Metabolism of Prostacyclin and 6-Keto-prostaglandin F\textsubscript{1α} in Man*

(Received for publication, April 2, 1980, and in revised form, June 26, 1980)

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Labeled and unlabeled prostacyclin and 6-keto-PGF\textsubscript{1α} were infused into healthy volunteers; urine was chromatographed on different systems including high pressure liquid chromatography. The peaks obtained by the latter method were derivatized to the methoxime methyl ester trimethyl silyl ether and analyzed by gasliquid chromatography-mass spectrometry. After infusion of prostacyclin the following metabolites could be identified: dinor-4-keto-7,9,13-trihydroxy-prosta-11,12-enoic acid (20.5%), dinor-4,13-diketo-7,9-dihydroxy-prostanoic acid (6.8%), dinor-4,13-diketo-7,9-dihydroxy-prostan-1,18-dioic acid (18.7%), and 6-keto-PGF\textsubscript{1α} (14.2%). The in vivo hydrolysis product of prostacyclin, 6-Keto-PGF\textsubscript{1α}, infusion resulted in the same metabolites with the relative amounts of 22.4, 5.4, 7.0, and 6.8%, respectively. Additionally, 6,15-diketo-13,14-dihydro-PGF\textsubscript{1α} (5.7%) could be identified. These data show that the metabolic pathway of prostacyclin involves hydrolysis to 6-keto-prostaglandin F\textsubscript{1α}, subsequent β-oxidation, dehydrogenation at C-15, reduction of the double bond between C-13 and C-14, and ω-oxidation to the dicarboxyl metabolite. We conclude that dinor-4-keto-7,9,13-trihydroxy-prosta-11,12-enoic acid and dinor-4,13-diketo-7,9-dihydroxy-prostan-1,18-dioic acid represent the major urinary metabolites of prostacyclin in man. 6-keto-PGF\textsubscript{1α} is a minor urinary excretory product following the administration of prostacyclin or 6-keto-PGF\textsubscript{1α}.

Prostacyclin (PG1\textsubscript{2})\textsuperscript{1} is a recently discovered prostanoid exerting biological activity in very small concentrations (1–4). Its platelet antiaggregating and vasodilator properties have stimulated investigations into its role in a number of pathologic conditions including atherosclerosis (5), diabetes (6), and uremia (7). Such investigations are hampered by the instability of PG1\textsubscript{2} at physiological pH leading to its conversion to PGF\textsubscript{2α} (8), which is almost completely metabolized on a single passage through the pulmonary circulation (10, 11). Consequently, it has been postulated that PG1\textsubscript{2} might represent a circulating hormone (12). Chemical measurements of PG1\textsubscript{2} have not been reported mainly because such measurements are difficult due to low concentrations and conversion to 6-keto-PGF\textsubscript{1α}. Measurement of prostacyclin metabolites might yield information on its rate of synthesis which is otherwise difficult to obtain.

Metabolism of PG1\textsubscript{2} has been described in several animal species (13–17). In a previous publication, we have already reported that dinor-4-keto-7,9,13-trihydroxy-prosta-11,12-enoic acid represents the main metabolite of prostacyclin and 6-keto-PGF\textsubscript{1α} (18). The present paper describes the major metabolites of both prostanoids in normal volunteers.

EXPERIMENTAL PROCEDURES

Infusion of Prostacyclin and 6-Keto-prostaglandin F\textsubscript{1α}.—PG1\textsubscript{2} and 6-keto-PGF\textsubscript{1α} were infused intravenously into four healthy male volunteers after informed consent was obtained. On separate occasions, [16, 17, 18, 19\textsuperscript{3H}]-PG1\textsubscript{2} in an amount of 100 μCi (specific activity 0.73 Ci/mmol) and 0.2 mg of unlabeled PG1\textsubscript{2} were infused at the rates of 1.0 ng/kg/min and 5 ng/kg/min, respectively. PG1\textsubscript{2} was infused in a glycine buffer solution (pH 10.5). A total amount of 75 μCi of [5, 8, 9, 11, 13, 14, 15\textsuperscript{3H}]-6-keto-PGF\textsubscript{1α} (specific activity 100 Ci/mmol) was administered at the rate of 0.04 ng/kg/min, whereas in a separate experiment, 2 mg of the unlabeled compound was infused at the rate of 420 ng/kg/min.

