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Biochemistry

Transformation of prostaglandin D_2 to 9α,11β-(15*S*)-trihydroxyprosta-(5*Z*,13*E*)-dien-1-oic acid (9α,11β-prostaglandin F_2): A unique biologically active prostaglandin produced enzymatically *in vivo* in humans

(11-ketoreductase/11-epiprostaglandin $F_{2\alpha}$/human liver/mastocytosis)

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ABSTRACT *In vitro* studies examining the metabolic transformation of prostaglandin D_2 (PGD_2) by human liver were conducted. PGD_2 was found to be converted by a NADPH-dependent enzyme in the 100,000 × *g* supernatant of human liver exclusively to a single more polar compound that had a mass spectrum essentially the same as that of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). However, this compound could be chromatographically separated from $PGF_{2\alpha}$ and failed to form a butylboronate derivative. The structure of this compound was established as 9α,11β-(15*S*)-trihydroxyprosta-(5*Z*,13*E*)-dien-1-oic acid (9α,11β-PGF_2) by comparison of its chromatographic and mass spectral characteristics with authentic 9α,11β-PGF_2. This compound was found to be biologically active by demonstrating increases in blood pressure in rats in a dose-related fashion following intravenous administration. By using a mass spectrometric assay, levels of this compound in plasma and urine from a normal volunteer were 6 pg/ml and 982 ng/24 hr, respectively. In a patient with systemic mastocytosis associated with overproduction of PGD_2, urinary excretion of 9α,11β-PGF_2 was 6634 ng/24 hr and a circulating plasma level as high as 490 ng/ml was found during a severe episode of systemic mast cell activation. 9α,11β-PGF_2 is structurally a unique prostaglandin, is enzymatically formed, is produced *in vivo* in humans, and is biologically active.

Recently we investigated the metabolism of prostaglandin D_2 (PGD_2) in a normal male volunteer and found that PGD_2 is converted predominantly to PGF-ring compounds (1). These data provided presumptive evidence for 11-ketoreductase activity *in vivo* in humans analogous to evidence for this pathway of metabolism of PGD_2 described in the monkey (2). Furthermore, almost all of the PGF-ring urinary metabolites of PGD_2 isolated failed to form a butylboronate derivative when treated with *n*-butylboronic acid, suggesting that the C-9 and C-11 hydroxyl groups of these metabolites were not coplanar (3). Intact PGF_2 was also excreted into the urine as a metabolite of PGD_2 and did not react with *n*-butylboronic acid. We have now undertaken *in vitro* studies of the conversion of PGD_2 to PGF_2 and report the finding that PGD_2 is converted stereospecifically to 9α,11β-(15*S*)-trihydroxyprosta-(5*Z*,13*E*)-dien-1-oic acid (9α,11β-PGF_2) by an enzyme in the 100,000 × *g* supernatant of human liver, that this compound is present in plasma and urine from humans, and that this compound is biologically active.

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EXPERIMENTAL PROCEDURES

Materials and Methods. Unlabeled PGD_2, $PGF_{2\alpha}$, and 9α,11β-PGF_2 were the generous gifts of John Pike and Gordon Bundy of Upjohn. Synthesis of 9α,11β-PGF_2 was accomplished with modifications of procedures described previously (4). The synthetic 9α,11β-PGF_2 contains ≈15% 5-*trans* isomer, which was removed by HPLC, yielding pure 5-*cis* 9α,11β-PGF_2. [5,6,8,9,11,12,14,15-$^2H_8$]Arachidonic acid and [$^2H_7$]PGD_2 were prepared following previously published procedures (5, 6).

Fractionation of Human Liver. Eighteen grams of human liver was homogenized (1:3, wt/vol) in 0.1 M potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 4°C at 10,000 × *g* for 30 min. The 10,000 × *g* supernatant was then centrifuged for 90 min at 100,000 × *g*. The 100,000 × *g* microsomal pellet was resuspended (1:3, vol/vol) in a 10 mM Tris acetate solution (pH 7.4) containing 1 mM EDTA and 20% glycerol and stored at −70°C. Protein was determined according to the method of Bradford (7) with bovine serum albumin as a standard.

