



(5Z,13E)-(15S)-9 α ,11 β ,15-trihydroxyprosta-5,13-dien-1-oic Acid (9 α ,11 β -prostaglandin F₂): Formation and Metabolism by Human Lung and Contractile Effects on Human Bronchial Smooth Muscle

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(5*Z*,13*E*)-(15*S*)-9 α ,11 β ,15-Trihydroxyprosta-5,13-dien-1-oic acid (9 α ,11 β -prostaglandin F₂): Formation and metabolism by human lung and contractile effects on human bronchial smooth muscle

(11-*epi*-prostaglandin F_{2 α} /prostaglandin D₂/11-ketoreductase/asthma/mast cell)

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ABSTRACT Prostaglandin D₂ (PGD₂) was recently found to be stereospecifically converted to the compound (5*Z*,13*E*)-(15*S*)-9 α ,11 β ,15-trihydroxyprosta-5,13-dien-1-oic acid (9 α ,11 β -PGF₂) by a human liver cytosolic NADPH-dependent 11-ketoreductase enzyme. Because PGD₂ is a potent bronchoconstrictor and is released into bronchoalveolar lavage fluid after allergen stimulation in patients with allergic asthma, the ability of human lung to metabolize PGD₂ to 9 α ,11 β -PGF₂ and the contractile effects of 9 α ,11 β -PGF₂ on human bronchial smooth muscle were investigated. The 100,000 \times *g* supernatant of human lung converted PGD₂ in the presence of an NADPH-generating system stereospecifically to 9 α ,11 β -PGF₂ at a rate of 3.46 ± 0.94 pmol per min per mg of protein. 9 α ,11 β -PGF₂ was found to contract human bronchial rings in a dose-dependent fashion with a potency virtually identical with that of both PGD₂ and PGF_{2 α} , known potent bronchial constrictors. PGD₂ was found to be a very poor substrate for human lung 15-hydroxyprostaglandin dehydrogenases and to be preferentially metabolized by lung to 9 α ,11 β -PGF₂. 9 α ,11 β -PGF₂ was also found to be a very poor substrate for the lung 15-hydroxyprostaglandin dehydrogenases. Thus, once formed, 9 α ,11 β -PGF₂ would not be expected to be rapidly inactivated *in situ* by these metabolic enzymes. These results suggest that 9 α ,11 β -PGF₂ may participate along with other putative mediators in the pulmonary allergic response in humans.

Prostaglandin D₂ (PGD₂) is the principal cyclooxygenase product produced by mast cells *in vitro* (1, 2). The finding of marked overproduction of PGD₂ in patients with increased proliferation of mast cells, mastocytosis, established that human mast cells also produce PGD₂ *in vivo* (3). Identification of PGD₂ as a product of the activation of mast cells, which are central to the acute allergic response, has led to speculation regarding its role in immediate hypersensitivity reactions. PGD₂ has been found to exert a variety of biological actions, some of which may be potentially relevant to processes involved in the pathophysiology of the pulmonary allergic response. PGD₂ is a fairly potent bronchoconstrictor following inhalation in patients with asthma (4). PGD₂ potentiates airway responsiveness to both histamine and methacholine (5). It has also been found to augment increased vascular permeability induced by histamine and leukocyte infiltration induced by leukotriene B₄ (6, 7). The possibility that PGD₂ may participate in the pulmonary allergic response has been greatly supported by the recent demonstration that PGD₂ is released *in vivo* into the lower respiratory tract of humans after acute allergic challenge (8).

Recently, it was demonstrated that PGD₂ is metabolized in humans predominantly via an 11-ketoreductase pathway to metabolites with a PGF ring (9, 10). More recently, it was

found that PGD₂ is converted stereospecifically to the biologically active prostaglandin (5*Z*,13*E*)-(15*S*)-9 α ,11 β ,15-trihydroxyprosta-5,13-dien-1-oic acid (9 α ,11 β -PGF₂) by a cytosolic NADPH-dependent 11-ketoreductase enzyme in human liver (11). Evidence was also obtained that 9 α ,11 β -PGF₂ is formed *in vivo* in humans and its production increases markedly following systemic mast-cell activation. Thus, 9 α ,11 β -PGF₂ is potentially also a mediator of biological events associated with mast-cell activation.

