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(5*Z*,13*E*)-(15*S*)-9α,11β,15-Trihydroxyprosta-5,13-dien-1-oic acid (9α,11β-prostaglandin F_2): Formation and metabolism by human lung and contractile effects on human bronchial smooth muscle

(11-epi-prostaglandin $F_{2\alpha}$/prostaglandin D_2/11-ketoreductase/asthma/mast cell)

KAREN SEIBERT, JAMES R. SHELLER, AND L. JACKSON ROBERTS II*

Departments of Pharmacology and Medicine, Vanderbilt University, Nashville, TN 37232

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ABSTRACT **Prostaglandin D_2 (PGD_2) was recently found to be stereospecifically converted to the compound (5*Z*,13*E*)-(15*S*)-9α,11β,15-trihydroxyprosta-5,13-dien-1-oic acid (9α,11β-PGF_2) by a human liver cytosolic NADPH-dependent 11-ketoreductase enzyme. Because PGD_2 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 PGD_2 to 9α,11β-PGF_2 and the contractile effects of 9α,11β-PGF_2 on human bronchial smooth muscle were investigated. The 100,000 × *g* supernatant of human lung converted PGD_2 in the presence of an NADPH-generating system stereospecifically to 9α,11β-PGF_2 at a rate of 3.46 ± 0.94 pmol per min per mg of protein. 9α,11β-PGF_2 was found to contract human bronchial rings in a dose-dependent fashion with a potency virtually identical with that of both PGD_2 and $PGF_{2\alpha}$, known potent bronchial constrictors. PGD_2 was found to be a very poor substrate for human lung 15-hydroxyprostaglandin dehydrogenases and to be preferentially metabolized by lung to 9α,11β-PGF_2. 9α,11β-PGF_2 was also found to be a very poor substrate for the lung 15-hydroxyprostaglandin dehydrogenases. Thus, once formed, 9α,11β-PGF_2 would not be expected to be rapidly inactivated *in situ* by these metabolic enzymes. These results suggest that 9α,11β-PGF_2 may participate along with other putative mediators in the pulmonary allergic response in humans.**

Prostaglandin D_2 (PGD_2) is the principal cyclooxygenase product produced by mast cells *in vitro* (1, 2). The finding of marked overproduction of PGD_2 in patients with increased proliferation of mast cells, mastocytosis, established that human mast cells also produce PGD_2 *in vivo* (3). Identification of PGD_2 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. PGD_2 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. PGD_2 is a fairly potent bronchoconstrictor following inhalation in patients with asthma (4). PGD_2 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 B_4 (6, 7). The possibility that PGD_2 may participate in the pulmonary allergic response has been greatly supported by the recent demonstration that PGD_2 is released *in vivo* into the lower respiratory tract of humans after acute allergic challenge (8).

Recently, it was demonstrated that PGD_2 is metabolized in humans predominantly via an 11-ketoreductase pathway to metabolites with a PGF ring (9, 10). More recently, it was found that PGD_2 is converted stereospecifically to the biologically active prostaglandin (5*Z*,13*E*)-(15*S*)-9α,11β,15-trihydroxyprosta-5,13-dien-1-oic acid (9α,11β-PGF_2) by a cytosolic NADPH-dependent 11-ketoreductase enzyme in human liver (11). Evidence was also obtained that 9α,11β-PGF_2 is formed *in vivo* in humans and its production increases markedly following systemic mast-cell activation. Thus, 9α,11β-PGF_2 is potentially also a mediator of biological events associated with mast-cell activation.

Because PGD_2 is released in the lung following antigen challenge in patients with allergic asthma, we examined the potential metabolic fate of PGD_2 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), PGD_2 may be further transformed to 9α,11β-PGF_2, which may be biologically active in the lung. Alternatively, PGD_2 could be inactivated by lung 15-hydroxyprostaglandin dehydrogenase (15-OH-PGDH) (13). We report the findings that the lung preferentially metabolizes PGD_2 to 9α,11β-PGF_2, that 9α,11β-PGF_2 is a poor substrate for human lung 15-OH-PGDH and that 9α,11β-PGF_2 contracts human bronchial smooth muscle *in vitro*.