***In Vitro* Assessment of Human 11-Ketoreductase Activity.** 11-Ketoreductase activity was determined by monitoring the conversion of [$^3H_7$]PGD_2 to tritiated compounds with a C-11 hydroxyl group. The reaction mixture contained 50 μg of PGD_2 (47 μM), 300,000 cpm of [$^3H_7$]PGD_2, NADP (0.5 mM), glucose 6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (9 units), 0.5 ml of liver supernatant (≈5 mg of protein), and 0.1 M phosphate buffer (pH 7.4) to a final volume of 3 ml. Reactions were carried out for 60 min at 37°C.

The methyl ester trimethylsilyl ether derivative (Me-Me_3Si) of products formed was prepared by treatment with diazomethane and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), respectively, and analyzed by using a Nermag R10-10C gas chromatograph/mass spectrometer interfaced with a DEC PDP-11/23 plus computer system. GC was performed on a 6-m DB-1 fused silica capillary column (inner diameter = 0.25 mm; coating thickness = 0.25 μm) programed from 190°C to 325°C at 25°C/min. Alternatively, mass spectra were obtained by using a LKB 9000 GC/MS with conditions as described (8).

Preparation of [$^2H_7$]9α,11β-PGF_2. [5,6,8,9,11,12,14,15-$^2H_8$]Arachidonic acid was converted to [$^2H_7$]PGD_2 as described (6). [$^2H_7$]9α,11β-PGF_2 was obtained by incubating

Abbreviations: PG, prostaglandin; 9α,11β-PGF_2 (11-epi-$PGF_{2\alpha}$), 9α,11β-(15*S*)-trihydroxyprosta-(5*Z*,13*E*)-dien-1-oic acid; Me, methyl ester; F_5Bzl, pentafluorobenzyl ester; Me_3Si, trimethylsilyl ether; Bu^tMe_2Si, *t*-butyldimethylsilyl ether; SP, straight phase; RP, reversed phase; BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide.

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$[^2H_7]PGD_2$ with the 100,000 × g supernatant of human liver as described above. After purification by reversed-phase HPLC (RP-HPLC) as described below, $[^2H_7]9\alpha,11\beta$-PGF_2 was standardized by selected ion-monitoring MS against $[^2H_0]9\alpha,11\beta$-PGF_2.

Assessment of Biological Activity of $9\alpha,11\beta$-PGF_2. Female Sprague–Dawley rats (≈500 g) were anesthetized with pentobarbital (30 mg/kg) and blocked ganglionicly with chlorisondamine chloride (Ecolid, CIBA–Geigy, 3 mg/kg). The femoral artery and vein were catheterized. Arterial blood pressure was monitored by a pressure transducer with a Hewlett–Packard 7754B recorder. $PGF_{2\alpha}$ and $9\alpha,11\beta$-PGF_2 were dissolved in 0.9% NaCl in water and administered i.v. as a bolus dose.

Assay of Urinary and Plasma $9\alpha,11\beta$-PGF_2. To ≈50 ml of urine and ≈3–10 ml of plasma was added 27.75 ng and 11.10 ng, respectively, of $[^2H_7]9\alpha,11\beta$-PGF_2. Samples were extracted by using C_{18} Sep-Pak cartridges and were further purified on Silica Sep-Pak (9). Urine and plasma samples were then subjected to straight-phase HPLC (SP-HPLC) and urine samples were further purified by RP-HPLC (for conditions, see Figs. 1 and 2). Urinary $9\alpha,11\beta$-PGF_2 and plasma $9\alpha,11\beta$-PGF_2 were then converted to a pentafluorobenzyl ester (F_5Bzl), t-butyldimethylsilyl ether (Bu^tMe_2Si) derivative and were further purified by TLC with a solvent of heptane/ethyl acetate, 10:1 (vol/vol) (R_f = 0.48).

Quantification of urinary and plasma $9\alpha,11\beta$-PGF_2 was accomplished by GC negative-ion chemical ionization MS using a Hewlett–Packard 5982A gas chromatograph/mass spectrometer (10). The negative-ion chemical ionization mass spectrum of the F_5Bzl-Bu^tMe_2Si derivative of $9\alpha,11\beta$-PGF_2 generates essentially a single intense ion at m/z 695 (M-181), loss of $CH_2C_6F_5$ (data not shown). Quantification of urinary and plasma $9\alpha,11\beta$-PGF_2 was accomplished by selected ion monitoring of the ratio of the ions m/z 695 (endogenous $9\alpha,11\beta$-PGF_2) and m/z 702 ($[^2H_7]9\alpha,11\beta$-PGF_2).