Because PGD₂ is released in the lung following antigen challenge in patients with allergic asthma, we examined the potential metabolic fate of PGD₂ after its release in the lung. If 11-ketoreductase activity is present in human lung, as has been found in animals such as the rat (12), PGD₂ may be further transformed to 9 α ,11 β -PGF₂, which may be biologically active in the lung. Alternatively, PGD₂ could be inactivated by lung 15-hydroxyprostaglandin dehydrogenase (15-OH-PGDH) (13). We report the findings that the lung preferentially metabolizes PGD₂ to 9 α ,11 β -PGF₂, that 9 α ,11 β -PGF₂ is a poor substrate for human lung 15-OH-PGDH and that 9 α ,11 β -PGF₂ contracts human bronchial smooth muscle *in vitro*.

EXPERIMENTAL PROCEDURES

Materials. Unlabeled PGD₂, PGF_{2 α} , and PGE₂ were purchased from Upjohn and radiolabeled PGs were from New England Nuclear. Acetylcholine, atropine, pyrilamine, NAD⁺, NADP⁺, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from Sigma. [3,3,4,4-²H₄]PGF_{2 α} was a gift from John Pike, Upjohn. [²H₇]9 α ,11 β -PGF₂ was prepared as described (11). FPL 55712 was a gift from Fisons (Loughborough, England).

Chemically synthesized 9 α ,11 β -PGF₂ methyl ester was a gift from John Pike and Gordon Bundy (Upjohn). The 9 α ,11 β -PGF₂ contained \approx 15% Δ (5*E*) isomer and pure Δ (5*Z*) 9 α ,11 β -PGF₂ was obtained by the following procedure. The mixture of Δ (5*Z*) and Δ (5*E*) 9 α ,11 β -PGF₂ was subjected to argentation TLC using the organic layer of the solvent system ethyl acetate/isooctane/methanol/water (180:50:35:100, vol/vol), Δ (5*E*) isomer *R_f* = 0.33 and Δ (5*Z*) 9 α ,11 β -PGF₂ *R_f* = 0.22. The compounds were visualized by spraying with distilled water. The Δ (5*Z*) 9 α ,11 β -PGF₂ zone was scraped, water was added, and the compound was extracted into ethyl acetate.

Hydrolysis of the methyl ester was accomplished by addition of equal volumes of methanol and 1 M KOH. After 1 hr at room temperature, 2 vol of phosphate buffer (pH 3)

Abbreviations: PG, prostaglandin; 9 α ,11 β -prostaglandin F₂, (5*Z*,13*E*)-(15*S*)-9 α ,11 β ,15-trihydroxyprosta-5,13-dien-1-oic acid; Me₃Si, trimethylsilyl ether; RP, reversed phase; SP, straight phase; 15-OH-PGDH, 15-hydroxyprostaglandin dehydrogenase.

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was added, the mixture was acidified to pH 3 with 1 N HCl, and $9\alpha,11\beta$ -PGF₂ was extracted into ethyl acetate. The compound was then subjected to TLC using the organic layer of the solvent system ethyl acetate/isooctane/methanol/acetic acid/water (180:50:35:20:100, vol/vol), $R_f = 0.42$. $9\alpha,11\beta$ -PGF₂ was visualized by spraying with water, scraped, and extracted from the silica gel with methanol. The compound was then dissolved in methanol/ethyl acetate (1:9, vol/vol) and recrystallized at -20°C . Crystals were filtered and washed with hexane yielding pure $\Delta(5Z)$ $9\alpha,11\beta$ -PGF₂ as judged by TLC.

Fractionation of Human Lung. Human lung was obtained from National Diabetes Research Interchange, Philadelphia. The lung was removed during operation from a transplant donor who did not smoke, was cut into ≈ 1 -g pieces, snap frozen immediately in liquid nitrogen, and shipped on dry ice. Ten grams of lung was allowed to thaw and then homogenized (1:3, wt/vol) in 0.1 M potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 4°C at $10,000 \times g$ for 30 min. The $10,000 \times g$ supernatant was then centrifuged for 90 min at $100,000 \times g$ and the supernatant was removed. Protein was determined according to the method of Bradford (14) with bovine serum albumin as a standard.

