SOME PROPERTIES OF PROSTACYCLIN SYNTHASE FROM PIG AORTA

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1. Introduction

Two potent bioregulators can be formed from the prostaglandin endoperoxide PGH_2. thromboxane A_2 (TXA_2), a platelet-aggregating and smooth muscle-contracting agent [1] and prostacyclin (PGI_2) which inhibits platelet aggregation and relaxes smooth muscles [2]. The thromboxane synthase has been partially purified from platelets [3] and lung [4], and shown to also catalyze the conversion of PGH_2 to 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) [5,6]. A possible mechanism of these transformations is shown in fig.1. It is postulated that the reactions are initiated by protonation of the oxygen at C-9 of PGH_2 and that the charged intermediate (I) rearranges to TXA_2 or cleaves to HHT plus malondialdehyde. On the other hand, protonation of the oxygen at C-11 could initiate a rearrangement of PGH_2 to PGI_2. The intermediate (II) might also undergo a similar cleavage as (I) to give HHT and malondialdehyde. This paper describes the solubilization and fractionation by DEAE cellulose chromatography of prostacyclin synthase. On the basis of these experiments, the use of enzyme inhibitors, and protein denaturing agents, it it concluded that PGI_2 synthase does not enzymatically form HHT.

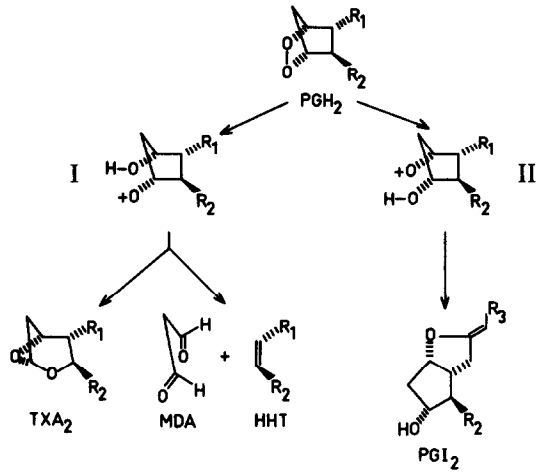

Fig.1. Possible mechanisms of thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) formation from PGH_2.

2. Materials and methods

2.1. *Chemicals*

Prostaglandins $F_{2\alpha}$, E_2, 6-keto-$PGF_{1\alpha}$ and EMPA (9α,11α-epoxymethano-15(*S*)hydroxyprosta-5(*cis*), 13(*trans*)-dienoic acid were obtained from the Upjohn Company, Kalamazoo, MI. 2-Iso-propyl-3-nicotinyl-indole (L-8027) and sodium *p*-benzyl-4-[1-oxo-2-(4-chlorobenzyl)-3-phenylpropyl]phenyl phosphonate (N-0164) were given by Labaz and Nelson Research, respectively. [1-^{14}C]PGH_2 (spec. act. 1.0 Ci/mol) and 12L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) were prepared by the methods in [7,8]. DEAE cellulose was from Whatman, Silica gel G, imidazole and $SnCl_2$ from Merck.

Abbreviations EMPA, 9α,11α-epoxymethano-15(*S*)hydroxy-prosta-5(*cis*)-13(*trans*)-dienoic acid, HHT, 12-hydroxy-5,8,10-heptadecatrienoic acid; L-8027, 2-isopropyl-3-nicotinylindole, N-0164, *p*-benzyl-4[1-oxo-2-(4-chlorobenzyl)-3-phenylpropyl]-phenyl phosphonate; PG, prostaglandin; TLC, thin-layer chromatography, TXB_2, thromboxane B_2, 15-HPETE,15-hydroperoxy 5,8,11,13-eicosatetraenoic acid

2.2. *Enzyme preparation*

Pig aorta was obtained from slaughter house and immediately frozen on dry ice. Portions of tissue (~50 g) were frozen in liquid nitrogen, crushed into a fine powder and homogenized in 2 vol. 0.1 M potassium phosphate buffer (pH 7.4). Subcellular fractions were obtained by differential centrifugation, as for bovine lung [4]. The mitochondrial and microsomal fractions (sedimented at 9000 × *g*, 15 min and 100 000 × *g*, 60 min, respectively) were resuspended in the same buffer to a concentration corresponding to 2 g tissue/ml.

Solubilization of microsomes and DEAE-cellulose chromatography were performed as for platelet [3] and bovine lung [4] thromboxane synthase.