RESULTS

***In Vitro* Metabolism of PGD_2 by Human Liver.** After incubation of $[^3H]PGD_2$ (47 μM) with the 100,000 × g supernatant of human liver in the presence of a NADPH-generating system and subsequent extraction of products formed $[^{14}C]PGF_{2\alpha}$ was added and the mixture was analyzed by SP-HPLC (Fig. 1). PGD_2, which characteristically elutes between 30 and 40 ml, was found to be converted essentially quantitatively to a single more polar product that was slightly separated chromatographically from added $[^{14}C]PGF_{2\alpha}$. Differences in the chromatographic characteristics of the ^3H-labeled polar hepatic metabolite and $[^{14}C]PGF_{2\alpha}$ were even more apparent on RP-HPLC (Fig. 2). This experiment has been repeated >20 times with identical results.

The conversion of PGD_2 by the liver supernatant was found to be NADPH dependent in that <10% conversion of PGD_2 to the polar product occurred in the presence of 2 mM NADH, NAD, or NADP (data not shown). Incubation of PGD_2 with boiled liver supernatant in the presence of NADPH also did not result in the conversion of PGD_2 to the polar compound, indicating that this transformation of PGD_2 is an enzymatic process. Incubation of PGD_2 with the microsomal pellet of human liver also did not result in the conversion of PGD_2 to the polar compound, indicating that the enzyme responsible for this conversion is a cytosolic enzyme. Conversion of PGD_2 to this more polar product by an enzyme in the hepatic supernatant preparation occurred in a time-dependent fashion, converting ≈0.43 nmol of PGD_2 per min/mg of protein to the more polar compound. Incubation of the polar compound produced from PGD_2 with the human liver supernatant in the presence of NADP resulted in the conversion of <5% of this compound to PGD_2, indicating only slow reversibility of this enzyme.

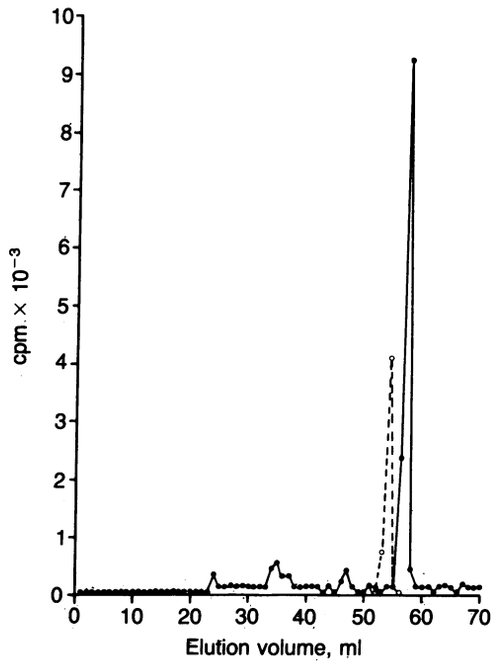

FIG. 1. SP-HPLC of $[^{14}C]PGF_{2\alpha}$ and hepatic supernatant metabolites of $[^3H_7]PGD_2$. Column: Alltech 5-μm silica; solvent systems chloroform/acetic acid, 100:0.1 (vol/vol) (A), and chloroform/methanol/acetic acid, 90:10:0.1 (vol/vol/vol) (B); solvent program A to 100% B over 2 hr, 1 ml/min, 1-ml fractions. ●—●, ^3H; ○--○, $[^{14}C]PGF_{2\alpha}$.