The labeled PG1\textsubscript{2} was stored as the methyl ester until 1 day before the experiment. Then it was demethylated in glycine buffer (pH 10.5). The purity of the demethylated compound was determined by silica gel thin layer chromatography (TLC) using the acidic solvent system chloroform/methanol/acetic acid (90:5:5) which converts PG1\textsubscript{2} to 6-keto-PGF\textsubscript{1α}. The radioactive material co-chromatographed with authentic 6-keto-PGF\textsubscript{1α} (R\textsubscript{T} = 0.28). Contamination of PG1\textsubscript{2} with 6-keto-PGF\textsubscript{1α} was excluded by derivatization to the more stable p-phenylphenacyl ester (19) and TLC at 4°C using ethyl acetate as solvent. The corresponding derivatives of authentic PG1\textsubscript{2} and 6-keto-PGF\textsubscript{1α} were used as reference compounds. The p-phenylphenacyl ester of the demethylated PG1\textsubscript{2} co-chromatographed with the PG1\textsubscript{2} ester (R\textsubscript{T} = 0.36) whereas the ester of 6-keto-PGF\textsubscript{1α} showed a R\textsubscript{T} value of 0.28.

The identity and purity of the labeled 6-keto-PGF\textsubscript{1α} was proven by TLC and scanning (Berthold Dünnschicht Scanner LB 2723, Berthold, Germany). The following solvent systems were used: chloroform/methanol/acetic acid (90:5:5), ethyl acetate/isoctane/acetate acid (water saturated; double developed) (44:20:8), and ethyl acetate/acetate acid (50:1). The radioactive material co-chromatographed with reference 6-keto-PGF\textsubscript{1α} showing R\textsubscript{T} values of 0.26, 0.23, and 0.22, respectively.

Blood pressure and pulse rate were not affected during the infusions. The urine was collected from the beginning until 2 h after termination of the infusion.

Chromatography of the Urine.—The urine was acidified to pH 3.0 with formic acid and subsequently percolated through a column packed with Amberlite XAD-2 (25 × 5 cm). The column was washed with 1000 ml of water, and then the radioactivity was eluted with tetrahydrofuran. The solvent was evaporated, and the residue dissolved in acetone/chloroform (1:1) and purified on an open bed silicic acid column (30 g of SilicAR CC-4, Mallinckrodt Chemical Works) with the same solvent system. The materials obtained from infusions with the labeled and the unlabeled prostanoids were combined, the solvent was evaporated, and the residue dissolved in 2 ml of acetonitrile. The sample then was chromatographed on an open bed reversed phase column packed with 20 g of C\textsubscript{18} phase-bonded HI-FLOSIL (80 to 100 mesh, Applied Science Laboratories). A gradient of 10%, 30%, 50%, and 80% acetonitrile in water was used as solvent system (16). The flow rate was adjusted to 10 to 20 ml/h. Fractions of 2 ml were collected.

* This work was supported by a grant of the Robert Bosch Foundation, Stuttgart, Germany (Federal Republic of Germany). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: PG, prostaglandin; GLC-MS, gas-liquid chromatography-mass spectrometry; HPLC, high pressure liquid chromatography; TLC, thin layer chromatography.
Several zones could be separated on this column. Most of the radioactivity could be extracted into chloroform and further purified on high pressure liquid chromatography (HPLC) using a µPorasil column (Waters Associates). A gradient was applied changing linearly from chloroform to chloroform/methanol/acetic acid (94:5:0.2:0.5) within 1 h (System A) at the flow rate of 1 ml/min. One-milliliter fractions were collected and the radioactivity determined by liquid scintillation counting. The fractions corresponding to one peak were collected and partly repurified on a reversed phase HPLC system changing linearly from 20% to 80% ethyl acetate in chloroform within 1 h (System A) at the flow rate 1 ml/min (System C). The electron energy was kept at 70 eV. The mass spectrum (Fig. 4) shows the prominent ions 350 (M+7 - 2 x 90 - 71), 390 (M+7 - 2 x 90 - 31), 421 (M+7 - 2 x 90), 440 (M+7 - 71 - 90), 480 (M+7 - 90 - 31), 511 (M+7 - 90), 530 (M+7 - 71), 570 (M+7 - 31), 586 (M+7 - 15), and 601 (M+7). This mass spectrum is identical with that of dinor-4-keto-7,9,13-trihydroxy-prosta-11,12-enoic acid (21). In addition, the fragments 394, 425, 484, and 574 can be detected. These fragments are not show analogous δ3 fragments could be explained by the low relative intensity of the parent ions.

