	3.02	0.77	5.47
3-Hydroxytyramine	2.14	3.88	0.87	6.89
5-Hydroxyindol-3-ylacetic acid	1.29	2.42	0.73	4.44
5-Hydroxytryptamine	1.34	3.22	1.01	5.57
N-Acetyl-5-methoxytrypt- amine	0.89	1.28	0.49	2.66
Quinol (0.2 mM)	0.62	3.40	0.88	4.90
L-Tri-iodothyronine (0.2 mM)	0.65	0.97	0.45	2.07
L-Thyroxine (0.2 mM)	0.73	1.37	0.59	2.69
Oestrone (0.2 mM)	0.57	1.03	0.36	1.96
Oestradiol (0.2 mM)	0.68	1.26	0.39	2.33
Ascorbic acid	0.50	0.46	0.30	1.26
Histamine	0.36	0.46	0.22	1.04
GSH	0.94	1.01	0.54	2.49

but only slightly by GSH. However, both GSH and adrenaline were required to achieve maximal enzyme activity.

The ability of adrenaline to enhance prostaglandin synthesis could be mimicked by a variety of aromatic compounds. Table 4 indicates that, of the aromatic compounds studied, catecholamines, dopamine, 5-hydroxytryptamine, quinol and hydroxyindole derivatives were especially potent activators of the synthase system. Other compounds, such as melatonin, thyroid hormones and oestrogens were weak activators, and L-ascorbate and histamine were inactive,

Fig. 5. Effect of L-adrenaline or *p*-aminophenol concentrations on the rate of synthesis of prostaglandins

Incubation and assay conditions were as described in the Methods section, except that the concentration of phenolic compounds was varied as indicated; 2.1 mg of microsomal protein was used for each assay.

Table 5. Effect of thiols on prostaglandin synthesis

The assays were carried out in the presence of 1 mM-L-adrenaline and 1 mM-thiol compounds as indicated. Other conditions were as described in Table 3.

Thiols	Prostaglandin synthesis (nmol)			
	F _{2a}	E ₂	D ₂	Total
None	1.37	3.78	1.15	6.30
GSH	3.32	3.96	1.92	9.20
Cysteine	1.39	4.59	1.23	7.21
Thioglycolic acid	0.96	3.88	1.11	5.95
Dithiothreitol	0.83	0.51	0.37	1.71
2-Mercaptoethanol	0.34	0.85	0.21	1.40

The effect of various concentrations of two representative compounds, i.e. adrenaline and *p*-aminophenol, are depicted in Fig. 5. Apparent K_a values of activation as determined were 6×10^{-5} M and 2×10^{-5} M respectively. Excess of *p*-aminophenol appeared to cause strong inhibition of prostaglandin synthesis.

GSH has also been shown to enhance the formation of prostaglandins in sheep and bovine seminal vesicles. Of the thiol compounds tested (Table 5), none was as effective as GSH in stimulating prostaglandin synthesis by the kidney-medulla enzyme system. Although cysteine had some stimulatory effect, other thiols were actually inhibitory. The apparent K_a of activation for GSH in the presence of near-saturating concentration of adrenaline is approx. 2×10^{-4} M.

Fig. 6. Effect of pH on the rate of synthesis of different prostaglandins

Incubation and assay conditions were as described in the Methods section, except that the pH was varied as indicated; 0.6 mg of microsomal protein was used for each assay. PG, Prostaglandin.

Effect of substrate concentration and pH

The dependence of the rate of the reaction on arachidonic acid concentrations was examined. An apparent K_m of $8.3 \mu\text{M}$ for arachidonic acid was estimated from a Lineweaver-Burk double-reciprocal plot of the reaction rate versus the substrate concentration. The pH profiles for the synthesis of different prostaglandins are shown in Fig. 6. The rate of prostaglandin formation is optimal at pH 8.0–8.5 for prostaglandin E₂ and prostaglandin D₂, and at pH 7.5–8.0 for prostaglandin F_{2a}. Therefore a pH value of 8.0 was chosen for routine assays.

Inhibitor studies

The effects of metal-chelating agents on the reaction are presented in Table 6. Little inhibition of the synthesis of total prostaglandins was observed with EDTA, EGTA, azide, $\alpha\alpha'$ -bipyridyl and *o*-phenanthroline at 1 mM. However, significant inhibition was obtained with cyanide, 8-hydroxyquinoline and diethyldithiocarbamate at the same concentration. Among a number of thiol-group inhibitors tested, none inhibited prostaglandin formation at 1 mM. On the contrary, they appeared to induce some stimulatory effect.

Table 6. *Effect of metal-chelating agents on prostaglandin synthesis*

The assay conditions were as described in the Methods section, except that further additions of various inhibitors were as indicated; 0.6mg of microsomal protein was used for each assay.

Metal-chelating agents	Prostaglandin synthesis (nmol)			
	F _{2a}	E ₂	D ₂	Total
None	3.38	3.69	1.44	8.51
EDTA (10mM)	3.02	3.11	1.73	7.86
EGTA (10mM)	3.46	3.37	1.43	8.23
NaN ₃ (1mM)	2.80	3.36	1.31	7.47
α,α' -Bipyridyl (1mM)	4.00	2.57	0.38	6.95
<i>o</i> -Phenanthroline (1mM)	4.13	1.61	0.59	6.33
NaCN (1mM)	1.92	2.18	0.88	4.98
NaCN (3mM)	0.42	0.56	0.34	1.22
8-Hydroxyquinoline (1mM)	0.00	0.14	0.00	0.14
Diethyldithiocarbamate (1mM)	0.90	3.38	0.94	5.22

Indomethacin and meclofenamic acid, the non-steroidal anti-inflammatory drugs, were reported to be potent inhibitors of prostaglandin synthase from various tissues (Vane, 1971; Takeguchi & Sih, 1973). They were also found to be potent inhibitors of kidney-medulla prostaglandin synthase. The concentrations required for 50% inhibition of prostaglandin synthesis under our assay conditions were 15.9 μ M and 3.2 μ M for indomethacin and meclofenamic acid respectively.