Structural Identification of the Product Formed from PGD_2 by Human Liver Supernatant. The hepatic metabolite obtained after RP-HPLC was initially converted to a Me-Me_3Si derivative and was analyzed by GC/MS. This yielded a single

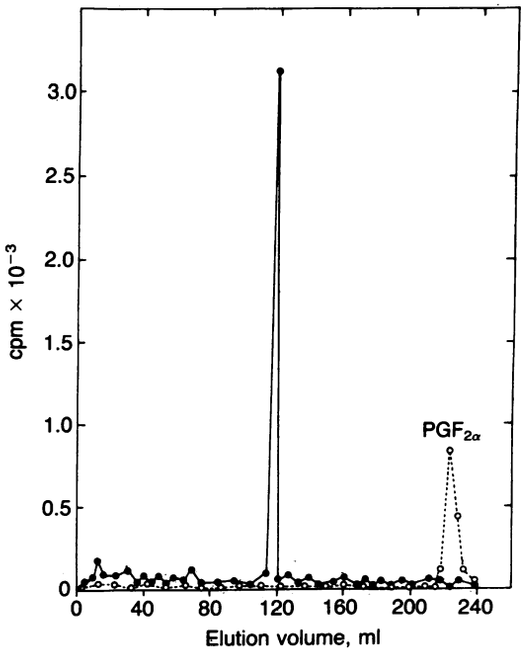

FIG. 2. RP-HPLC of $[^{14}C]PGF_{2\alpha}$ and the polar hepatic supernatant metabolite of $[^3H_7]PGD_2$. Column: Alltech 5-μm C_{18}; solvent system acetonitrile/water/acetic acid, 23:77:0.1 (vol/vol/vol), run isocratically, 1 ml/min, 1-ml fractions. ●—●, ^3H; ○--○, ^{14}C.

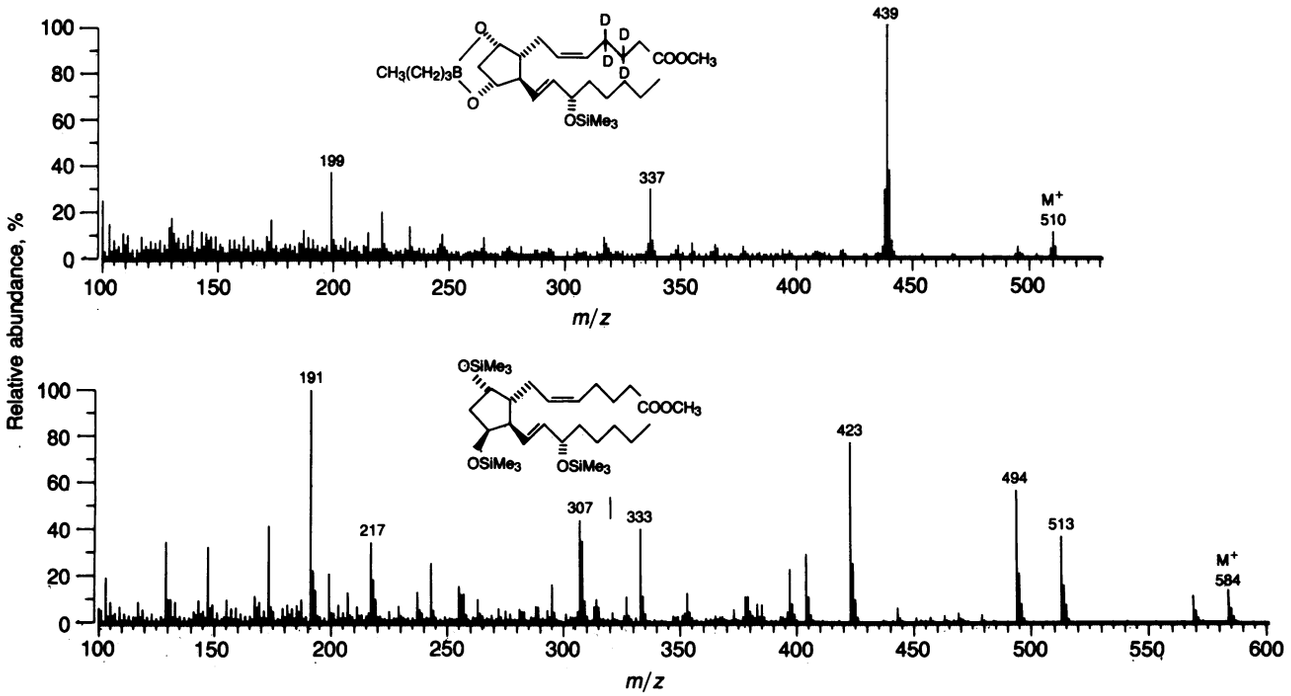

FIG. 3. Mass spectra of the hepatic supernatant 11-ketoreductase metabolite of PGD_2 (*Lower*) and $[^2H_4]PGF_{2\alpha}$ (*Upper*) following coderivatization by treatment sequentially with diazomethane, *n*-butylboronic acid, and BSTFA.

