Vol. 83, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 28, 1978 Pages 731-738

PULMONARY METABOLISM OF PROSTACYCLIN (PGI_2) IN THE RABBIT

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Received June 16,1978

SUMMARY: Metabolism of prostacyclin, [9-^{3}H]PGI_2, was examined in the isolated perfused rabbit lung and the post-microsomal supernate of rabbit lung homogenate. Two major metabolites of [9-^{3}H]PGI_2 from the lung perfusate were separated by thin-layer chromatography and radiometric gas-chromatography. These two products were identified as 6 keto-$PGF_{1\alpha}$ and 6,15 diketo-13,14 dihydro $PGF_{1\alpha}$ by mass-spectrometry; they represented 65% and 14% of the total radioactivity. When [9-^{3}H]PGI_2 was incubated with the lung homogenate in the presence of either NAD^+ or $NADP^+$, more than 36% and 25%, respectively, was converted to the 6,15 diketo-13,14 dihydro metabolite.

INTRODUCTION: Moncada and his co-workers have described an enzyme system in the blood vessel wall that converts prostaglandin endoperoxides to prostacyclin (PGI_2) (1). Prostacyclin is relatively short-lived; its biological activity in aqueous solution disappears within several minutes during the course of its transformation to a more stable product, 6-keto-$PGF_{1\alpha}$ (1,2). The latter compound has been reported by Pace-Asciak and Wolfe to be produced by rat stomach homogenates (3). Although many mammalian tissues including the lung possess the capacity for PGI_2 biosynthesis (4), little is known about its metabolism. PGI_2 released from various tissues could function as a circulating hormone if it is not degraded on passage across the pulmonary circulation (5). In this study, we have examined the metabolic fate of [9-^{3}H]PGI_2 in the rabbit lung and the post microsomal supernate of the lung homogenate and have identified its metabolite as 6,15 diketo-13,14 dihydro $PGF_{1\alpha}$** and

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**PGI_2: Prostacyclin, 6 keto-$PGF_{1\alpha}$: 6 keto prostaglandin $F_{1\alpha}$, 6,15 diketo $PGF_{1\alpha}$: 6,15 diketo-prostaglandin $F_{1\alpha}$, 6,15 diketo-13,14 dihydro $PGF_{1\alpha}$: 6,15 diketo-13,14 dihydro prostaglandin $F_{1\alpha}$, TLC: Thin layer chromatography. GC - gas chromatography

0006-291X/78/0832-0731$01.00/0

its stable hydrolytic product, 6 keto-$PGF_{1\alpha}$.

MATERIALS AND METHODS: Radiolabeled prostacyclin was prepared chemically as [9-^{3}H]-prostacyclin from [9-^{3}H]-prostaglandin $F_{2\alpha}$ (10 Ci/mmole, New England Nuclear, Boston, Mass.), according to the method of Johnson et. al. (6). The purity of the resulting prostacyclin methyl ester was established by thin-layer chromatography (TLC plate, 0.25 mm thick, 5 x 20 cm silica gel precoated plastic sheets, Brinkman NY) with hexane:acetone (1:1 v/v) as solvent. Radiochromatogram scans showed a single peak of prostacyclin methyl ester on TLC plates with an Rf value of 0.68. The methyl ester of [9-^{3}H]-prostacyclin was converted to the prostacyclin sodium salt the day before use by mild alkaline hydrolysis, and diluted with authentic prostacyclin sodium salt (Upjohn) to a specific activity of 15.5 µCi/mmole.

Lung perfusion: Male New Zealand rabbits were anesthetized with sodium pentobarbital (30 mg/kg) and the lungs and heart were isolated from the animal. The pulmonary artery and trachea were cannulated. Most of the heart was cut away and the left atrium was opened. The lungs were then placed in a chamber and perfused through the pulmonary artery with Tyrode's solution at 37°C at a rate of 5 ml/min. This method of perfusion was similar to that reported by others (7). [9-^{3}H]-PGI_2 (15.5 µCi/mmole) was injected into the arterial inflow over a period of 5 minutes. The venous effluent was collected during the administration of [9-^{3}H]PGI_2 and for an additional 25 min after the termination of the infusion of [9-^{3}H]PGI_2. The perfusate was acidified with 1N HCl to pH 3.0 and then extracted three times with ten volumes of ethyl acetate. The ethyl acetate extract was evaporated to dryness *in vacuo*.