EXPERIMENTAL PROCEDURES

Materials. Unlabeled PGD_2, $PGF_{2\alpha}$, and PGE_2 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-$^2H_4$]$PGF_{2\alpha}$ was a gift from John Pike, Upjohn. [$^2H_7$]9α,11β-PGF_2 was prepared as described (11). FPL 55712 was a gift from Fisons (Loughborough, England).

Chemically synthesized 9α,11β-PGF_2 methyl ester was a gift from John Pike and Gordon Bundy (Upjohn). The 9α,11β-PGF_2 contained ≈15% Δ(5*E*) isomer and pure Δ(5*Z*) 9α,11β-PGF_2 was obtained by the following procedure. The mixture of Δ(5*Z*) and Δ(5*E*) 9α,11β-PGF_2 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*) 9α,11β-PGF_2 R_f = 0.22. The compounds were visualized by spraying with distilled water. The Δ(5*Z*) 9α,11β-PGF_2 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)

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Abbreviations: PG, prostaglandin; 9α,11β-prostaglandin F_2, (5*Z*, 13*E*)-(15*S*)-9α,11β,15-trihydroxyprosta-5,13-dien-1-oic acid; Me_3Si, trimethylsilyl ether; RP, reversed phase; SP, straight phase; 15-OH-PGDH, 15-hydroxyprostaglandin dehydrogenase.

*To whom reprint requests should be addressed.

was added, the mixture was acidified to pH 3 with 1 N HCl, and 9α,11β-PGF_2 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. 9α,11β-PGF_2 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 Δ(5Z) 9α,11β-PGF_2 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 × *g* for 30 min. The 10,000 × *g* supernatant was then centrifuged for 90 min at 100,000 × *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 PGD_2 to PGF_2. The reaction mixture contained 25 μg of PGD_2 (3.0 μM), 700,000 cpm of [$^3H_7$]PGD_2, $NADP^+$ (0.5 mM), glucose 6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (72 units), 24 ml of the 100,000 × *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 PGD_2, 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. [$^2H_7$]9α,11β-PGF_2 (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 PGD_2. The incubation mixture was washed twice with petroleum ether and discarded and the remaining acetone was evaporated under N_2. Both samples were then diluted to 3 ml with phosphate buffer (pH 3) and extracted using a Sep-Pak C_{18} 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), 9α,11β-PGF_2 R_f = 0.17. The zone containing 9α,11β-PGF_2 was scraped and the compound was extracted with methanol. The compound was then converted to a trimethylsilyl (Me_3Si) 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 ≈250°C with other conditions as described (15). Quantification was accomplished by selected ion monitoring of the ratios of the M-$CH_2C_6F_5$ ions for [$^2H_0$]9α,11β-PGF_2 (*m/z*, 569) and [$^2H_7$]9α,11β-PGF_2 (*m/z*, 576). The net amount of 9α,11β-PGF_2 formed from incubation of PGD_2 was calculated after subtracting the amount present in the lung supernatant that was not incubated with PGD_2.

Comparative Metabolism of Prostaglandins by Human Lung. Comparative studies of the metabolism of PGE_2, PGD_2, and 9α,11β-PGF_2 by the 100,000 × *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 ≈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 PGE_2 for 2 hr and 20 μg of 9α,11β-PGF_2 for 3 hr.

Isolation and Identification of Metabolites. Metabolites formed in the incubation with the 100,000 × *g* supernatant of human lung were extracted by using Sep-Pak C_{18} (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 C_{18} 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. Me_3Si 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°C/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; CaCl, 2.5; $MgSO_4$, 1.2; NaH_2O_4, 1.2; $NaHCO_3$, 25.5; glucose, 5.6) bubbled with a 95% O_2/5% CO_2 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 $PGF_{2\alpha}$, PGD_2, or 9α,11β-PGF_2 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 9α,11β-PGF_2 and the effect of sequentially adding 1 μM atropine, 1 μM pyrilamine, and the leukotriene receptor antagonist FPL 55712 (10 μg/ml) was determined.

Dose–response curves for PGD_2 and $PGF_{2\alpha}$ were also determined for bronchial rings precontracted with 0.13 μM and 0.59 μM 9α,11β-PGF_2.