prominent total ion current peak at C-23.8 with a mass spectrum similar to previously published mass spectra of the Me-Me_3Si derivative of $PGF_{2\alpha}$, except for some minor variation in relative ion abundances (11). To determine whether the stereochemical configuration of the prostane ring hydroxyl groups of the hepatic metabolite and $PGF_{2\alpha}$ differed, the ability of these compounds to form a butylboronate derivative when treated with *n*-butylboronic acid was examined. A butylboronate derivative will form bridging the hydroxyl oxygens at C-9 and C-11 only if both hydroxyl groups are coplanar in relation to the prostane ring (3). Approximately equal amounts of $[^2H_4]PGF_{2\alpha}$ and the hepatic metabolite were mixed and coderivatized initially to a methyl ester followed by sequential treatment with *n*-butylboronic acid and BSTFA and subsequent analysis by GC and GC/MS. $[^2H_4]PGF_{2\alpha}$ was coderivatized with the hepatic metabolite to provide a means to determine the completion of the boronation reaction when analyzed by GC/MS. When analyzed by GC, two prominent peaks were present at C-23.8 and C-25.4. When analyzed by GC/MS, the peak at C-23.8 yielded a mass spectrum essentially the same as published mass spectra of the Me-Me_3Si derivative of $[^2H_0]PGF_{2\alpha}$ (11) (Fig. 3 *Lower*). The peak at C-25.4 yielded a mass spectrum consistent with the Me-butylboronate-Me_3Si derivative of $[^2H_4]PGF_{2\alpha}$ (Fig. 3 *Upper*). Importantly, there were no doublet ion peaks in either mass spectrum differing by four atomic mass units, indicating that the $[^2H_4]PGF_{2\alpha}$ was quantitatively converted to a butylboronate derivative and that none of the compound at C-23.8 was converted to a butylboronate derivative.

The above data suggested that the structure of the compound formed from PGD_2 by the liver supernatant was $9\alpha,11\beta$-PGF_2. To further define the structure of this compound, we compared the chromatographic characteristics, mass spectra, and biological activity of the compound with authentic chemically synthesized $9\alpha,11\beta$-PGF_2. The Me-Me_3Si derivatives of both compounds had identical mass spectra when analyzed on the same mass spectrometer (Fig. 4). When approximately equal quantities of the two compounds were subjected to RP-HPLC using conditions described previously (see Fig. 2), both compounds eluted in a single smooth symmetrical peak at ≈120 ml (data not shown). Compound elution was detected by monitoring UV absorbance at 205 mm. Both compounds also perfectly coeluted when cochromatographed as a Me-Me_3Si derivative on a 183-cm GC column of SP-2250 (Supelco) operated isothermally at 240°C (data not shown). Although $PGF_{2\alpha}$ and $9\alpha,11\beta$-PGF_2 coelute on a GC column of SP-2100, they are almost baseline-separated on SP-2250, with $PGF_{2\alpha}$ eluting at a slightly longer retention time than $9\alpha,11\beta$-PGF_2.

Blood Pressure Response in the Rat to Administered $PGF_{2\alpha}$, Authentic $9\alpha,11\beta$-PGF_2, and the Product Formed from PGD_2 by Human Liver Supernatant. The biological effects of authentic $9\alpha,11\beta$-PGF_2 and the hepatic metabolite on rat blood pressure were next compared. Rats that were blocked ganglionicly were prepared as described under *Experimental Procedures* and varying doses of $PGF_{2\alpha}$, authentic $9\alpha,11\beta$-PGF_2, and the hepatic metabolite were administered by i.v. bolus while the blood pressure responses recorded from an arterial catheter were monitored (Fig. 5). Authentic $9\alpha,11\beta$-PGF_2 and the hepatic metabolite were both found to cause a rise in systemic blood pressure in a dose-related fashion and both compounds were approximately equivalent in potency. Injection of saline alone was not associated with a change in blood pressure. The relative potency of $9\alpha,11\beta$-PGF_2 was also compared to the vasopressor effects of $PGF_{2\alpha}$ and to the vasodepressor effects of PGD_2, potent vasoactive prostaglandins. $9\alpha,11\beta$-PGF_2 and $PGF_{2\alpha}$ were approximately equipotent in raising blood pressure in the rat and this rise in blood pressure was approximately equal in magnitude to the fall in blood pressure that occurred following injection of equivalent doses of PGD_2 (data not shown).