Thin-layer and radiometric gas-chromatography: The residue was redissolved in 100 µl of acetone; aliquots of 20 µl of the extract was applied to a thin-layer chromatographic plate (Brinkman) with co-migration of authentic 6,15 diketo $PGF_{1\alpha}$, 6 keto-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ standards (Upjohn) and developed twice with iso-octane/ethyl acetate/acetic acid/water (25:55:10:55, v/v) (8). The radioactive products were detected on a Packard radiochromatogram scanner, Model 7201. The zones corresponding to 6,15-diketo-$PGF_{1\alpha}$ and 6,15 diketo-13,14 dihydro-$PGF_{1\alpha}$ (Rf values: 0.46 and 0.47) and 6-keto $PGF_{1\alpha}$, (Rf: 0.23) were cut out and suspended in 10 ml of 0.4% omniflour and 20% triton X-100 toluene liquid scintillation fluid and counted in a Searle Mark III liquid scintillation counter. The observed cpm were converted to dpm using a quench correction curve and external standards. The remaining portion of the sample was converted to the methyl ester with diazomethane in ether:methanol (9:1) followed by treatment with methoxyamine HCl in pyridine (5 mg/ml). Finally, it was converted to trimethylsilyl ether derivatives by treatment with bistrimethylsilyl triflouroacetamide before analyses with radiometric gas chromatography and gas chromatography-mass spectrometry. Gas chromatography was carried out on a Varian 2700 gas chromatograph coupled with a Packard 894 radioactivity detector for simultaneous recording of mass and radioactivity. GC-MS analysis were carried out in a LKB-9000 mass spectrometer. The 6 ft column (1% SE-30) on Chromosorb-W (HP) was kept at 210°C, the flash-heater at 240°C and the separator at 250°C. Electron energy was set at 22.5 eV.

Radiometric assay: A rabbit lung weighing 20 g was homogenized in five volumes of ice-cold 0.05 M Tris buffer, pH 8.5, containing 0.1 mM dithiothreitol with a Polytron homogenizer operated at top speed for 2 min. The homogenate was centrifuged at 7,000 x g for 20 min and the supernatant fluid was centrifuged at 105,000 x g for 60 min in a Beckman Model L ultracentrifuge. The post-microsomal supernate was used in all assays for 15-hydroxyprostaglandin dehydrogenase activity using [9-^{3}H] prostacyclin as substrate (specific activity of 36.8 µCi/µmole) as

described (12). The assay mixture contained NAD^+ or $NADP^+$ (4mM) and [9-^{3}H]-prostacyclin sodium salt (80,000 dpm/1.8μM), post microsomal supernate of rabbit lung and Tris buffer, pH 8.5, in a final volume of 1 ml. Assay mixtures without the post microsomal supernate were run simultaneously as controls. After incubation at 37° for 2 minutes, the reaction was stopped by acidification of the mixture with 1M citric acid to pH 3.0. The reaction mixture was extracted 3 times with 3 ml of ethyl acetate and the extract dried under a stream of N_2. The residue was redissolved in 100 μl of acetone. An aliquot of 50 μl of the extract was applied on a thin-layer chromatographic plate (Brinkman) and developed twice with iso-octane/ethyl acetate/acetic acid/water (25:55:10:55, v/v) (8). Identification was made by comparison with the mobilities of authentic 6,15 diketo-$PGF_{1\alpha}$, 6-keto $PGF_{1\alpha}$, and PGE_2 and $PGF_{2\alpha}$ (Upjohn). The zones corresponding to 6,15 diketo-$PGF_{1\alpha}$ + 6,15 diketo-13,14 dihydro $PGF_{1\alpha}$, (Rf values: 0.46 and 0.47) and 6-keto $PGF_{1\alpha}$, (Rf: 0.23) were cut out and suspended in 10 ml of 0.4% Omniflour and 20% triton X-100 toluene liquid scintillation fluid and counted in a Searle Mark III liquid scintillation counter. The observed cpm were converted to dpm using a quench correction curve and external standards. The results were expressed as pmole of 6,15-diketoprostaglandin $F_{1\alpha}$ formed per min per mg of protein. Protein was determined by the method of Lowry et. al. using bovine albumin as a standard (9).

