METABOLISM AND ACTION OF THE PROSTAGLANDIN ENDOPEROXIDE PGH_2 IN RAT KIDNEY

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SUMMARY: Kidney membrane fractions metabolized [1-^{14}C]PGH_2 to TXB_2, PGE_2, $PGF_{2\alpha}$, PGD_2, 6-keto $PGF_{1\alpha}$, and HHT. TXA_2, as measured by TXB_2, was enzymatically formed in cortex microsomes and was identified by thin layer chromatography and gas chromatography - mass spectrometry. PGH_2 caused a labile inhibition of cortical PGE_2-stimulated adenylate cyclase. PGE_2, $PGF_{2\alpha}$, and PGD_2 are stimulators of cortical adenylate cyclase. The inability of two thromboxane synthetase inhibitors, imidazole and 9,11-azoprosta-5,13 dienoic acid, to block PGH_2 inhibition suggested that TXA_2 was not an obligatory intermediate in this process. Therefore, a potential function of cortical PGH_2 is inhibition of adenylate cyclase.

INTRODUCTION

Recent experiments with platelets (1), vascular tissue (2), and stomach (2) indicate thromboxane (TX)A_2 and PGI_2, like PGE_2 and $PGF_{2\alpha}$, are metabolic products of the cyclic endoperoxide prostaglandin PGH_2. TXA_2 is a potent vasoconstrictor (3) and, like PGH_2, inhibits PGE_1-stimulated platelet adenylate cyclase, and promotes platelet aggregation (4). PGI_2 has opposite effects. It is a vasodilator (2); stimulates adenylate cyclase (5); and inhibits platelet aggregation (5). Since the identification of medullin, an antihypertensive material prepared from the renal medulla, as a prostaglandin (6), there has been a sustained interest in the biological significance of renal production and responsiveness to prostaglandins. Prostaglandins synthesized by the kidney are PGE_2, $PGF_{2\alpha}$, and PGD_2 (7). Because of the potentially important relationships between renal function and prostaglandin synthesis, further characterization of that synthesis and renal responses to various prostaglandins are important. The present study characterizes the products of PGH_2 metabolism in the kidney and examines some interactions of prostaglandins with renal adenylate cyclase.

METHODS

Thin Layer Chromatography. Metabolites of [1-^{14}C]PGH_2, prepared biosynthetically as previously described (8), were identified by thin layer chromatography on silica gel plates. Plates were developed with either solvent

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system A which contained the organic phase from ethyl acetate, acetic acid, 2,2,4-trimethylpentane, and water (110:20:50:100) or solvent system B containing 1% acetic acid in ethyl acetate. The plates were then scanned with a Vangard Strip Scanner to determine radioactivity. Solvent system A separates 6-keto $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGD_2, and HHT (12L-hydroxy-5,8,10-heptadecatrienoic acid) but not PGE_2 and TXB_2. Using solvent system B, PGE_2 and TXB_2 are separated, but not 6-keto $PGF_{1\alpha}$ and $PGF_{2\alpha}$. The distribution of PGD_2 and HHT were qualitatively as well as quantitatively similar in both solvent systems (Table I).

Gas Chromatography - Mass Spectrometry: Identification of Thromboxane B_2 Cortical 100,000 xg fraction (25 mg protein) was incubated with 500 mg of PGH_2 under the assay conditions used for adenylate cyclase. The reaction mixture was extracted with diethyl ether and separated on a silicic acid column. Fractions containing TXB_2 were pooled and treated with ethereal diazomethane and silanized with a 3:1 mixture of bis (N,O-trimethylsilyl) trifluoroacetamide and dimethyl formimide. The mixture was allowed to stand at room temperature for 1 hr before being subjected to GC/MS analysis. GC/MS analyses were done on an LKB-9000 GC/MS equipped with a 6 ft. column of 1% SE-30 on GasChrom Q (80-100 mesh) operated at 210°. Both the flash heater and the separator were operated at 240° and the carrier helium gas flow was set at 30 ml/min. Electronic energy was kept at 22.5 ev and trap current was 60 μA. Unknown material gave a single GC peak with a C-value of 24.8.

Preparation of Renal Fractions and Assay of Adenylate Cyclase Activity. Tissue slices from cortex, outer medulla, and inner medulla were collected separately, minced, and homogenized as previously described (9). This homogenate was separated into 1,000 xg, 40,000 xg, and 100,000 xg fractions. Adenylate cyclase reaction mixture was as previously described (9), except 8 μM GTP was included, and cyclic AMP was isolated by a 2-step column procedure (10). Protein was estimated by the Lowry method (11). Basal activity represents that activity observed in the absence of test agents and was not altered by the ethanol or acetone diluents for PGE_2 and PGH_2, respectively.