Assessment of 11-Ketoreductase Activity. The presence of 11-ketoreductase activity in human lung was assessed by monitoring the conversion of PGD₂ to PGF₂. The reaction mixture contained 25 μg of PGD₂ (3.0 μM), 700,000 cpm of [³H]₇PGD₂, NADP⁺ (0.5 mM), glucose 6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (72 units), 24 ml of the $100,000 \times g$ lung supernatant (480 mg of protein). Reactions were carried out for 60 min at 37°C and terminated by placing on ice. Products were subsequently analyzed by HPLC and GC/mass spectrometry (GC/MS) as described below.

To quantitate the lung 11-ketoreductase activity, the reaction mixture contained 100 μl of lung supernatant, 2 mM PGD₂, and the same concentrations of the components of the NADPH-generating system described above. Incubations were carried out at 37°C for 15 min and the reaction was terminated by addition of 1 vol of cold acetone. [²H]₇ $9\alpha,11\beta$ -PGF₂ (12 ng) was then added to the incubation mixture and also to a 100- μl aliquot of lung supernatant that had not been incubated with PGD₂. The incubation mixture was washed twice with petroleum ether and discarded and the remaining acetone was evaporated under N₂. Both samples were then diluted to 3 ml with phosphate buffer (pH 3) and extracted using a Sep-Pak C₁₈ column (Waters Associates) (10). The extract was converted to a pentafluorobenzyl ester by treatment with a mixture of 30 μl of acetonitrile, 40 μl of 10% pentafluorobenzyl bromide in acetonitrile, and 10% diisopropylethylamine in acetonitrile at room temperature for 30 min. After evaporation of reagents, the residue was subjected to TLC by using the solvent system chloroform/ethanol (93:7, vol/vol), $9\alpha,11\beta$ -PGF₂ $R_f = 0.17$. The zone containing $9\alpha,11\beta$ -PGF₂ was scraped and the compound was extracted with methanol. The compound was then converted to a trimethylsilyl (Me₃Si) ether derivative as described below. Samples were then analyzed by negative ion chemical ionization GC/MS using a Hewlett-Packard 5982A gas chromatograph mass spectrometer with a 2-ft column of SP2250 maintained at $\approx 250^\circ\text{C}$ with other conditions as described (15). Quantification was accomplished by selected ion monitoring of the ratios of the M-CH₂C₆F₅ ions for [²H₀] $9\alpha,11\beta$ -PGF₂ (m/z , 569) and [²H]₇ $9\alpha,11\beta$ -PGF₂ (m/z , 576). The net amount of $9\alpha,11\beta$ -PGF₂ formed from incubation of PGD₂ was calculated after subtracting the amount present in the lung supernatant that was not incubated with PGD₂.

Comparative Metabolism of Prostaglandins by Human Lung. Comparative studies of the metabolism of PGE₂, PGD₂, and $9\alpha,11\beta$ -PGF₂ by the $100,000 \times g$ supernatant of

human lung were conducted by using substrate concentrations of 2.8 μM and cofactor concentrations (NAD⁺, NADP⁺) of 2 mM or an NADPH-generating system as described. Incubations also contained $\approx 500,000$ cpm of tritiated substrate. Incubations were carried out at 37°C for 60 min. Reactions were stopped by placing on ice and acidifying to pH 3 by addition of one drop of 1 N HCl. Products were then extracted and analyzed by straight phase (SP) HPLC as described below. To obtain sufficient quantities of metabolites formed for analysis by GC/MS, incubations were carried out containing 5 μg of PGE₂ for 2 hr and 20 μg of $9\alpha,11\beta$ -PGF₂ for 3 hr.

Isolation and Identification of Metabolites. Metabolites formed in the incubation with the $100,000 \times g$ supernatant of human lung were extracted by using Sep-Pak C₁₈ (16), purified by HPLC, and subsequently analyzed by GC/MS. SP-HPLC was done on a 5- μm Alltech (Deerfield, IL) silica column; solvent A, chloroform/acetic acid (100:0.1, vol/vol); and solvent B, chloroform/methanol/acetic acid (90:10:0.1, vol/vol); solvent program A to 100% B over 2 hr, 1 ml/min, 1-ml fractions. Reversed-phase (RP) HPLC was performed on a 5- μm Alltech C₁₈ column with the solvent system acetonitrile/water/acetic acid (29:71:0.1, vol/vol), 1 ml/min, 1-ml fractions.