RESULTS

Human Lung 11-Ketoreductase Metabolism of PGD_2. After incubation of [$^3H_7$]PGD_2 (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 PGD_2 that eluted at 30–34 ml had been converted to a more polar compound with an elution volume characteristic for PGF_2 (48–52 ml). The [^{3}H]PGF_2 peak was pooled, [^{14}C]$PGF_{2\alpha}$ was added, and the mixture was rechromatographed on RP-HPLC (Fig. 1), which widely separates 9α,11β-PGF_2 and $PGF_{2\alpha}$ (11). The tritiated metabolite eluted at 33–37 ml and was clearly separated from the added [^{14}C]$PGF_{2\alpha}$, 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 N_2. 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 [$^2H_4$]$PGF_{2\alpha}$. The mixture was converted to a methyl ester, treated with *n*-butylboronic acid, and subsequently converted to a Me_3Si 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). [$^2H_4$]$PGF_{2\alpha}$ 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-Me_3Si ether derivative of 9α,11β-PGF_2 and the Me-butylboronate-Me_3Si ether derivative of [$^2H_4$]$PGF_{2\alpha}$ (11). Importantly, there were no doublet ion peaks 4 atomic mass units apart in either mass spectrum, indicating that the [$^2H_4$]$PGF_{2\alpha}$ 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 PGD_2 metabolite was 9α,11β-PGF_2.

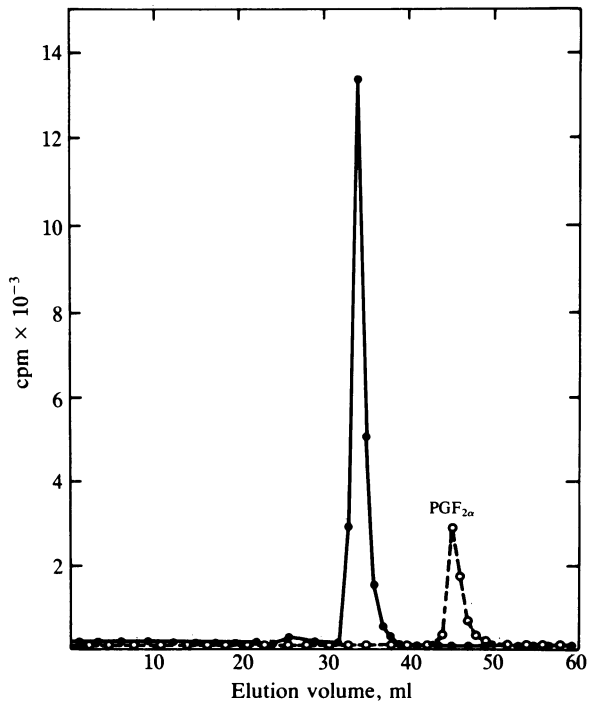

FIG. 1. RP-HPLC of [1-^{14}C]$PGF_{2\alpha}$ (○) and the ^{3}H metabolite (●) formed after incubation of PGD_2 with the 100,000 × *g* supernatant of human lung in the presence of an NADPH-generating system. The characteristic elution volume for 9α,11β-PGF_2 in this system is 33–37 ml.

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 PGD_2 to 9α,11β-PGF_2.

Comparative Metabolism of Prostaglandins by Human Lung. The ability of the 100,000 × *g* supernatant of human lung to metabolize PGD_2, 9α,11β-PGF_2, and PGE_2 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 PGE_2 in the presence of NAD^+ is shown in Fig. 2. PGE_2 characteristically elutes in this SP-HPLC system between 40 and 45 ml. In the presence of NAD^+, 67.1% ± 3.7% of PGE_2 was converted to 15-keto-13,14-dihydro-PGE_2, which eluted between 20 and 30 ml. In contrast, <10% of PGD_2 and 9α,11β-PGF_2 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-PGF_2 in the presence of NAD^+ is shown in Fig. 3. When the incubation time of 9α,11β-PGF_2 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-PGF_2. In contrast, in the presence of NADPH alone or both NADPH and NAD^+, >50% of PGD_2 was metabolized to 9α,11β-PGF_2. These data indicate that PGD_2 is preferentially metabolized by the 100,000 × *g* supernatant of human lung to 9α,11β-PGF_2 and that 9α,11β-PGF_2 is a poor substrate for human lung 15-OH-PGDH.