2.3. *Enzyme assay*

Incubations were performed in 0.1 M potassium phosphate buffer (pH 7.4) and details are shown in appropriate legends. The reaction was stopped with 5 vol. ethanol containing 5 mg/ml $SnCl_2$ and products were extracted with ethyl acetate [9]. They were analyzed by TLC in ethyl acetate/2,2,4-trimethylpentane/acetic acid/water, 55.25·10:50 (organic phase, solvent I). When the plate was developed twice in the same solvent, excellent separation of products was obtained, with the following R_F values: 6-keto-$PGF_{1\alpha}$ (the stable end-product of PGI_2), 0.24; $PGF_{2\alpha}$, 0.37; PGE_2, 0.55; HHT, 0.90. Unlabelled compounds were used as internal (6-keto-$PGF_{1\alpha}$) or external ($PGF_{2\alpha}$, PGE_2 and HHT) references. In solvent II (ethyl acetate/2,2,4-trimethylpentane/water 5:10:10) all prostaglandins (methyl esters) remained close to the origin and HHT had an R_F of about 0.5.

The reference compounds were visualized by iodine vapor and the chromatograms were scanned for radioactivity with a Berthold Dünnschicht Scanner II. The radioactive zones were scraped off and counted in a Packard Tri-Carb liquid scintillation spectrometer model 3375.

3. Results

3.1. *Subcellular distribution of PGI_2 synthase*

Table 1 shows the conversion of PGH_2 by subcellular fractions of pig aorta.

Table 1
Conversion of PGH_2 by subcellular fractions of pig aorta – effect of boiling

	Product formation (% conversion)				
	6-Keto-$PGF_{1\alpha}$	$PGF_{2\alpha}$	PGE_2	PGD_2	HHT
Homogenate	72	3	12	3	10 (11)
Boiled homogenate	3	16	7	11	63 (62)
Mitochondria	7	29	28	13	21 (20)
Boiled mitochondria	4	13	45	21	17 (15)
Microsomes	35	17	19	6	22 (20)
Boiled microsomes	5	33	16	11	35 (36)
100 000 × *g* supernatant	10	25	26	11	28 (25)
Boiled supernatant	6	19	9	12	54 (56)
Buffer alone	3	9	55	24	9

Last column (in parentheses) shows HHT determinations by TLC in solvent II

All samples contained material corresponding to 0.5 g tissue in 1 ml 0.1 M potassium phosphate buffer, pH 7.4. Incubation was for 10 min, at 37°C with 5 μg PGH_2 (~30 000 cpm) and terminated with 5 ml ethanol containing 25 mg $SnCl_2$. Products were extracted and analyzed as in section 2

PGI_2 forming activity was localized mainly in the microsomes as in [10]. However, the microsomes produced less PGI_2 (determined as 6-keto-$PGF_{1\alpha}$), compared to an equivalent amount of homogenate, suggesting the presence in the latter of an activating or stabilizing factor. Table II shows that addition of supernatant to the microsomes stimulated PGI_2 formation. The factor responsible for this effect was not destroyed by boiling nor removed by dialysis (table 2).

3.2. *Effects of denaturing agents on PGI_2 and HHT formation*

Boiling (5–7 min at 100°C) destroyed the PGI_2 forming activity in all preparations. Formation of HHT was, however, either not affected (mitochondria) or 2–6-fold enhanced (high-speed supernatant and homogenate, respectively). High production of HHT in those preparations contrasted with the small amount of HTT formed in buffer alone, under the same experimental conditions. Treatment with 6 M guanidine–HCl had similar effects as boiling: using microsomes, the formation of PGI_2 was abolished whereas HHT formation increased from 31–45%. In the supernatant guanidine–HCl raised the HHT formation from 31–66%.

Table 2
Effect of supernatant on 6-keto-$PGF_{1\alpha}$ formation by aorta microsomes

Addition	Products	
	6-Keto-$PGF_{1\alpha}$ (%)	HHT (%)
None	32	23
Native supernatant (0.25 ml)[a]	62	17
Boiled supernatant (0.25 ml)[a]	54	34
Dialyzed supernatant (0 25 ml)[a]	47	19

[a] Corresponding to 0.125 g tissue

Experimental details same as in table 1

3.3. *Effects of inhibitors*

Four inhibitors of thromboxane synthase were used in concentrations which inhibit TXB_2 formation by platelet microsomes by 80–90% [5]. Table 3 shows that none of these compounds appreciably influenced PGI_2 formation. On the other hand, 15-HPETE, an inhibitor of PGI_2 synthesis [10], prevented the formation of 6-keto-$PGF_{1\alpha}$, but had no effect on HHT formation.