RESULTS AND DISCUSSION: The thin-layer chromatographic scan of the extract of the lung perfusate is shown in Fig. 1. Two major radioactive peaks were observed. The major product (compound I) had the mobility of 6-keto $PGF_{1\alpha}$; the minor radioactive peak (compound II) was similar in mobility to 6,15 diketo $PGF_{1\alpha}$ and 6,15 diketo-13,14 dihydro $PGF_{1\alpha}$; these substances represented 65% and 14% of the total radioactivity, respectively. The remaining radioactivity was not extracted from the effluent.

The radiometric gas-chromatographic profile of the derivatized sample also showed two major GC peaks which co-chromatographed with the methyl ester methyloxime TMSi ether derivatives of 6 keto-$PGF_{1\alpha}$ and 6,15 diketo-13,14 dihydro-$PGF_{1\alpha}$. Compound II which was inseparable from both 6,15 diketo-$PGF_{1\alpha}$ and 6,15 diketo-13,14 dihydro-$PGF_{1\alpha}$ on TLC was further analysed by radiometric gas-chromatography. A single radioactive peak corresponding to standard 6,15 diketo-13,14 dihydro $PGF_{1\alpha}$ was observed on the GC.

The trimethylsilyl derivative of compound I (MeMOTMSi) was identified as 6 keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, by mass spectrometry. The molecular ion and major fragment ions of compound II (Fig. 2) at m/e: 586 [M^+], 555 [M^+-CH_3O], 496 [M^+-TMSiOH],

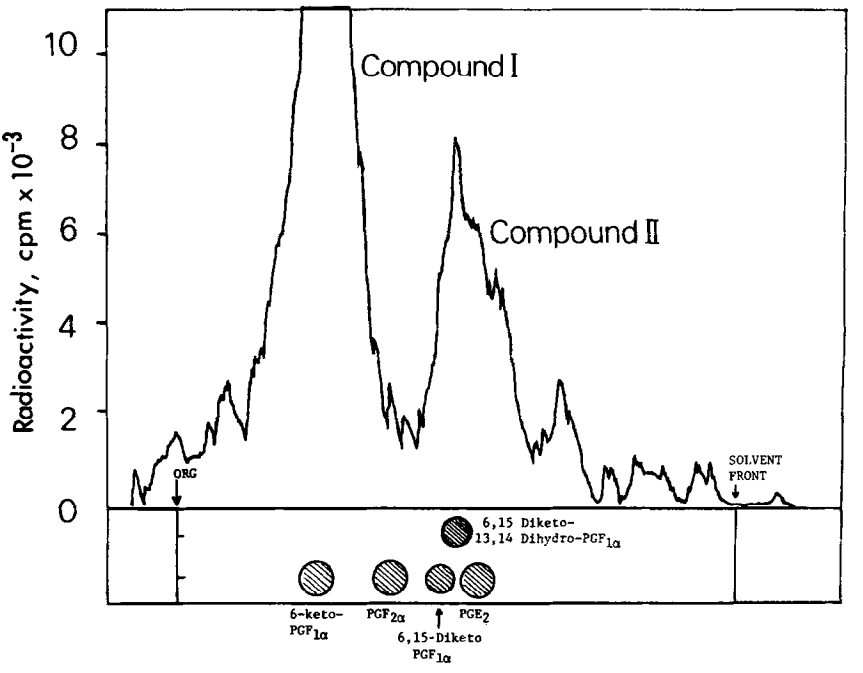

Fig. 1: Thin layer chromatogram of the radioactive products in the lung perfusate.