Methyl esters of compounds were formed by treatment with excess ethereal diazomethane. Me₃Si ethers were formed by treatment with 20 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide (Regis, Morton Grove, IL) and 20 μl of dimethylformamide at room temperature for 15 min. Butylboronate derivatives were formed by treatment with 50 μl of butylboronic acid (Aldrich) in pyridine (10 mg/ml) at 60°C for 45 min. *O*-methyloxime derivatives were formed by treatment with 2% methoxyamine hydrochloride (Regis) in pyridine at 70°C for 60 min followed by evaporation of the pyridine, addition of water, and extraction with ethyl acetate.

GC/MS analysis was performed with a Nermag (Houston, TX) R10-10C gas chromatograph mass spectrometer interfaced with a DEC PDP-11/23 plus computer system. GC was done on a 6-m DB-1 fused silica capillary column programmed from 190°C – 325°C at $25^\circ\text{C}/\text{min}$. Helium was used as the carrier gas at a flow rate of 1 ml/min. Ion source temperature was 200°C , electron energy was 87 eV, and filament current was 0.25 mA.

In Vitro Contractile Effects of PGs on Human Bronchial Rings. Bronchi were dissected from macroscopically normal areas of lung obtained within 2 hr of resection for carcinoma, and were placed in room temperature Krebs solution or normal saline, and were transported to the laboratory within 20 min. The tissue was placed in a modified Krebs–Ringer solution (in mM: NaCl, 118; KCl, 5.9; CaCl₂, 2.5; MgSO₄, 1.2; NaH₂O₄, 1.2; NaHCO₃, 25.5; glucose, 5.6) bubbled with a 95% O₂/5% CO₂ gas mixture. Bronchial rings 3–6 mm long were partially cleaned of adherent lung parenchyma and tied with silk threads to a moveable glass rod and to an isometric force transducer (Grass FT03). Baseline tension was set at 1–4 g depending on the size of the ring. Isometric tension was recorded on a direct writing oscillograph (Grass 7D). After a 2-hr incubation at 37°C and three exchanges of fresh Krebs–Ringer solution, the peak response to 50 μM acetylcholine was determined, and subsequent contractile responses were normalized to this value. PGs were added in 100- μl quantities or less to the 20-ml tissue chambers to construct cumulative dose–response curves for either PGF_{2 α} , PGD₂, or $9\alpha,11\beta$ -PGF₂ in single bronchial rings. Concentrations of PGs are expressed as the final molar concentration in the chamber. To test the effect of muscarinic, histaminergic, and leukotriene receptor blockade, rings were contracted with 5.9 μM $9\alpha,11\beta$ -PGF₂ and the effect of sequentially adding 1 μM atropine, 1 μM pyrilamine, and the leukotriene receptor antagonist FPL 55712 (10 $\mu\text{g}/\text{ml}$) was determined.

Dose-response curves for PGD₂ and PGF_{2α} were also determined for bronchial rings precontracted with 0.13 μM and 0.59 μM 9α,11β-PGF₂.

RESULTS

Human Lung 11-Ketoreductase Metabolism of PGD₂. After incubation of [³H]PGD₂ (25 μg) with the 100,000 × *g* supernatant of human lung in the presence of a NADPH-generating system and extraction of products formed, the mixture was analyzed by SP-HPLC. This revealed that ≈67% of the PGD₂ that eluted at 30–34 ml had been converted to a more polar compound with an elution volume characteristic for PGF₂ (48–52 ml). The [³H]PGF₂ peak was pooled, [¹⁴C]PGF_{2α} was added, and the mixture was rechromatographed on RP-HPLC (Fig. 1), which widely separates 9α,11β-PGF₂ and PGF_{2α} (11). The tritiated metabolite eluted at 33–37 ml and was clearly separated from the added [¹⁴C]PGF_{2α}, which eluted at 44–48 ml.