Contractile Effects of 9α,11β-PGF_2 on Human Bronchial Rings *in Vitro*. Contractile responses of human bronchial rings to various doses of 9α,11β-PGF_2 were compared to that of $PGF_{2\alpha}$ and PGD_2. 9α,11β-PGF_2 caused slowly developing contractions, which were sustained for 30 min or longer and were similar in intensity to contractions caused by PGD_2 (Fig. 4). The contractile dose–response curve for $PGF_{2\alpha}$ (n = 8) was virtually identical and superimposable with that of 9α,11β-PGF_2 (data not shown). Supraadditive effects of contractions to PGD_2 and $PGF_{2\alpha}$ (n = 3) were not observed in rings precontracted with small doses of 9α,11β-PGF_2 (data not shown). Contractions caused by 9α,11β-PGF_2 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).

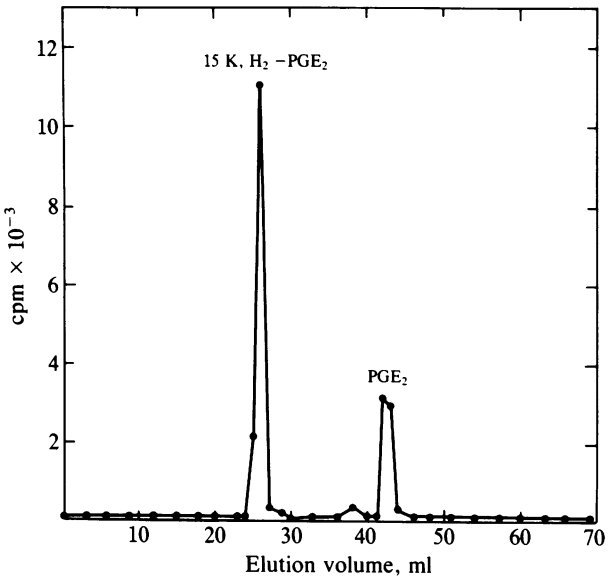

FIG. 2. SP-HPLC analysis after incubation of PGE_2 (2.8 μM) with the 100,000 × *g* supernatant of human lung for 60 min in the presence of NAD^+ (2 mM). 15K, H_2-PGE_2, 15-keto-13,14-dihydro-PGE_2.

DISCUSSION

Recently, we reported the finding that PGD_2 is stereospecifically converted to 9α,11β-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 $PGF_{2\alpha}$, the same group has recently reexamined the metabolism of PGD_2 by rabbit liver and found that PGD_2 is transformed to 9α,11β-PGF_2 rather than $PGF_{2\alpha}$ (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 enzyme (20). More recently, in collaboration with Watanabe and co-workers, we have demonstrated that this bovine lung enzyme stereospecifically reduces PGD_2 to 9α,11β-PGF_2, whereas PGH_2 is stereospecifically converted to $PGF_{2\alpha}$ (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 $PGF_{2\alpha}$ has not been examined.

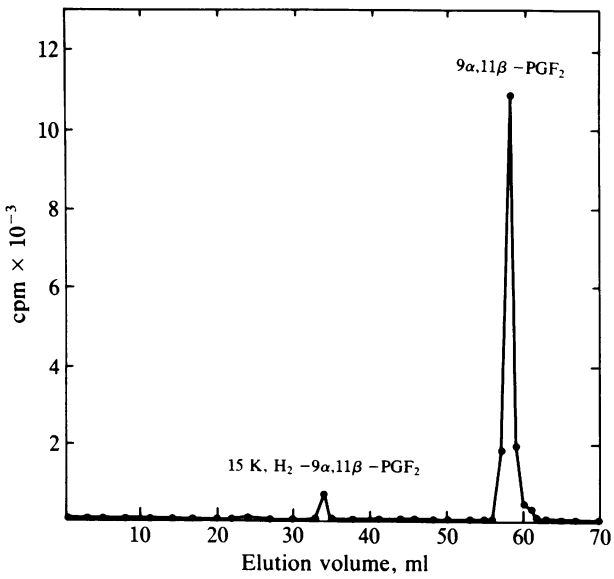

FIG. 3. SP-HPLC analysis after incubation of 9α,11β-PGF_2 (2.8 μM) with the 100,000 × *g* supernatant of human lung for 60 min in the presence of NAD^+ (2 mM). 15K, H_2-9α,11β-PGF_2, 15-keto-13,14-dihydro-9α,11β-PGF_2.