465 [M^+-TMSiOH-CH_3O], 425 [M^+-TMSiOH-C_5H_{11}], 475 [M^+-2XTMSiOH-CH_3O], 310 [M^+-C_1 to C_7-TMSiOH], 278 [M^+-2XTSiOH-C_{15} to C_{20}], 187 [H_2C=C (-NHOMe) $(CH_2)_4CO_2Me$] and 115 $[(CH_2)_4CO_2Me]^+$ were identical to the structure proposed for 6,15 diketo-13,14 dihydro-$PGF_{1\alpha}$ (10,11). Table I shows that the post-microsomal fraction of rabbit lung possesses both NAD^+ and $NADP^+$ dependent 15-hydroxyprostaglandin dehydrogenase and prostaglandin Δ^{13} reductase activities which metabolize [9-^{3}H]-PGI_2 to a single product which comigrated with 6,15 diketo-13,14 dihydro-$PGF_{1\alpha}$ on TLC. In this study, the NAD^+ dependent dehydrogenase was more active than the $NADP^+$ dependent enzyme.

This study demonstrates that PGI_2 was metabolized to the same product, 6,15 diketo-13,14 dihydro-$PGF_{1\alpha}$, whether it was perfused through the isolated rabbit perfused lung or added to the lung homogenates. Cockerill et. al. (11) reported the occurrence of 6,15 diketo-13,14-dihydro $PGF_{1\alpha}$ in

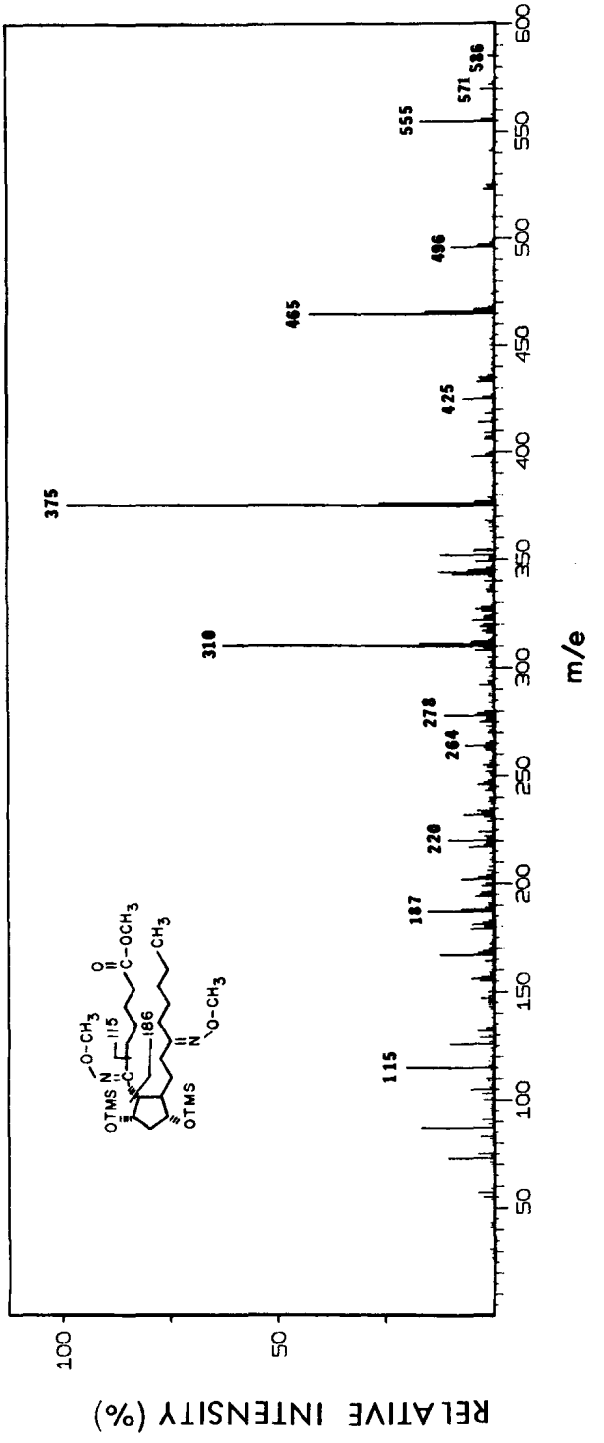

Fig. 2: Mass-spectrum of compound II eluted from the 1% SE-30 gas chromatography column.

Table I

NAD^+ and $NADP^+$-DEPENDENT 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE ACTIVITIES IN RABBIT LUNG

Enzyme activities given in pmol of 6,15 diketo-13,14-dihydro $PGF_{1\alpha}$ formed per min per mg protein.