The tritiated metabolite from RP-HPLC was extracted from the RP-HPLC solvent into ethyl acetate, and the ethyl acetate was evaporated under N₂. Structural identification of this compound was accomplished as described (11). Approximately 2 μg of the compound was combined and co-derivatized with 3 μg of [²H₄]PGF_{2α}. The mixture was converted to a methyl ester, treated with *n*-butylboronic acid, and subsequently converted to a Me₃Si ether derivative. A butylboronate derivative will form, bridging the C-9 and C-11 oxygens upon treatment of PGF ring compounds with *n*-butylboronic acid only if the C-9 and C-11 hydroxyls are *cis* in the prostane ring (17). [²H₄]PGF_{2α} was co-derivatized with the metabolite to permit an assessment of the completion of the boronation reaction when analyzed by GC/MS. When analyzed, mass spectra were obtained that were identical to those previously published for the Me-Me₃Si ether derivative of 9α,11β-PGF₂ and the Me-butylboronate-Me₃Si ether derivative of [²H₄]PGF_{2α} (11). Importantly, there were no

doublet ion peaks 4 atomic mass units apart in either mass spectrum, indicating that the [²H₄]PGF_{2α} was quantitatively converted to a butylboronate derivative, whereas none of the metabolite reacted with *n*-butylboronic acid. These data established that the structure of the PGD₂ metabolite was 9α,11β-PGF₂.

Incubations were then carried out as described in *Experimental Procedures* to quantitate the 11-ketoreductase activity in human lung. The lung supernatant was found to convert 3.46 ± 0.94 pmol per min per mg of protein (mean ± SEM; *n* = 6) of PGD₂ to 9α,11β-PGF₂.

Comparative Metabolism of Prostaglandins by Human Lung. The ability of the 100,000 × *g* supernatant of human lung to metabolize PGD₂, 9α,11β-PGF₂, and PGE₂ was compared using conditions, substrate, and cofactor concentrations described in *Experimental Procedures*. Product formation was analyzed by SP-HPLC and identification was accomplished by GC-MS. Results are listed in Table 1. A representative chromatogram obtained after incubation of PGE₂ in the presence of NAD⁺ is shown in Fig. 2. PGE₂ characteristically elutes in this SP-HPLC system between 40 and 45 ml. In the presence of NAD⁺, 67.1% ± 3.7% of PGE₂ was converted to 15-keto-13,14-dihydro-PGE₂, which eluted between 20 and 30 ml. In contrast, <10% of PGD₂ and 9α,11β-PGF₂ were converted to 15-keto-13,14-dihydro metabolites in the presence of either NAD⁺ or NADP⁺. A representative chromatogram obtained after incubation of 9α,11β-PGF₂ in the presence of NAD⁺ is shown in Fig. 3. When the incubation time of 9α,11β-PGF₂ was extended to 3 hr to allow further metabolism to obtain sufficient quantities of products for analysis by GC-MS, a small peak also appeared on SP-HPLC eluting at 47–48 ml, which was identified as 13,14-dihydro-PGF₂. In contrast, in the presence of NADPH alone or both NADPH and NAD⁺, >50% of PGD₂ was metabolized to 9α,11β-PGF₂. These data indicate that PGD₂ is preferentially metabolized by the 100,000 × *g* supernatant of human lung to 9α,11β-PGF₂ and that 9α,11β-PGF₂ is a poor substrate for human lung 15-OH-PGDH.

Contractile Effects of 9α,11β-PGF₂ on Human Bronchial Rings *in Vitro*. Contractile responses of human bronchial rings to various doses of 9α,11β-PGF₂ were compared to that of PGF_{2α} and PGD₂. 9α,11β-PGF₂ caused slowly developing contractions, which were sustained for 30 min or longer and were similar in intensity to contractions caused by PGD₂ (Fig. 4). The contractile dose-response curve for PGF_{2α} (*n* = 8) was virtually identical and superimposable with that of 9α,11β-PGF₂ (data not shown). Supraadditive effects of contractions to PGD₂ and PGF_{2α} (*n* = 3) were not observed in rings precontracted with small doses of 9α,11β-PGF₂ (data not shown). Contractions caused by 9α,11β-PGF₂ were not relaxed by atropine, pyrilamine, or FPL 55712.