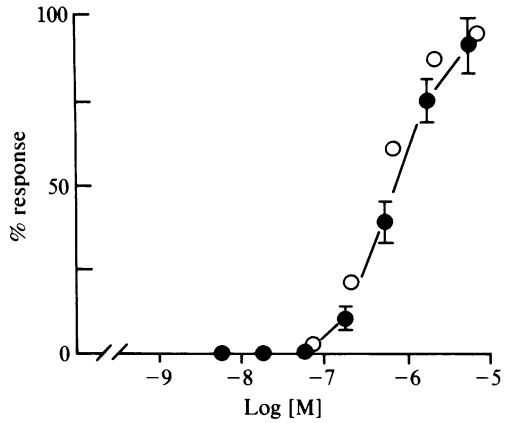

FIG. 4. Effect of increasing concentrations of 9α,11β-PGF_2 and PGD_2 on isometric tension development in human bronchial rings, measured as % response to 50 μM acetylcholine. ●, 9α,11β-PGF_2; ○, PGD_2. Each point represents the mean ± SEM (n = 9).

The complete spectrum of the biological activity of 9α,11β-PGF_2 remains to be elucidated. 9α,11β-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 9α,11β-PGF_2 also contracts human bronchial smooth muscle *in vitro*. The potency of the contractile effects of 9α,11β-PGF_2 on human bronchial smooth muscle *in vitro* was virtually identical to that of both PGD_2 and $PGF_{2\alpha}$. 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 $PGF_{2\alpha}$ (and 9α,11β-PGF_2) *in vitro*, PGD_2 administered by inhalation was found to be a more potent bronchoconstrictor than $PGF_{2\alpha}$. Future studies examining the bronchoconstrictor effects of inhaled 9α,11β-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 $PGF_{2\alpha}$ in lung as a consequence of conversion of PGs to their respective 15-keto-13,14-dihydro metabolites by a 15-OH-PGDH and Δ13 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, 9α,11β-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 9α,11β-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, PGD_2 has been found to be a poor substrate for these enzymes (26, 27). More recently, however, PGD_2 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 PGD_2 has not been investigated, nor has the ability of $9\alpha,11\beta$-PGF_2 to act as a substrate for 15-OH-PGDHs been examined. We found that both PGD_2 and $9\alpha,11\beta$-PGF_2 are metabolized poorly by 15-OH-PGDHs in human lung in the presence of either NAD^+ or $NADP^+$ and that PGD_2 is preferentially metabolized via the 11-ketoreductase pathway to $9\alpha,11\beta$-PGF_2.

In light of the previous demonstration that PGD_2 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 PGD_2 to $9\alpha,11\beta$-PGF_2 and PGD_2 was shown to be preferentially metabolized via this pathway rather than inactivated by lung 15-OH-PGDH. Furthermore, $9\alpha,11\beta$-PGF_2 was found to contract human bronchial smooth muscle. Collectively, these findings suggest the possibility that $9\alpha,11\beta$-$PGF_{2\alpha}$ may participate in the pathophysiology of the pulmonary allergic response in humans. This possibility seems even more attractive in view of the demonstration that $9\alpha,11\beta$-PGF_2 is a very poor substrate for human lung 15-OH-PGDH. Thus, once formed in the lung, $9\alpha,11\beta$-PGF_2 would not be expected to be rapidly inactivated *in situ*. Future studies involving direct quantitative assessment of the formation of $9\alpha,11\beta$-PGF_2 *in vivo* in the lung during allergic bronchoconstriction in humans will be of importance.

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