		Enzyme activities	
Substrate	n	NAD^+ dependent	$NADP^+$ dependent
[9-^{3}H]-PGI_2	4	56.0 ± 2.0*	38.0 ± 2.1*

* p value less than 0.001, n= number of determination.

Post-microsomal supernate of the rabbit lung (4.6 mg protein) was incubated with NAD^+ or $NADP^+$ (4mM) [9-^{3}H]-PGI_2 (110,000 dpm) for 2 min at 37°C in 0.05 M Tris Buffer with 0.1 mM DTT, pH 8.5 (final volume 1 ml). The reaction was stopped by acidifying to pH 3.0 with 1M citric acid. The products were extracted and analyzed as described in Methods.

the effluent of sensitized challenged guinea pig lungs; they suggested that it resulted from the reduction of 6,15 diketo-$PGF_{1\alpha}$ to 6,15 diketo-13,14 dihydro $PGF_{1\alpha}$ by the lung prostaglandin Δ^{13} reductase. Despite the high capacity of the lung post-microsomal supernate enzyme to metabolize PGI_2, less than 20% of PGI_2 was metabolized on passage through the perfused lung of the rabbit. This may be due to low affinity of PGI_2 for the transport system of the lung (12) which determines accessibility of prostaglandins to the intracellular catabolizing enzymes and thereby degradation of prostaglandins in the venous blood in its transit through the pulmonary circulation (12). For example, PGA_2, although having a relatively low Km for the lung 15-hydroxyprostaglandin dehydrogenase (13), escapes destruction in the lung because of its low affinity for the pulmonary transport system (14).

Armstrong et. al. (15) have shown that the vasodepressor effect of PGI_2 is not reduced after passage through the rat and rabbit lung. Further,

Waldman and his co-workers (16) have demonstrated that the systemic vascular effect of PGI_2 is not altered after single passage through the pulmonary circulation of the dog. These authors concluded that PGI_2 is not metabolized on transit through the pulmonary circulation. However, in view of the present demonstration of PGI_2 is metabolized by 14%, the failure of these investigators to show metabolism of PGI_2 may be due to the relative insensitivity of non-specificity of the methods employed which could not detect the loss of less than 20% of PGI_2. The biochemical evidence presented here suggests that PGI_2 may be able to pass through the pulmonary circulation with little loss of activity and therefore may be a circulating hormone. However, this study does not rule out that under more physiological conditions, pulmonary metabolism of PGI_2 may be more efficient.

Recently, McGuire and Sun (17) have identified several metabolites of $[^3H]$-PGI_2 in the urine of rats after administration of $[11-^3H]PGI_2$. They found that most of the PGI_2 metabolites possess a keto-group at the C-15 position, which suggests that the metabolism of PGI_2 in vivo is initiated by a 15-hydroxyprostaglandin dehydrogenase catalyzed oxidation. Since 6 keto-$PGI_{1\alpha}$ is a poor substrate for 15-hydroprostaglandin dehydrogenase (17,18), these results and the present study indicate that PGI_2 is oxidized to 15-keto PGI_2 prior to its hydrolysis to the 6-keto derivative. The formation of 6,15 diketo-13,14-dihydro $PGI_{1\alpha}$ as the only enzymic product of PGI_2 metabolism present in the venous effluent of the lung indicated that the level of Δ^{13} reductase activity in the lung is higher than in blood vessels where the most abundant $[9-^3H]$-PGI_2 metabolite is 6,15 diketo-$PGF_{1\alpha}$ (18).

Acknowledgements:

This work was supported by NIH, Heart and Lung Institute Grants HL-22075 and HL-18845, The American Heart and Memphis Heart Association. P. Y-K. W. and K. U. M. are recipients of NIH, Heart and Lung Institute Young Investigator and Career Development Awards.

Prostacyclin, 6 keto-$PGF_{1\alpha}$ and their metabolites were gifts of Dr. U. Axen of the Upjohn Company. We thank Dr. L. Cagen for helpful criticism and Mrs. Patsy Wong and Ms. Patricia Goldstein for performing the enzyme assays.

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