Table 1. Metabolism of prostaglandins by the 100,000 × *g* supernatant of human lung

Cofactor	Substrate	% substrate converted to 15-keto metabolites	% substrate converted to 9α,11β-PGF ₂
NAD ⁺	PGE ₂	76.1 ± 3.7	—
	PGD ₂	7.4 ± 3.4	—
	9α,11β-PGF ₂	6.8 ± 0.5	—
NADP ⁺	PGD ₂	8.8 ± 1.0	—
	9α,11β-PGF ₂	8.7 ± 1.0	—
NADPH	PGD ₂	1.6 ± 0.5	64.7 ± 6.8
NAD ⁺ + NADPH	PGD ₂	3.0 ± 0.2	50.2 ± 5.5

Incubations were carried out for 60 min at 37°C. Cofactor concentration was 2 mM and substrate concentration was 2.8 μM. Data are expressed as mean ± SEM (*n* = 3 or 4).

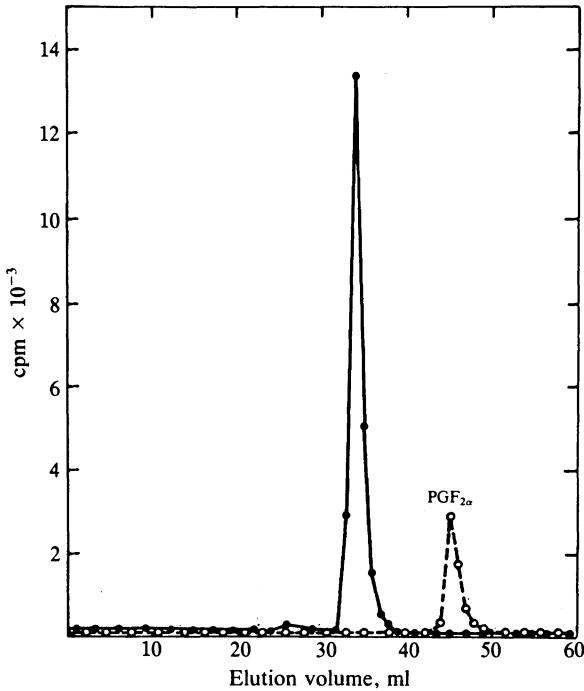


FIG. 1. RP-HPLC of [¹⁴C]PGF_{2α} (○) and the ³H metabolite (●) formed after incubation of PGD₂ with the 100,000 × *g* supernatant of human lung in the presence of an NADPH-generating system. The characteristic elution volume for 9α,11β-PGF₂ in this system is 33–37 ml.

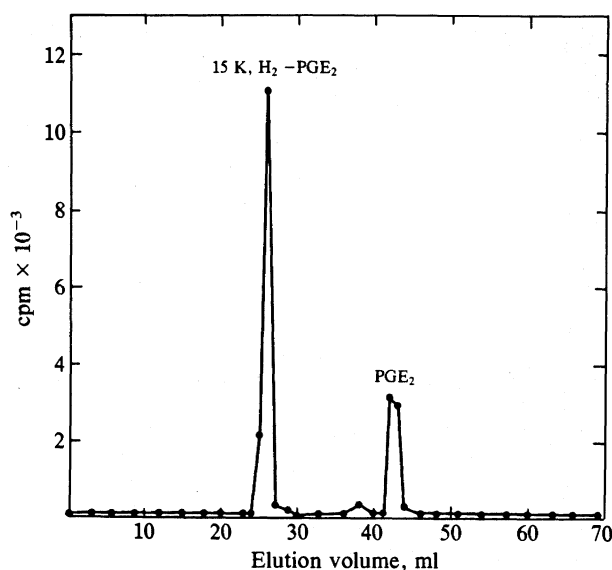


FIG. 2. SP-HPLC analysis after incubation of PGE_2 ($2.8 \mu\text{M}$) with the $100,000 \times g$ supernatant of human lung for 60 min in the presence of NAD^+ (2 mM). 15K, H_2 - PGE_2 , 15-keto-13,14-dihydro- PGE_2 .