Levels of $9\alpha,11\beta$-PGF_2 in Human Plasma and Urine. To determine whether $9\alpha,11\beta$-PGF_2 is actually produced *in vivo* in humans, levels of this compound were quantified in plasma and urine obtained from a normal volunteer and from a patient with systemic mastocytosis (Table 1). This patient experienced severe attacks of mast cell activation with attendant release of large quantities of the mast cell mediators, histamine and PGD_2 (12).

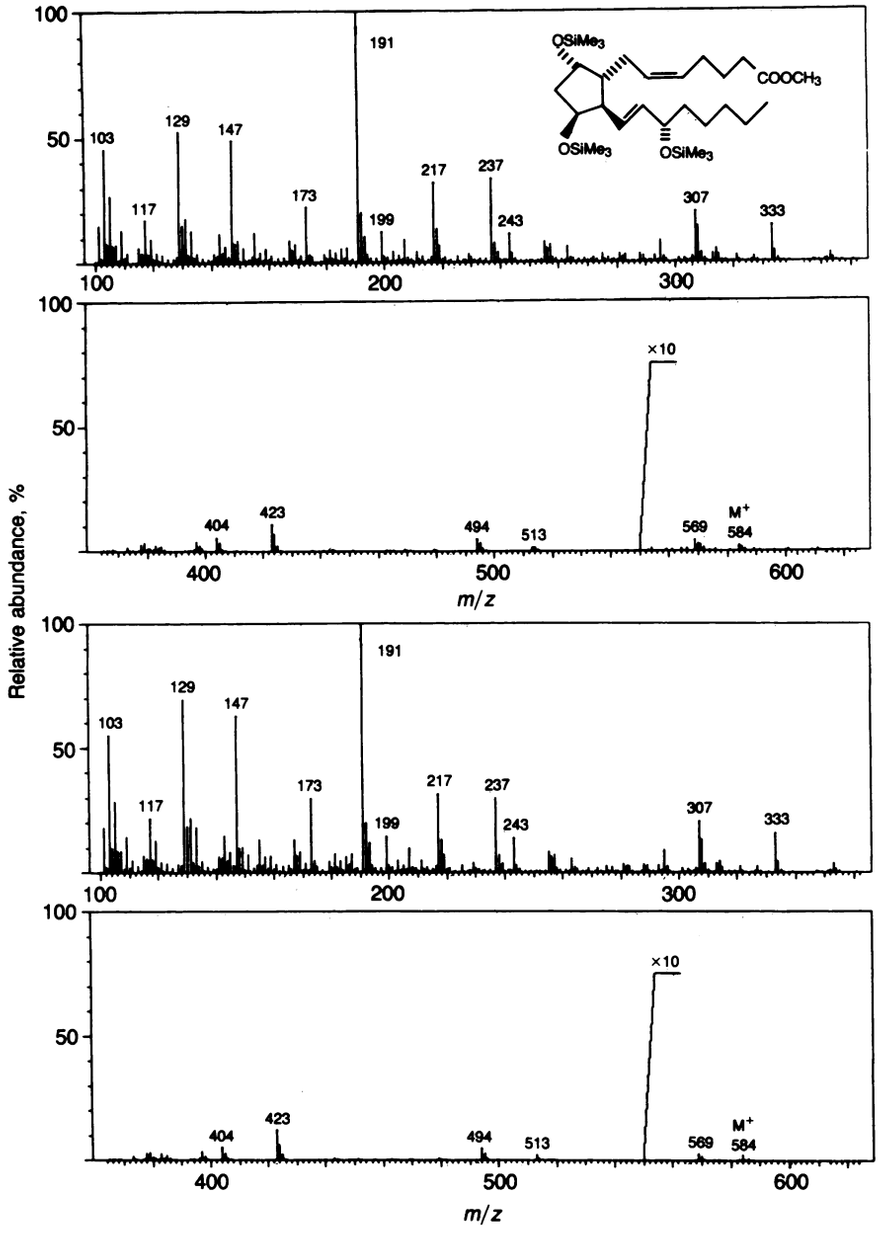

FIG. 4. Mass spectra of the Me-Me_3Si derivatives of authentic chemically synthesized 9α,11β-PGF_2 (*Upper*) and the hepatic 11-ketoreductase metabolite of PGD_2 (*Lower*).

Urinary and plasma levels of 9α,11β-PGF_2 found in the normal volunteer (T.L.) were 982 ng/24 hr and 6 pg/ml, respectively. The level of urinary excretion of 9α,11β-PGF_2 in a random 24-hr urine collection obtained from the patient with mastocytosis (F.T.) was 6634 ng/24 hr. In this patient, a plasma level of 9.24 ng/ml was found during an episode of mast cell activation of moderate severity. During an unusually severe episode of mast cell activation in this patient, an enormously increased plasma level of 490 ng/ml was measured. Fig. 6 *A* and *B* show selected ion current chromatograms obtained from analysis of urine from F.T. and plasma B from F.T., respectively. The peaks *m/z* 695 represent the endogenous levels of 9α,11β-PGF_2 and the peaks *m/z* 702 represent the added internal standard [$^2H_7$]9α,11β-PGF_2. As is apparent, both plasma and urine samples were free of interfering impurity peaks.