RESULTS

PGE_2, $PGF_{2\alpha}$, and HHT (a product of PGH_2 thought to be formed non-enzymatically) each represented approximately a 20-30% metabolism of PGH_2 (Table I). In each fraction, less 6-keto $PGF_{1\alpha}$ (the stable metabolite of PGI_2) or PGD_2 were produced than either PGE_2 or $PGF_{2\alpha}$. Cortical fractions demonstrated the highest conversion of PGH_2 to 6-keto $PGF_{1\alpha}$ while the inner medulla yielded the highest percentage conversion to PGD_2. The inner medulla produced the least amount of TXB_2 (a stable hydrolysis product of TXA_2) with the 100,000 xg fraction of the cortex producing 4 to 8 times as much TXB_2 as the corresponding fraction of the inner medulla. Considerable TXB_2 synthesis was also observed in the 40,000 xg fraction of outer medulla. TXB_2 formed by the 100,000 xg cortical fraction was structurally identified by gas chromatography-mass spectrometry. TXB_2 mass spectrum gave a base peak at m/e 256 and other major ions at m/e 510, 420, 366, 295, 225, and 217. These results confirmed

TABLE I. METABOLISM OF [1-^{14}C] PGH_2 IN RENAL CORTEX, OUTER MEDULLA AND INNER MEDULLA

	1,000 x g		40,000 x g		100,000 x g	
	System A	System B	System A	System B	System A	System B
	% total counts on TLC plate					
CORTEX						
6-keto $PGF_{1\alpha}$	10		8		12	
$PGF_{2\alpha}$	32		18		23	
6-keto $PGF_{1\alpha}$ + $PGF_{2\alpha}$		39		23		31
PGE_2		25		32		14
TXB_2		9		12		24
PGE_2- TXB_2	31		40		36	
PGD_2	8	5	14	11	5	6
HHT	21	24	22	24	25	27
OUTER MEDULLA						
6-keto $PGF_{1\alpha}$	7		7		3	
$PGF_{2\alpha}$	26		22		28	
6-keto $PGF_{1\alpha}$ + $PGF_{2\alpha}$		28		21		27
PGE_2		24		20		21
TXB_2		9		20		14
PGE_2- TXB_2	31		38		34	
PGD_2	13	7	9	7	12	7
HHT	24	34	27	35	25	32
INNER MEDULLA						
6-keto $PGF_{1\alpha}$	3		3		2	
$PGF_{2\alpha}$	24		27		25	
6-keto $PGF_{1\alpha}$ + $PGF_{2\alpha}$		18		26		22
PGE_2		27		22		27
TXB_2		3		6		3
PGE_2-TXB_2	33		29		32	
PGD_2	23	18	13	11	26	21
HHT	20	37	30	38	18	30

that the compound was thromboxane B_2, as both the retention time and the fragmentation pattern were identical to those reported by Hamberg and Samuelsson (12).

In the 1,000 xg and 100,000 xg fractions, PGE_2 ($8x10^{-4}$M) caused an increase

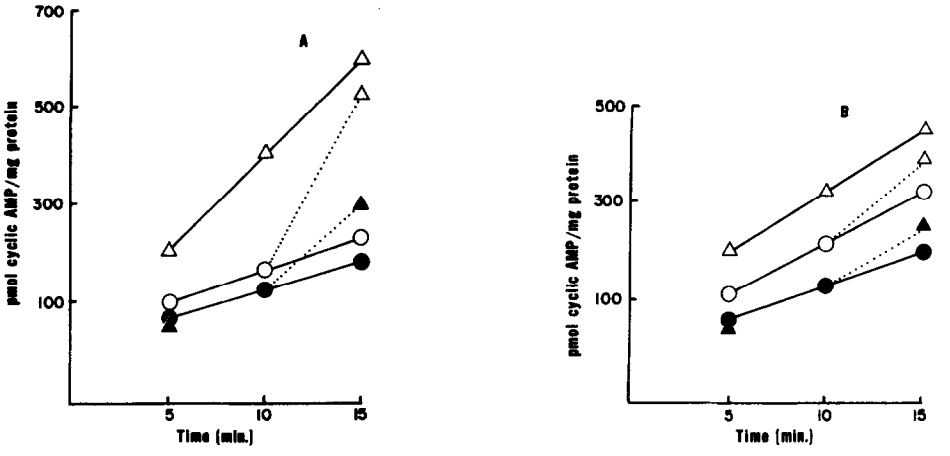

Figure 1. Effects of PGE_2 and PGH_2 on renal cortical a) 1,000xg and b) 100,000xg adenylate cyclase activity. Dotted lines indicate values for PGE_2 following addition after 10 min. of incubation in the presence or absence of PGH_2. Basal and PGE_2 adenylate cyclase values are represented by open circles and triangles, respectively, with dark symbols (●,▲) representing the presence of 1 x 10^{-4}M PGH_2. Each value represents the mean of triplicate determinations with a standard error of less than 10%.