DISCUSSION

Recently, we reported the finding that PGD_2 is stereospecifically converted to $9\alpha,11\beta$ - PGF_2 by an NADPH-dependent human liver cytosolic enzyme and that this PG is produced *in vivo* in humans and is biologically active (11). These observations have now been extended with the identification of identical 11-ketoreductase enzyme activity in human lung. Wong has previously reported purification of 11-ketoreductase from rabbit liver (18). Although it was originally thought that this enzyme converted PGD_2 to $\text{PGF}_{2\alpha}$, the same group has recently reexamined the metabolism of PGD_2 by rabbit liver and found that PGD_2 is transformed to $9\alpha,11\beta$ - PGF_2 rather than $\text{PGF}_{2\alpha}$ (19). Watanabe *et al.* have reported the purification to apparent homogeneity of a PGF-synthetase enzyme from bovine lung that catalyzes the reduction of both PGD_2 and PGH_2 to PGF_2 , but at different active sites on the

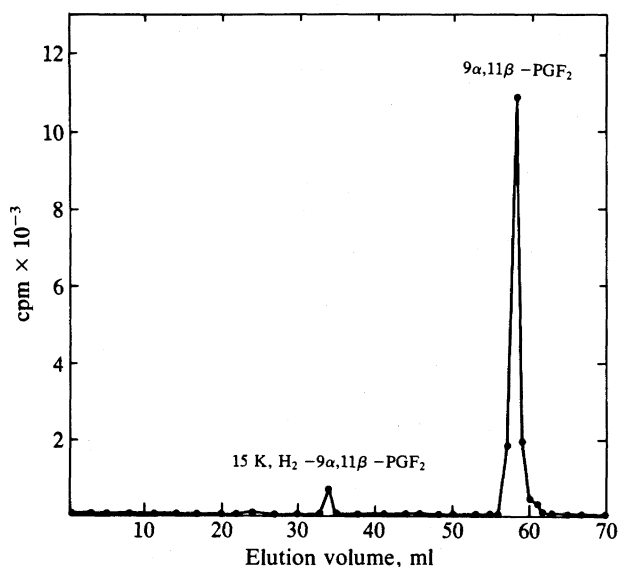


FIG. 3. SP-HPLC analysis after incubation of $9\alpha,11\beta$ - PGF_2 ($2.8 \mu\text{M}$) with the $100,000 \times g$ supernatant of human lung for 60 min in the presence of NAD^+ (2 mM). 15K, H_2 - $9\alpha,11\beta$ - PGF_2 , 15-keto-13,14-dihydro- $9\alpha,11\beta$ - PGF_2 .

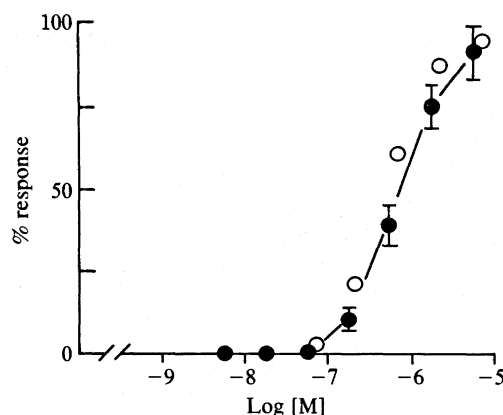


FIG. 4. Effect of increasing concentrations of $9\alpha,11\beta$ - PGF_2 and PGD_2 on isometric tension development in human bronchial rings, measured as % response to $50 \mu\text{M}$ acetylcholine. \bullet , $9\alpha,11\beta$ - PGF_2 ; \circ , PGD_2 . Each point represents the mean \pm SEM ($n = 9$).

enzyme (20). More recently, in collaboration with Watanabe and co-workers, we have demonstrated that this bovine lung enzyme stereospecifically reduces PGD_2 to $9\alpha,11\beta$ - PGF_2 , whereas PGH_2 is stereospecifically converted to $\text{PGF}_{2\alpha}$ (21). Whether the human lung enzyme with 11-ketoreductase activity is similar to the bovine lung PGF synthetase in that it also catalyzes the reduction of PGH_2 to $\text{PGF}_{2\alpha}$ has not been examined.

