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Research Article

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9α,11β-Prostaglandin F_2, a Novel Metabolite of Prostaglandin D_2 Is a Potent Contractile Agonist of Human and Guinea Pig Airways

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Abstract

Prostaglandin (PG) D_2, the predominant prostanoid released from activated mast cells in humans is initially metabolized by reduction of the C-11 keto function to yield 9α,11β-PGF_2. In this study the airway effects of 9α,11β-PGF_2 were compared with those of its epimer 9α,11α-PGF_2 ($PGF_{2\alpha}$) and PGD_2. 9α,11β-PGF_2 was a potent contractile agonist of isolated guinea pig trachea and 4-mm human airways in vitro; the potencies of the PGs relative to PGD_2 (= 1.00) being 0.65 (NS) and 4.08 ($P < 0.001$) for 9α,11β-PGF_2, and 0.52 ($P < 0.01$) and 2.40 ($P < 0.001$) for $PGF_{2\alpha}$, respectively. When inhaled by asthmatic subjects, 9α,11β-PGF_2 was a potent bronchoconstrictor agent, being approximately equipotent with PGD_2 and 28–32 times more potent than histamine ($P < 0.01$). These studies suggest that 9α,11β-PGF_2 is at least equipotent with PGD_2 as a bronchoconstrictor agonist, and in being a major metabolite of PGD_2, could contribute to the bronchoconstrictor effect of this mast cell-derived mediator in asthma.

Introduction

There is considerable interest in the role of prostaglandin D_2 (PGD_2)[1] in the pathogenesis of asthma. Not only is PGD_2 the most potent bronchoconstrictor prostaglandin studied so far in humans, being 3.5 times more potent than prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (1), but it is also the predominant cyclooxygenase product generated during immunologic activation of human pulmonary mast cells in vitro (2). In addition to its bronchospastic effect, PGD_2 may contribute actively to airway inflammation by stimulating neutrophil chemokinesis (3), causing vasodilatation, and increasing postcapillary venule permeability (4). PGD_2 has been shown to synergize with leukotriene B_4 in promoting neutrophil infiltration in human skin (4), to potentiate histamine-induced vascular permeability in rat skin (5), and reduce the threshold at which airways respond to inhaled histamine in asthmatic subjects (6).

It has been suggested that PGD_2 is initially metabolized by reduction of the 3-hydroxycyclopentanone ring to yield the cyclopentane-1,3-diol ring of F-series prostaglandins (PGs), with subsequent metabolism by PG 15-hydroxydehydrogenase and Δ^{13}-reductase (7, 8). We have recently shown that inhalation of $PGF_{2\alpha}$ by either normal or asthmatic subjects resulted in a twofold increase in plasma concentrations of the 13,14-dihydro-15-keto metabolite of $PGF_{2\alpha}$. However, when the same concentration of PGD_2 was inhaled, the plasma levels of this metabolite remained unchanged (9). This suggests that degradation of PGD_2 to $PGF_{2\alpha}$ with subsequent C-15 oxidation and Δ^{13}-reduction is unlikely to represent a major metabolic pathway for PGD_2 immunologically released from mast cells of human airways.

Recent investigations into the fate of PGD_2 in humans have shown that the predominant route of metabolism is via initial formation of the 11β-hydroxyl epimer of $PGF_{2\alpha}$, 9α,11β-PGF_2 (10–12). On examining the metabolic transformation of PGD_2 by human liver in vitro, Roberts and co-workers have provided convincing evidence that PGD_2 is converted exclusively to 9α,11β-PGF_2 (10). They have also shown that after infusion of radiolabeled PGD_2 in a normal volunteer, at least 13 of the PGF ring compounds isolated in the urine had 9α,11β geometry (11). Furthermore, in a patient with systemic mastocytosis with an established overproduction of PGD_2, plasma levels of 9α,11β-PGF_2 correlate closely with the clinical exacerbations of the disease. A concentration of 490 ng/ml has been reported during a severe exacerbation of the disease, a level that is > 80,000 times greater than that present in plasma from a normal subject (10).

In vitro, 9α,11β-PGF_2 is a contractile agonist of human coronary artery strips and is a weak inhibitor of ADP-induced platelet aggregation (13). When injected intravenously into rats it causes hypertension (10). Thus, in view of the biological activities of 9α,11β-PGF_2 and its potential for being generated in large amounts from PGD_2 derived from mast cells of human airways, we have investigated its ability to cause bronchoconstriction in asthmatic and normal subjects. In further experiments we have compared its effects with those of PGD_2 and $PGF_{2\alpha}$ on isolated guinea pig and human airways.