**RESULTS**

**Urinary Metabolites of Prostacyclin**—Chromatography of the urine on XAD-2 and subsequent silicic acid column showed a recovery of 94%. This material was split into two aliquots and separately eluted on open reversed phase column chromatography (Hi-FLOSIL) with a recovery of 60 and 51%, respectively. One chromatogram is shown in Fig. 1. The two zones obtained were separated further on µPorasil HPLC (System A) (see Fig. 2, A and B). Because the material belonging to the peaks with a retention volume of 32 to 36 ml showed two peaks on subsequent gas-liquid chromatography, it was repurified by reversed phase HPLC (System B) as shown in Fig. 3. The fractions corresponding to the main peak (20.5% of the radioactivity) were derivatized and analyzed by GLC-MS. The mass spectrum (Fig. 4) shows the prominent ions 350 (M+7 - 2 x 90 - 71), 390 (M+7 - 2 x 90 - 31), 421 (M+7 - 2 x 90), 440 (M+7 - 71 - 90), 480 (M+7 - 90 - 31), 511 (M+7 - 90), 530 (M+7 - 71), 570 (M+7 - 31), 586 (M+7 - 15), and 601 (M+7). This mass spectrum is identical with that of dinor-4-keto-7,9,13-trihydroxy-prosta-11,12-enoic acid (21). In addition, the fragments 394, 425, 484, and 574 can be detected. These fragments are not expected to have a corresponding deuterated ion because the position of labeling was at C-16 to C-19. The fact that the ions 511, 586, and 601 did not show analogous δ3 fragments could be explained by the low relative intensity of the parent ions.

**Fig. 1.** Open bed reversed phase column chromatography of prostanoids obtained from the urine on XAD-2, 1 ml fractions were collected.

**Fig. 2.** High pressure liquid chromatography (µPorasil column) of an extract of plasma. The two peaks indicated are (A) 6-keto-PGF1α and (B) 6-keto-PGF2α.

**Fig. 3.** Reversed phase high pressure liquid chromatography of Peak 3 recovered from the µPorasil column (Fig. 2). The flow rate was reduced to 2 ml/min, and 0.2-ml fractions were collected.
The material belonging to the peak with a retention volume of 16 to 19 ml (Fig. 2B), and accounting for 6.8% of the radioactivity eluted from HPLC, gave a mass spectrum with the prominent ions 347 ($M^+ - 31 - 2 \times 90$), 437 ($M^+ - 31 - 90$), 468 ($M^+ - 90$), and 527 ($M^+ - 31$) (Fig. 5). This mass spectrum could be identified as dinor-4,13-diketo-7,9-dihydroxyprostanoic acid (15, 16). The deuterated fragments show that the mass spectrum corresponded to the infused labeled prostacyclin as described above.

GLC-MS of the fractions with a retention volume of 38 to 40 ml (15.5% of the radioactivity) (Fig. 2B) resulted in a mass spectrum with the ions 354 ($M^+ - 158 - 90$), 391 ($M^+ - 31 - 2 \times 90$), 472 ($M^+ - 115 - 15$), 481 ($M^+ - 31 - 90$), 512 ($M^+ - 90$) and 571 ($M^+ - 31$) (Fig. 6). This mass spectrum could be identified as dinor-4,13-diketo-7,9-dihydroxy-prostan-1,18-dioic acid (16). Fragment 472 was not observed by Sun and Taylor (16). In addition, a fragment characterized by ($M^+ - 15 - C_{19}$ to $C_{20}$) could not be obtained from the other metabolites of prostacyclin or 6-keto-prostaglandin $F_{1\alpha}$. Therefore, it is likely that this ion results from contamination from other biological material contained in the urine. Such peaks are occasionally observed in the mass spectra obtained from biological sources (14). This spectrum does not show any deuterated fragments. However, the carbonyl function at the upper chain indicates that this prostanoid represents a metabolite of PGL$_2$ or of 6-keto-PGF$_{1\alpha}$. Therefore, it might be speculated that the rate of endogenous production of this metabolite is so high that the deuterated compound could not be detected.