The complete spectrum of the biological activity of $9\alpha,11\beta$ - PGF_2 remains to be elucidated. $9\alpha,11\beta$ - PGF_2 was initially shown to be a vasopressor agent in the rat (11). Subsequently, it has also been found to contract human coronary arteries *in vitro* (22) and to inhibit human platelet aggregation (19, 23). In this report, we have now demonstrated that $9\alpha,11\beta$ - PGF_2 also contracts human bronchial smooth muscle *in vitro*. The potency of the contractile effects of $9\alpha,11\beta$ - PGF_2 on human bronchial smooth muscle *in vitro* was virtually identical to that of both PGD_2 and $\text{PGF}_{2\alpha}$. This is significant in that PGD_2 has been demonstrated to be a potent bronchoconstrictor following inhalation in humans, and patients with allergic asthma exhibit enhanced sensitivity to its spasmogenic effects (4). Interestingly, although we found no difference in the potency of the contractile effects of PGD_2 and $\text{PGF}_{2\alpha}$ (and $9\alpha,11\beta$ - PGF_2) *in vitro*, PGD_2 administered by inhalation was found to be a more potent bronchoconstrictor than $\text{PGF}_{2\alpha}$. Future studies examining the bronchoconstrictor effects of inhaled $9\alpha,11\beta$ - PGF_2 in normal volunteers and in patients with allergic asthma will be of considerable interest.

Rapid metabolic inactivation of PGs such as PGE_2 and $\text{PGF}_{2\alpha}$ in lung as a consequence of conversion of PGs to their respective 15-keto-13,14-dihydro metabolites by a 15-OH-PGDH and $\Delta 13$ reductase is well recognized (13). Following release of PGD_2 in the lung, therefore, further metabolism of PGD_2 could occur either by the 11-ketoreductase pathway leading to the formation of the biologically active metabolite, $9\alpha,11\beta$ - PGF_2 , or PGD_2 could be converted to 15-keto-13,14-dihydro- PGD_2 , which is essentially devoid of biological activity at least in regard to inhibition of platelet aggregation (24). Which pathway predominates in the lung would be dependent on the relative concentrations of 15-OH-PGDH and the 11-ketoreductase and the K_m values of the enzymes for PGD_2 . Which pathway predominates has obvious relevance as to whether participation of $9\alpha,11\beta$ - PGF_2 in physiological or pathophysiological events in the lung is a potentially important consideration.

Two different 15-OH-PGDHs have been identified in the cytosol of a variety of mammalian tissues; one that utilizes NAD^+ as a cofactor more effectively than NADP^+ , and one that utilizes NADP^+ more effectively than NAD^+ (25).

However, PGD₂ has been found to be a poor substrate for these enzymes (26, 27). More recently, however, PGD₂ has been found to be a very good substrate for NADP⁺-linked 15-OH-PGDHs identified in the cytosol of swine brain and human platelets (24, 28). Whether there are 15-OH-PGDHs in human lung that efficiently metabolize PGD₂ has not been investigated, nor has the ability of 9 α ,11 β -PGF₂ to act as a substrate for 15-OH-PGDHs been examined. We found that both PGD₂ and 9 α ,11 β -PGF₂ are metabolized poorly by 15-OH-PGDHs in human lung in the presence of either NAD⁺ or NADP⁺ and that PGD₂ is preferentially metabolized via the 11-ketoreductase pathway to 9 α ,11 β -PGF₂.

In light of the previous demonstration that PGD₂ is released *in vivo* in the lung following antigen challenge in patients with allergic asthma, the current findings described here have potentially important pathophysiological relevance. 11-Ketoreductase activity has now been identified in human lung, which converts PGD₂ to 9 α ,11 β -PGF₂ and PGD₂ was shown to be preferentially metabolized via this pathway rather than inactivated by lung 15-OH-PGDH. Furthermore, 9 α ,11 β -PGF₂ was found to contract human bronchial smooth muscle. Collectively, these findings suggest the possibility that 9 α ,11 β -PGF₂ may participate in the pathophysiology of the pulmonary allergic response in humans. This possibility seems even more attractive in view of the demonstration that 9 α ,11 β -PGF₂ is a very poor substrate for human lung 15-OH-PGDH. Thus, once formed in the lung, 9 α ,11 β -PGF₂ would not be expected to be rapidly inactivated *in situ*. Future studies involving direct quantitative assessment of the formation of 9 α ,11 β -PGF₂ *in vivo* in the lung during allergic bronchoconstriction in humans will be of importance.

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