Methods

In vitro studies

Segments of trachea from male Dunkin-Hartley strain guinea pigs (500–750 g) and 4-mm diameter bronchi from human lung tissue obtained from patients undergoing resection for bronchial carcinoma, were used for these experiments. The tissues were cut spirally, divided into sections 1–2 cm long, and suspended in an organ bath containing Krebs' solution, (composition, in millimoles, KCl, 4.69; KH_2PO_4, 1.18; $MgSO_4$, 1.03; NaCl, 118.1; $NaHCO_3$, 25.0; glucose, 11.1; and $CaCl_2$, 2.5), maintained at 37°C and aerated with 95% O_2 and 5% CO_2. Tension developed by the tissues was measured isometrically, using a Lectromed isometric (force) transducer (type 4155) and an Ormed Multitrace six-channel re-

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1. *Abbreviations used in this paper:* 9α,11β-PGF_2, 9α,11β-prostaglandin F_2; EC_{50}, response level corresponding to 50% of prostaglandin D_2 maximum; FEV_1, forced expiratory volume in 1 s; PC, provocation concentration; PGs, prostaglandins; PGD_2, prostaglandin D_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$, sGaw, specific airways conductance; TLC, total lung capacity; $\dot{V}max_{30}$, maximum airflow rate at a lung volume of 30% of initial vital capacity.

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corder. Tissues were suspended with initial tension of 5 *g* and allowed to equilibrate for 1 h.

All of the guinea pig tracheal spirals and 70% of the human bronchial spiral preparations gave consistent contractile responses to the initial supramaximal (1×10^{-6} M) concentration of methacholine, and these were therefore considered suitable for analysis of PG effects. Cumulative dose–response curves were constructed for PGD_2, $9\alpha,11\beta$-PGF_2, and $PGF_{2\alpha}$ ($0.1–32.0 \times 10^{-6}$ M). Preliminary experiments had demonstrated that the human bronchial spiral was resistant to desensitization by all three PGs used, and that the guinea pig trachea exhibited desensitization only when exposed to higher doses of PGs ($> 32 \times 10^{-6}$ M) for longer periods (45 min) than those used in these experiments. PGs were made up freshly for each study in Krebs' solution and tested in a random order.

In vivo studies

Subjects. Eight men with mild allergic asthma participated in this study. All gave histories of episodic dyspnea with wheezing and had at least a 15% improvement in forced expiratory volume in 1 s (FEV_1) in response to albuterol. Their mean age (±SEM) was 29±2 yr, and all were nonsmokers. The mean baseline FEV_1 (±SEM) was 70.1±5.9% of the predicted normal values. All eight subjects were being treated with inhaled albuterol, and in addition, two were receiving inhaled beclomethasone diproprionate. No other medications were taken by any of the subjects during the study. Inhaled treatment was withheld for at least 8 h before the inhalation tests.

Five normal nonallergic men participated as matched control subjects. Their mean age (±SEM) was 25±2 yr, and all were nonsmokers on no medication. Their mean baseline FEV_1 (±SEM) was 91.8±1.6% of the predicted normal values.

The study was approved by the Southampton University Hospitals Ethical Committee and subjects gave their informed consent.

Airway measurements. Airway resistance and thoracic gas volume were measured in a pressure-compensated, volume displacement whole body plethysmograph (Fenyves & Gut, Basle, Switzerland), with subjects panting at 2 Hz over 12 s. The signal was automatically computed to mean values for specific airways conductance (sGaw) by an on-line microprocessor, to take account of variations in lung volume at which measurements of airway resistance were made (14). FEV_1 and the maximum expiratory flow rate at a lung volume of 30% of the initial vital capacity ($\dot{V}max_{30}$) were measured by a rolling seal spirometer (Morgan Spiroflow 12L, P. K. Morgan Ltd., Gillingham, Kent, UK) linked to a Hewlett-Packard 85B microcomputer. Subjects were instructed to perform a partial expiratory maneuver beginning at 50% total lung capacity (TLC), which was followed by a maximum forced expiration from TLC. $\dot{V}max_{30}$ was measured during the initial partial expiration to avoid changes in airway caliber that may occur immediately following both a forced expiration and inspiration to TLC. For this reason also, the forced maneuvers were performed after measurement of sGaw and the second test repeated 1 min after the first.