Of the radioactivity, 14.2% was eluted with the same retention volume as reference 6-keto-prostaglandin $F_{1\alpha}$ (44 to 47 ml) (Fig. 2, A and B). The ions expected for 6-keto-prostaglandin $F_{1\alpha}$ could be detected, i.e. 378 ($M^+ - 71 - 2 \times 90$), 418 ($M^+ - 31 - 2 \times 90$), 468 ($M^+ - 71 - 90$), 508 ($M^+ - 31 - 90$), 558 ($M^+ - 71$), 598 ($M^+ - 31$), 629 ($M^+ - 90$), as well as the deuterated fragments 422, 512, and 602.

**Urinary Metabolites of 6-Keto-prostaglandin $F_{1\alpha}$**—The overall recovery during the chromatography of urine was 48% while the recovery of the subsequent open reversed phase column was 60%. On the latter column, four zones (A, B, C, and D) could be defined. These zones were recovered from Fractions 20 to 24, 76 to 159, 160 to 191, and 192 to 214, respectively. Zones B, C, and D were chromatographed further on Porasil HPLC. Zone B could be separated essentially into nine peaks while Zone C resulted in one large peak with the retention volume of 32 to 36 ml and two smaller peaks. Zone D could be separated into two peaks with the retention volumes of 17 to 20 and 85 to 86 ml, respectively.

The material which was eluted with a retention volume of 32 to 36 ml from this HPLC (System A) was further chromatographed on reversed phase HPLC (System B).

Four peaks could be separated. The main peak containing 29.0% of the radioactivity eluted from HPLC was identified as dinor-4-keto-7,9,13-trihydroxy-prosta-11,12-enoic acid by GLC-MS showing the same characteristic fragments as described above. The fractions with a retention volume of 7 to 12 ml on reversed phase HPLC containing 4.8% of the radioactivity were collected and analyzed by GLC-MS. The mass fragmentation was identical with that described for dinor-4,13-diketo-7,9-dihydroxy-prostan-1,18-dioic acid.

The material eluting with a retention volume of 17 to 20 ml represented 4.7% of the radioactivity; it was identified by
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Metabolism of Prostacyclin as dinor-4,13-diketo-7,9-dihydroxy-prostanoic acid characterized by the same fragmentation ions as shown earlier.

Five per cent of the radioactivity was eluted with a retention volume of 28 to 30 ml. The mass spectrum (Fig. 7) showed the ions 375 (M<sup>+</sup> - 31 - 2 × 90), 425 (M<sup>+</sup> - 71 - 90), 465 (M<sup>+</sup> - 31 - 90), 496 (M<sup>+</sup> - 90), 555 (M<sup>+</sup> - 31), and 571 (M<sup>+</sup> - 15). This spectrum was identical with that described for 6,15-diketo-13,14-dihydro-prostaglandin F<sub>1α</sub>(22).

The fractions with a retention volume of 43 to 47 ml showed the same mass spectrum as authentic 6-keto-prostaglandin F<sub>1α</sub>. Only a small amount of the radioactivity recovered from Zone A of the HI-FLOSIL column could be extracted into chloroform. Therefore, these polar metabolites eluting in Zone A were chromatographed further on HPLC using a different solvent system (System C) than described for the other metabolites. This chromatography resulted in several peaks indicating that this fraction did not correspond to one single polar metabolite. These polar metabolites, possibly glutathione conjugates (23), were not analyzed further.