Discussion

The synthesis of prostaglandin E₂ and prostaglandin F_{2a} from arachidonic acid by rabbit kidney medulla has previously been demonstrated by Hamberg (1969) and Crowshaw (1971, 1973) using crude homogenates or tissue slices. We were able to confirm the synthesis of these two prostaglandins by using microsomal fractions. However, the third product, prostaglandin D₂, which was consistently detected under our assay conditions, has not been reported by other workers. Prostaglandin D₂ has been well established as one of the reaction products of the prostaglandin synthase system from seminal vesicles (Granstrom *et al.*, 1968; Foss *et al.*, 1972). Failure to detect this product in the kidney-medulla system by other laboratories was probably due to the poor enzyme activity of the crude homogenates used.

The time-course of the formation of each of the prostaglandins did not appear to be precisely linear at any given period of time. This might be related to the instability of the microsomal prostaglandin synthase system at the assay temperature, since pre-incubation of the microsomal fraction at 37°C indicated that gradual loss of enzyme activity was observed even in the early phase of the reaction (H.-H. Tai & C. S. Hollander, unpublished work).

The rate of formation of any prostaglandin also did not correlate linearly with the amount of particulate enzyme preparation. The same properties have also been observed in the bovine seminal-vesicular system (Flower *et al.*, 1973). The presence of inhibitory substances in the microsomal fractions, such as soluble fraction and thiol compounds, may partially account for this non-linearity. Because of these peculiar kinetic properties of the enzyme system, assay conditions, such as the length of incubation and the amount of microsomal protein used, were somewhat arbitrary. In general, estimation of the initial velocity was carried out in such conditions that no more than 20% of the substrate was converted into products.

The presence of inhibitory macromolecular substances in the soluble fraction has also been demonstrated in both bovine seminal-vesicular and guinea-pig pulmonary systems (Takeguchi *et al.*, 1971; Parkes & Eling, 1974). The inhibitory factor(s) was not likely to be 15-hydroxyprostaglandin dehydrogenase, since no 15-oxoprostaglandin metabolites could be detected on t.l.c. The fact that serum albumin, added to either the microsomal fraction or the microsomal fraction plus boiled soluble fraction, showed considerable inhibition of prostaglandin synthase activity suggested that rabbit serum albumin present in the soluble fraction was likely to be one of the factors contributing to the inhibitory effect.

When the rabbit kidney-medulla microsomal fraction was separated from the inhibitor(s) present in the soluble fraction, its properties with respect to the stimulation by phenolic compounds and GSH differed in some respects from seminal-vesicular systems. GSH alone appeared to have a slight stimulatory effect on kidney-medulla prostaglandin synthase. However, when GSH was added along with a phenolic effector, a synergistic stimulation was observed. The stimulation was reflected in the synthesis of all three prostaglandins. This is in contrast with the seminal-vesicular system, in which GSH stimulates the synthesis of prostaglandin E at the expense of other products (Foss *et al.*, 1972; Lands *et al.*, 1971). A variety of phenolic compounds stimulated the synthesis of prostaglandins in the kidney-medulla system similarly to that in seminal-vesicular systems. However, in contrast with the seminal-vesicular system (Sih *et al.*, 1970), variations of phenolic effectors did not appear to alter significantly the ratio of prostaglandins, except that quinol seemed to enhance the synthesis of prostaglandin E₂. The fact that various phenolic hormones can stimulate prostaglandin synthesis in kidney-medulla tissue supports the proposition that these hormones might be the natural coenzymes in regulating the biosynthesis of prostaglandins *in vivo*.

Rabbit kidney-medulla prostaglandin synthase was not sensitive to a variety of metal-chelating

agents at 1 mM, except for cyanide, 8-hydroxyquinoline and diethyldithiocarbamate. Inhibition by cyanide and diethyldithiocarbamate may be due to metal-ion complexing, as proposed for seminal-vesicular prostaglandin synthase (LeTellier *et al.*, 1973). However, the strong inhibition by 8-hydroxyquinoline appeared to be unrelated to the metal chelation, since quinidine, which is a poor metal chelator but has a moiety structurally similar to 8-hydroxyquinoline, also inhibited prostaglandin synthesis to a comparable degree (H.-H. Tai & C. S. Hollander, unpublished work). It is likely that 8-hydroxyquinoline inhibits the system by binding to a hydrophobic site which is essential for the enzyme activity, as suggested for *m*-phenanthroline (LeTellier *et al.*, 1973).

Kidney prostaglandin synthase was found to be inhibited by a variety of thiol compounds except for GSH and cysteine, but was slightly activated by thiol-group inhibitors. The inhibitory effect of some mercaptans has also been observed in the seminal-vesicular system by Takeguchi *et al.* (1971). Lands *et al.* (1971) have demonstrated that GSH peroxidase in the presence of mercaptan inhibits the oxygenation of arachidonic acid by sheep vesicular homogenates. It is possible that some endogenous peroxidase activity, which can utilize endogenous or externally added thiol compounds to inhibit oxygenation, is still present in the microsomal fraction of rabbit kidney medulla. This possibility is strengthened by the fact that addition of thiol-group inhibitors, which could exhaust all the endogenous thiol content, produced somewhat higher amounts of prostaglandins.

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