Drug administration. Histamine acid monophosphate (BDH Chemicals Ltd., Poole, Dorset, UK) was made up freshly each day in 0.9% saline to produce a range of concentrations from $0.10–13.0 \times 10^{-3}$ M. PGD_2 and $9\alpha,11\beta$-PGF_2 (Salford Ultrafine Chemicals and Research Ltd., Salford, UK) were dissolved as stock solutions in methanol at a concentration of 113.6 and 113.0×10^{-3} M, respectively, and stored at − 20°C under nitrogen. The $9\alpha,11\beta$-PGF_2 was prepared from the Corey lactone using methods previously described (15). The identity and purity was verified by 360-MHz proton magnetic resonance spectroscopy and capillary column gas chromatography/mass spectrometry in the electron impact mode using the methyl ester, methoxine, trimethylsilyl ether derivatives. The mass spectrum contained fragments identical to those reported by Liston and Roberts (10) and was chromatographically distinct from $PGF_{2\alpha}$ using a 10-meter DB-5 column (i.d. 0.32 mm, helium flow 1 ml/min). Further confirmation was obtained by high performance liquid chromatography on two separate reversed phase systems and inclusion complexing on cyclodextrin beta, in which the purity was measured as 95%. $PGF_{2\alpha}$ (The Upjohn Co., Crawley, Sussex, UK) was obtained as a sterile aqueous solution of 14.1×10^{-3} M of the tromethamine salt in 0.9% benzyl alcohol. Immediately before use, aliquots of the PGs were diluted with 0.15 M sodium phosphate vehicle (pH 7.4) to achieve a range of concentrations from 0.0028 to 11.3×10^{-3} M. Placebo consisted of 0.9% saline before the inhalation of histamine, and phosphate buffer containing 0.01% of methanol before the PG inhalation. The drugs were nebulized from a starting volume of 1.0 ml using an Inspiron nebuliser (CR Bard International Ltd., Sunderland, UK) driven by compressed air and triggered by a Rosenthal-French dosimeter with a delivery time of 0.74 s. Aerosols with a mass median particle diameter of 4.7 μm were inhaled using a method modified from that of Chai et al. (16). The subjects took five breaths from functional residual capacity to TLC through a mouthpiece while wearing a noseclip. Under these conditions 34 μl of solution leaves the nebulizer with a coefficient of variation of 11%.

Protocol. In the dose–response studies, the asthmatic subjects attended the laboratory on four occasions, separated by at least 4 d, to inhale placebo followed by increasing concentrations of either histamine, PGD_2, $9\alpha,11\beta$-PGF_2, or $PGF_{2\alpha}$. The control subjects attended the laboratory once to inhale placebo followed by increasing concentrations of $9\alpha,11\beta$-PGF_2. Six measurements of sGaw and three measurements of FEV_1 and $\dot{V}max_{30}$ were made at 1-min intervals to obtain mean baseline values. The appropriate vehicle placebo was then administered and followed by two measurements of sGaw, FEV_1, and $\dot{V}max_{30}$. The agonists were then administered in a series of doubling concentrations at 6-min intervals. After each dose, sGaw was measured at 1 and 2.5 min, and then FEV_1 and $\dot{V}max_{30}$ at 3 and 4 min. The inhalations and measurements were continued until FEV_1 had fallen by > 20% of the post-placebo value or until the maximum concentration of agonist had been administered. The PGs were administered double-blind and in random order.

For the time course study, a single concentration (0.71×10^{-3} M) of PGD_2, $9\alpha,11\beta$-PGF_2, and $PGF_{2\alpha}$ was administered to six subjects on three separate occasions at least 4 d apart. Two patients (Nos. 2 and 4) demonstrated to have highly reactive airways in the dose–response studies, were not studied in the time course study for ethical reasons, because the concentration of PG inhaled was likely to cause severe bronchoconstriction. Six measurements of sGaw were made at 1-min intervals to obtain a mean baseline value. Placebo was then inhaled and sGaw measured at 3-min intervals for 15 min. The PG solution was then administered and single measurements of sGaw were made at regular intervals up to 45 min post-challenge.