A summary of the relative amounts of urinary metabolites of PGI<sub>2</sub> and 6-keto-PGF<sub>1α</sub> in man is given in Table I. The radioactivity obtained for the diketo metabolites of 6-keto-prostaglandin F<sub>1α</sub> has been corrected for the loss of 1 tritium atom during oxidation at C-15. 6,15-Diketo-13,14-dihydro-prostaglandin F<sub>1α</sub> could not be detected in the urine after prostacyclin infusion. However, 1.4% of the radioactivity was eluted in a peak with the same retention volume as found for this prostanoid after chromatography of the urine obtained from 6-keto-prostaglandin F<sub>1α</sub> infusion. It is possible that the material under this peak is identical with this metabolite. Dinor-4,13-diketo-7,9-dihydroxy-prostan-1,18-dioic acid could be detected in the urine obtained from 6-keto-prostaglandin F<sub>1α</sub> infusion only in one peak recovered from rechromatography of the fractions which had a retention volume of 32 to 36 ml on ρPorasil HPLC. However, after chromatography of the urine of prostacyclin infusion, the same prostanoid was found only at a retention volume of 38 to 40 ml. Therefore, for determination of the relative amounts of this metabolite of prostacyclin or 6-keto-prostaglandin F<sub>1α</sub> in urine in both cases the radioactivities of these two peaks were added.

**DISCUSSION**

Infusion of prostacyclin and 6-keto-PGF<sub>1α</sub> in man results in the metabolites shown in Fig. 8. Both prostanoids give a similar pattern of metabolites which results from the activities of enzymes affecting β-oxidation, Δ13-reduction, 15-hydroxy-dehydrogenation, and ω-oxidation. It is conceivable that the
similarity of the pattern of metabolites of the two prostanoids is the result of early conversion of prostacyclin to 6-keto-PGF$_1\alpha$ in the circulation.

The sequence of the various metabolic events for PGE$_2$, PGF$_2\alpha$, and PGI$_2$ has been elucidated previously and found to start with 15-OH-dehydrogenation followed by 13-reduction (24-26). We have been able to identify metabolites of PGI$_2$ and 6-keto-PGF$_1\alpha$ resulting from these metabolic steps. However, simple $\beta$-oxidation and subsequent elimination is a prominent pathway of PGI$_2$ and 6-keto-PGF$_1\alpha$ metabolism. In addition to dinor-4-keto-7,9,13-trihydroxy-prosta-11,12-enoic acid ("dinar-6-keto-PGF$_1\alpha$"), two metabolites resulting from $\beta$-oxidation were identified, dinor-4,13-diketo-7,9-dihydroxy-prostanoic acid and its $\omega$-oxidized analogue. We suggest that metabolism proceeds from 6-keto-PGF$_1\alpha$ via $\beta$-oxidation, 15-hydroxy-dehydrogenation and 13-reduction to the dicarboxyl metabolite.

In vitro studies have shown that some $\beta$-oxidized prostaglandins are not as good a substrate for 15-hydroxy-prostaglandin-dehydrogenase as the parent compounds (27). Therefore, the possibility cannot be excluded that metabolism starts with 15-dehydrogenation and 13-reduction followed by $\beta$- and $\omega$-oxidation.

Dinor-4-keto-7,9,13-trihydroxy-prosta-11,12-enoic acid has also been detected as a metabolite of PGI$_2$ and 6-keto-PGF$_1\alpha$ in the rat (16, 17). Furthermore, this metabolite was observed during perfusion of the isolated rabbit kidney (15). Thus, $\beta$-oxidation of PGI$_2$ and 6-keto-PGF$_1\alpha$ occurs in the organ of their synthesis (28) and excretion.

The reason for the difference in the relative amounts of metabolites observed after prostacyclin or 6-keto-PGF$_1\alpha$ infusion is not obvious. Even though infusion rates for these metabolites observed after prostacyclin or 6-keto-PGF$_1\alpha$, in addition to biochemical determination of the rate of synthesis of PGI$_2$ in addition to bioassays. It could be shown that 6-keto-PGF$_1\alpha$, is not the major urinary metabolite of PGI$_2$. Measurement of dinor-4-keto-7,9,13-trihydroxy-prosta-11,12-enoic acid should provide a better parameter of endogenous prostacyclin synthesis.

Acknowledgment—We would like to express our gratitude to Mrs. I. Koch for preparation of the manuscript.

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