in adenylate cyclase activity that was linear between 5 and 15 minutes (Fig. 1, a and b). PGH_2 ($1x10^{-4}$M) reduced basal activity at 10 and 15 minutes and blocked PGE_2 stimulation at 5 min. However, there was no inhibition of the PGE_2 effect when the PGH_2 was incubated with the membranes for 10 min. prior to addition of PGE_2. Preincubation of membranes with two different thromboxane synthetase inhibitors, 1 mM imidazole (Table II) or 1 mM 9,11 azoprosta-5,13 dienoic acid (Azo analog I) did not alter adenylate cyclase activity nor block PGH_2 inhibition.

DISCUSSION

[1-^{14}C]PGH_2 was converted to TXB_2, PGE_2, $PGF_{2\alpha}$, PGD_2, 6-keto $PGF_{1\alpha}$, and HHT. Synthesis of TXB_2 was verified by thin layer chromatography and mass spectral analysis. TXB_2 synthesis was primarily in the cortex and outer medulla with the largest percent conversion in the cortical 100,000 xg particulate fraction. Synthesis was probably enzymatic, since boiling the 100,000 xg fraction eliminated TXB_2 production. The percentage of conversion

TABLE II. EFFECT OF IMIDAZOLE ON PGH_2 INHIBITION OF PGE_2-STIMULATED ADENYLATE CYCLASE

	Imidazole	
	-	+
	pmol cyclic AMP/mg protein/5 min	
1,000 x g		
Basal	57 ± 14	51 ± 5
+PGH_2	56 ± 9	55 ± 11
PGE_2	145 ± 9	132 ± 14
+PGH_2	60 ± 2	56 ± 9
100,000 x g		
Basal	122 ± 3	120 ± 2
+PGH_2	86 ± 3	68 ± 3
PGE_2	191 ± 4	181 ± 3
+PGH_2	81 ± 2	84 ± 3

Membrane fractions were preincubated 5 min at 30°C with or without 3mM imidazole and then incubated for 5 min at 30°C in the presence or absence PGH_2 ($1x10^{-4}$M) or PGE_2 ($8X10^{-4}$M). The final concentration of imidazole in the adenylate cyclase incubation was 1mM. Each value represents the mean ± S.E. of triplicate determinations.

of PGH_2 to its natural prostaglandin metabolites was the following: $PGE_2 = PGF_{2\alpha} > PGD_2 > PGI_2$.

The PGH_2 metabolites formed have been reported to affect adenylate cyclase differently. PGE_2 stimulates renal cortical (9) and PGI_2 stimulates platelet adenylate cyclase (5). By contrast, $PGF_{2\alpha}$ and PGD_2 are only weak stimulators

of renal cortical adenylate cyclase (9). HHT is a stable PGH_2 metabolite which does not affect platelet adenylate cyclase (4). PGH_2 is a labile inhibitor of hormone-stimulated adenylate cyclase activity in fat cell ghosts (13), and both PGH_2 and TXA_2 inhibit PGE_1-stimulated cyclic AMP accumulation in platelets (4). In the present study, PGH_2 inhibited basal and PGE_2 activation of adenylate cyclase in cortex. The inhibition appears to be due to an unstable substance because addition of PGE_2 to preparations preincubated for 10 min. with PGH_2 resulted in significant increases in adenylate cyclase activity. PGH_2 is unstable in aqueous solution as are two of its metabolites, PGI_2 and TXA_2. Although PGI_2 was not tested in this study, previous studies with platelets indicate PGI_2 stimulates adenylate cyclase (5). Imidazole and Azo analog I inhibit thromboxane synthetase activity (14,15). Preliminary experiments demonstrated that 1 mM imidazole or 28 μM Azo analog I completely inhibit TXB_2 formation from 50 nM[1-^{14}C]PGH_2 in the 100,000 xg cortical fraction. Neither altered the PGH_2 inhibition of PGE_2-stimulated adenylate cyclase activity. Although relatively high concentrations of PGH_2 were used, the lack of any observable inhibition by imidazole or Azo analog I suggests that the labile inhibition demonstrated with PGH_2 was probably not dependent upon PGH_2 conversion to TXA_2, but rather to PGH_2 itself. This is different from human platelets where the cyclic AMP lowering activity of PGH_2 appears to be inhibited by imidazole and Azo analog I (R.R. Gorman, unpublished). These experiments do not rule out TXA_2 inhibition of cortical adenylate cyclase. The persistent inhibition of basal activity compared to the labile inhibition of PGE_2-stimulation is not understood. The antagonistic action of precursor, PGH_2, and product, PGE_2, could represent an important system for modulating cortical adenylate cyclase activity.

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