Data analysis. For the isolated airway preparations, contractile responses for each PG were expressed as a percentage of the maximum achieved with methacholine. Dose–response curves were constructed by plotting the contractile response against the agonist concentration on a logarithm scale. To compare the relative potencies of the PGs, the concentrations required to produce a response 50% of the maximum for PGD_2 (EC_{50}) and associated 95% confidence limits were determined by linear regression analysis. The significance of differences in the relative potencies were determined by analysis of variance, and probability values of 0.05 or less were considered to represent statistically significant differences.

For the in vivo dose–response studies, the airway response to provocation with a test substance was expressed as percentage change from the post-placebo baseline value for each of the three indices of airway caliber. For FEV_1, the lower of the two values recorded at each concentration was expressed as a percentage of the lower of the post-placebo baseline measurements. To accommodate for the greater intrasubject variation for the measurement of sGaw and $\dot{V}max_{30}$, the mean of two values recorded at each concentration was expressed as a percentage of the mean of the two post-placebo baseline measurements. Dose–response curves were constructed by plotting the percentage fall against the logarithm of the agonist concentration. Provocation concentration (PC) values were obtained from each dose–response curve by linear interpolation from the last two points, and geometric mean values calculated for the group. The PC_{20} FEV_1 was the concentration of drug producing a 20% fall in FEV_1; the PC_{35} sGaw, a 35% fall in sGaw; and the PC_{50} $\dot{V}max_{30}$, a 50% fall in $\dot{V}max_{30}$. Covariant analysis was used to assess whether the concentration effect curves differed significantly from parallel,

and the significance of differences in PC values evaluated by Wilcoxon signed rank test. Correlations between the PC values for the different agonists were established using mean least squares linear regression.

For the time course study, the percentage fall in sGaw from the mean baseline value measured 15 min after administration of placebo was calculated at each time point. The resultant maximum percentage fall in sGaw, the area under the time–response curve (calculated by trapezoid integration), and the rate of recovery from maximum fall to 50% of maximum were compared between agonists by the Wilcoxon signed rank test.

Results

PGD_2, $9\alpha,11\beta$-PGF_2, and $PGF_{2\alpha}$ all caused concentration-related contractions of both isolated guinea pig trachea and human bronchus (Fig. 1). There was considerable variability in the responsiveness of both guinea pig and human airway preparations to the PGs (Table I).

In the guinea pig trachea, the maximal responses of the PGs did not differ significantly from each other, and were small when compared with that of methacholine. The relative potencies of the PGs were compared at a response level corresponding to 10% of the methacholine maximum, which corresponds to the EC_{50} for PGD_2. The potencies of the PGs relative to PGD_2 (= 1.00) were $9\alpha,11\beta$-PGF_2 0.65 (NS) and $PGF_{2\alpha}$ 0.52 ($P < 0.01$) (Table I).

In human bronchus, the maximum responses for the PGs relative to that of MCh were similar being 92.2±1.7% at 32 μM PGD_2, 95.1±2.0% at 10 μM $9\alpha,11\beta$-PGF_2, and 93.1±1.0% at 10 μM $PGF_{2\alpha}$ ($n = 3$). Because these values were not significantly different, subsequent PG concentration–response studies were performed to obtain responses up to 75% of the MCh maximum in the same tissue. The relative potencies of PGs compared at 50% of the maximum response to MCh (which corresponds to the EC_{54} for PGD_2) were 1.00 for PGD_2, 4.08 for $9\alpha,11\beta$-PGF_2 ($P < 0.001$), and 2.40 for $PGF_{2\alpha}$ ($P < 0.001$) (Table I).

In vivo

Asthmatic subjects. There was no significant difference between the mean baseline measurements of FEV_1, sGaw, and $\dot{V}max_{30}$ on the different treatment days during either the dose–response or time course studies, either before or after administration of placebo.

Table I. Concentration of PGD_2, $9\alpha,11\beta$-PGF_2, and $PGF_{2\alpha}$ ($\times 10^{-6}$ M) Producing Contractile Responses Equivalent to 10% of the Maximum Response to MCh for Guinea Pig Trachea and 50% of the Maximum Response to MCh for Human Bronchial Spirals In Vitro

Agonist	Guinea pig trachea	*n*	Human bronchial spiral	*n*
PGD_2	0.25 (0.11, 0.55)*	17	4.90 (1.05, 22.91)	9
$9\alpha,11\beta$-PGF_2	0.38 (0.18, 0.78)	19	1.20 (0.74, 1.95)	10
$PGF_{2\alpha}$	0.47 (0.28, 0.78)	18	2.04 (1.35, 3.09)	8

n, No. of preparations tested.
* Geometric mean (95% confidence limits).