No evidence for formation of 9α,11β-PGF_2 *ex vivo* was found when blood was drawn into a syringe containing [$^3H_7$]PGD_2 and subsequently analyzed by HPLC. This indicates that the plasma levels of 9α,11β-PGF_2 measured represent actual circulating levels of this PG.

DISCUSSION

This report describes the discovery that PGD_2 is converted by an enzyme in the 100,000 × *g* supernatant from human liver stereospecifically to a structurally unique PG, 9α,11β-PGF_2. Evidence is also presented that this PG is produced *in vivo* in humans and is biologically active.

It is of interest that the human hepatic 11-ketoreductase stereospecifically reduces the C-11 keto group of PGD_2 to a C-11 hydroxyl, which is in the β configuration, whereas, in a different species, sheep, a 11-ketoreductase present in the cellular fraction of sheep blood converts PGD_2 exclusively to $PGF_{2\alpha}$ (13). A 11-ketoreductase enzyme has been purified from rabbit liver and 11-ketoreductase activity has been found in a variety of tissues of the rat (14–16). As these studies did not employ highly resolving chromatography procedures that would separate $PGF_{2\alpha}$ from 9α,11β-PGF_2

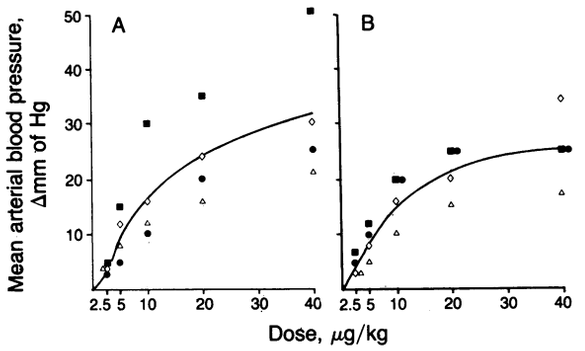

FIG. 5. Mean arterial blood pressure responses of anesthetized, ganglionicly blocked rats to the bolus i.v. administration of the hepatic supernatant 11-ketoreductase metabolite (*A*) and authentic 9α,11β-PGF_2 (*B*). Individually obtained dose–response curves are denoted by the same symbol.