3.4. *Partial purification of PGI_2 synthase*

Aorta microsomes were treated with Triton X-100 as for platelet [3] and lung [4] microsomes. The 100 000 × *g* supernatant, which contained most of the PGI_2 forming activity, was chromatographed on a DEAE-cellulose column (table 4). Prostacyclin syn-

Table 3
Conversion of [1-^{14}C]PGH_2 by aorta microsomes in the presence and absence of additions

Addition	Conversion of [1-^{14}C]PGH_2			
	6-Keto-$PGF_{1\alpha}$		HHT	
	%	relative[a]	%	relative[a]
None	42	100	26	100
L-8027 (10^{-4} M)	35	84	23	88
N-0164 (2×10^{-4} M)	33	80	25	96
EMPA (10^{-4} M)	36	86	24	92
Imidazole (10^{-3} M)	37	89	23	88
15-HPETE (6 μg/ml)	6	14	24	92

[a] Relative to the conversion by microsomes alone, taken as 100

Each sample contained 0 25 ml microsomal suspension (corresponding to 0.5 g tissue) in potassium phosphate buffer and the total volume was 0.5 ml. Other details as in table 1

Table 4
Product formation from [1-^{14}C]PGH_2 by aorta microsomal preparations

	Products	
	6-Keto-$PGF_{1\alpha}$ (%)	HHT (%)
Microsomal	49	24
Solubilized microsomes (supernatant)	43	28
Solubilized microsomes (sediment)	8	30
Fraction 1	5	18
Fraction 2	6	10
Fraction 3	37	29
Boiled fraction 3	4	32

Microsomal suspension 2 ml (corresponding to 4 g tissue) were solubilized with 0.5% Triton X-100 and the 100 000 × *g* supernatant was applied to a DEAE-cellulose column (5 ml). Elution 30 ml 10 mM potassium phosphate buffer (pH 7.4), 0.1% Triton X-100 (fraction 1), 10 ml 20 mM buffer; 0.1% Triton X-100 (fraction 2) and 10 ml 0 2 M buffer, 0.1% Triton X-100 (fraction 3). Eluates were concentrated by ultrafiltration and portions corresponding to 0.5 g initial tissue were incubated with 3 μg [1-^{14}C]PGH_2 (~20 000 cpm) for 15 min at 37°C in a total volume of 0 5 ml. Products were extracted and analyzed as in section 2

thase was eluted from the column with 0.2 M buffer and almost no activity appeared in the preceding two fractions. Boiling of the partially purified enzyme (fraction 3) abolished 6-keto-$PGF_{1\alpha}$ formation, but had no effect on HHT production.

4. Discussion

The two bioregulators, thromboxane and PGI_2, are formed from PGH_2 by the action of enzymes differently distributed in animal tissues [11]. While the thromboxane synthesizing enzyme systems in human platelets [3,5,6] and bovine lung [4] have been studied in some detail, less is known, so far, about the properties of PGI_2 synthase. The present results show that aorta microsomal PGI_2 synthase is stimulated by the high-speed supernatant. In contrast, lung thromboxane synthase was more active in isolated microsomes than in the unfractionated homogenate [4]. The effect of the supernatant on PGI_2 synthesis by pig aorta microsomes resembles the activation by supernatant of PGE_2 formation from arachidonic acid by sheep vesicular gland microsomes [in the absence of reduced glutathione, (unpublished observations)]. In either case, the soluble factor was resistant to boiling and non-dialyzable.

Four inhibitors of thromboxane synthase had almost no effect on PGI_2 formation by aorta microsomes. This indicates that these compounds can inhibit thromboxane formation selectively in systems which produce both thromboxanes and PGI_2. 15-HPETE inhibited PGI_2 formation, as has been reported [10] but had no effect on HHT formation. PGI_2 formation and HTT formation are therefore not coupled in the way that thromboxane and HHT formation are. In the latter case several structurally unrelated compounds inhibited TXB_2 and HHT formation identically [6]. HHT production from PGH_2 by aorta preparations was furthermore not prevented by boiling (see also [12]). Instead, its formation in certain fractions was enhanced by heating (table 1). In contrast to [12], we did not find much HHT formation from PGH_2 in buffer (table 1 and [4]), even when the incubation period was prolonged to 30 min (not shown). The major products were PGE_2 and PGD_2. It seems probable that a factor is present in the soluble fraction of the cell, which promotes HHT formation from PGH_2. When enzymes which utilize PGH_2 are denatured by heating, more PGH_2 becomes available for conversion to HHT. It has also been reported [13] that boiled homogenate of rat kidney has several-fold

increased HHT formation relative to the fresh homogenates which enzymatically form mostly PGE_2. Thus, thromboxane synthase is, so far, the only enzyme described which forms HHT.

Acknowledgements

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