Histamine, PGD_2, and $9\alpha,11\beta$-PGF_2 all caused concentration-related falls in FEV_1, sGaw, and $\dot{V}max_{30}$. $PGF_{2\alpha}$ caused a concentration-related fall in measurements of airway caliber in four asthmatic subjects (Nos. 2, 4, 5, and 8) three of whom had the lowest PC_{20} FEV_1 values for histamine. The remaining four subjects responded to $PGF_{2\alpha}$ with a bi- or triphasic response (Nos. 1, 3, 6, and 7). These reactions were characterized by initial bronchoconstriction at concentrations up to 1.0×10^{-4} M, followed by reversal at intermediate concentrations, and in subjects 1 and 6 further bronchoconstriction at concentrations $> 1.0 \times 10^{-3}$ M. When this type of response occurred with $PGF_{2\alpha}$, it was observed with all three indices of airway caliber. Fig. 2 shows the different patterns of airway response to inhaled $PGF_{2\alpha}$ for patients 3, 4, and 6, and Fig. 3 shows the corresponding dose–response curves to histamine, PGD_2, and $9\alpha,11\beta$-PGF_2 for these same patients.

Table II depicts geometric mean provocation concentrations and relative potency ratios (histamine = 1.0) for the three different methods of measuring airway caliber. PGD_2 and $9\alpha,11\beta$-PGF_2 were 26–54 and 28–32 times more potent than histamine as bronchoconstrictor agents. Due to the complex nature of the concentration–response curves to $PGF_{2\alpha}$ in four of the eight subjects, no direct comparisons were possible between $PGF_{2\alpha}$ and the other constrictor agonists. There was a close correlation

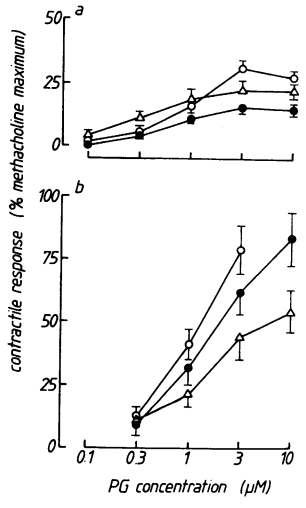

Figure 1. (*a*) Guinea-pig trachea. Contractile response observed with increasing concentrations of PGD_2 (*open triangles*), $9\alpha,11\beta$-PGF_2 (*open circles*), and $PGF_{2\alpha}$ (*filled circles*) for guinea-pig trachea spirals. Each point represents the mean (±SEM) contractile response as percent of methacholine maximum, 17–19 preparations. (*b*) Human bronchus. Contractile response observed with increasing concentrations of PGD_2 (*open triangles*), $9\alpha,11\beta$-PGF_2 (*open circles*), and $PGF_{2\alpha}$ (*filled circles*) for human bronchial segments. Each point represents the mean (±SEM) contractile response as percent of methacholine maximum, 8–10 preparations.

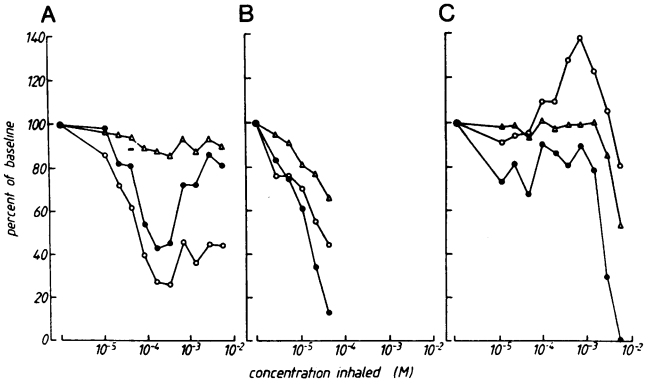

Figure 2. The changes in airway caliber after the inhalation of increasing concentrations of $PGF_{2\alpha}$ in three asthmatic subjects (Nos. 3, 4, and 6; *A*, *B*, and *C*, respectively). Each point represents the percent of baseline for FEV_1 (*open triangles*), sGaw (*open circles*), and $\dot{V}max_{30}$ (*filled circles*).