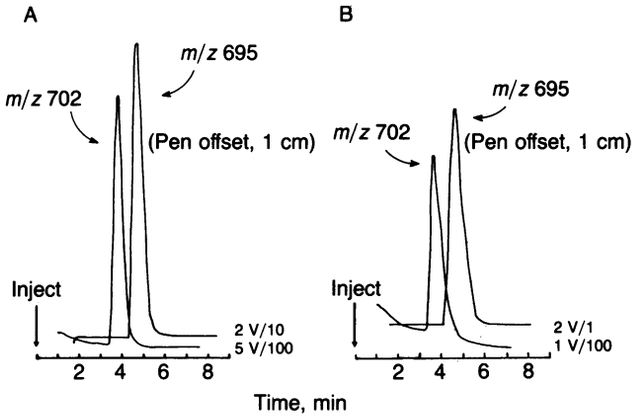

FIG. 6. Selected ion current chromatograms of *m/z* 695 (endogenous 9α,11β-PGF_2) and *m/z* 702 ($[^2H_7]$9α,11β-PGF_2) from the analysis of 9α,11β-PGF_2 in urine (*A*) and plasma (*B*) obtained from patient F.T. with systemic mastocytosis.

and did not assess the stereochemistry of the C-11 hydroxyl group in the PGF_2 product formed from PGD_2, it is not certain whether the human 11-ketoreductase is unique in comparison to the enzyme present in other animal species.

The finding that 9α,11β-PGF_2 circulates in plasma is of potential significance in regard to the possibility that this PG may act as a circulating hormone at least in certain pathophysiological situations, such as during systemic mast cell activation. This possibility is supported by the finding of a level as high as 490 ng/ml (1.4 μM) circulating in plasma in our patient with mastocytosis during a severe episode of mast cell activation. This circulating concentration of 9α,11β-PGF_2 would likely be sufficient to exert a systemic biological effect in that similar concentrations have been found to cause marked contraction of human vascular smooth muscle strips (coronary arteries) *in vitro* (unpublished data).

Since it is likely that the 11-ketoreductase is distributed widely throughout various human organs as it is in the rat (16), 9α,11β-PGF_2 can also probably act as a local hormone. Since PGD_2 and 9α,11β-PGF_2 apparently can have opposing biological activity—e.g., blood pressure effects—knowledge of the distribution of 11-ketoreductase activity in human organs and knowledge of the full spectrum of biological activity of 9α,11β-PGF_2 would contribute importantly to our understanding of the local biological consequences of PGD_2 release.

A final possibility that emerges from these studies that seems worthy of mention concerns the reliability and accuracy of some methods currently employed for quantification of $PGF_{2\alpha}$ in human biological fluids. It would seem necessary to assess whether antibodies used for radioimmunoassay of $PGF_{2\alpha}$ have associated crossreactivity with 9α,11β-PGF_2. Furthermore, unless appropriate highly resolving chromatographic procedures are employed to separate 9α,11β-PGF_2 from $PGF_{2\alpha}$, physical methods of analysis of $PGF_{2\alpha}$ such as GC/MS may simultaneously measure both $PGF_{2\alpha}$ and at least some fraction of 9α,11β-PGF_2 present in human biological fluids. The same general considerations apply to quantification of $PGF_{2\alpha}$ metabolites.

Table 1. Urinary and plasma 9α,11β-PGF_2

Urine		Plasma	
Subject	ng/24 hr	Subject	ng/ml
T.L.	982	T.L.	0.006
F.T.	6634	F.T., A*	9.24
		F.T., B†	490.09

T.L. is a normal male volunteer. F.T. is a female with systemic mastocytosis associated with overproduction of PGD_2.
*F.T. plasma A was obtained during an episode of systemic mast cell activation of moderate severity.
†F.T. plasma B was obtained during an extremely severe episode of mast cell activation.

In summary, this report describes the discovery of a biologically active, structurally unique PG (9α,11β-PGF_2) that is formed enzymatically and is produced *in vivo* in humans. Future studies determining the complete spectrum of the biological activity of 9α,11β-PGF_2 and its potential role in human physiology and pathophysiology will be of importance.

Gratitude is extended to Drs. John Pike and Gordon Bundy of the Upjohn Company for their generous gift of authentic chemically synthesized 9α,11β-PGF_2. The expert technical assistance of C. H. Kelly and J. L. Morgan was greatly appreciated. This work was supported by Grant GM15431 from the National Institutes of Health. T. E. Liston was supported by Training Grant GM07628 from the National Institutes of Health. Dr. Roberts is a Burroughs Wellcome Scholar in Clinical Pharmacology.

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