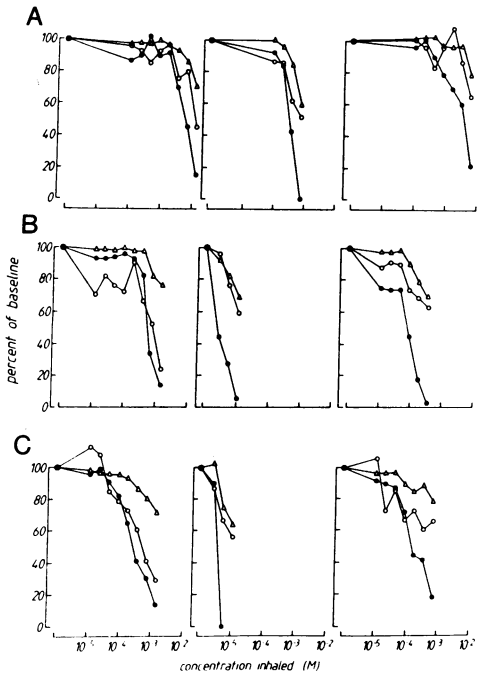

Figure 3. The changes in airway caliber after the inhalation of increasing concentrations of histamine (*top*), PGD_2 (*middle*), and $9\alpha,11\beta$-PGF_2 (*bottom*), in three asthmatic subjects (Nos. 3, 4, and 6; *A, B,* and *C,* respectively). Each point represents the percent of baseline for FEV_1 (*open triangles*), sGaw (*open circles*), and $\dot{V}max_{30}$ (*filled circles*).

between the airway responsiveness to histamine, PGD_2, and $9\alpha,11\beta$-PGF_2 (Table III).

In the time course study, all three PGs caused a fall in sGaw with the maximum airway response occurring between 3 and 7 min after aerosol inhalation (Fig. 4). The mean (±SEM) maximum fall for $9\alpha,11\beta$-PGF_2 was 48%±14% and this was significantly greater than the fall caused by PGD_2 (39%±12%, $P < 0.01$) and that after $PGF_{2\alpha}$ (32±14%, $P < 0.01$). The areas under the time course-sGaw response curves for $9\alpha,11\beta$-PGF_2 were significantly greater than that of $PGF_{2\alpha}$ ($P < 0.01$), but were not significantly different from those of PGD_2. Bronchoconstriction with $9\alpha,11\beta$-PGF_2 persisted for longer than the 45 min of the study period, whereas return to baseline sGaw for PGD_2 and $PGF_{2\alpha}$ had occurred by 45 and 25 min, respectively. Despite the greater duration and magnitude of bronchoconstriction observed with $9\alpha,11\beta$-PGF_2, the rates of recovery of airway caliber were not significantly different between the PGs.

Nonasthmatic subjects. Inhalation of $9\alpha,11\beta$-PGF_2 caused concentration-related falls in all three indices of airway caliber. After inhalation of the maximum concentration of $9\alpha,11\beta$-PGF_2 (11.3×10^{-3} M), the FEV_1 did not fall by > 20% of the post-placebo value in any of the five control subjects (range, +3 to −19%). The mean maximum fall (±SEM) at this maximum concentration was 23.0±8.2, 6.6±8.1, and 39.2±17.3% for sGaw, FEV_1, and $\dot{V}max_{30}$, respectively. This contrasts with the response observed with all the asthmatic subjects in which a fall in FEV_1 of at least 20% was achieved before reaching this maximum concentration of $9\alpha,11\beta$-PGF_2.

Discussion

This study demonstrates for the first time that a product of the principal pathway of PGD_2 metabolism in humans, $9\alpha,11\beta$-PGF_2 (10–12), is a contractile agonist of isolated guinea pig and human airways. In vitro it was more potent than PGD_2 on human airways, and more potent than its 11α-epimer $PGF_{2\alpha}$ on both guinea pig and human airways. In accordance with the in vitro studies, $9\alpha,11\beta$-PGF_2 constricted the airways of normal and asthmatic subjects in vivo. In the asthma group it was approximately equipotent with PGD_2, and 28–32 times more potent than histamine as a bronchoconstrictor agent.

It has been suggested that the measurements $\dot{V}max_{30}$ and sGaw reflect dimensions of the airways at different levels in the bronchial tree, $\dot{V}max_{30}$ comprising a major small airway component (17) and sGaw a large component being derived from the more proximal airways (18). The greater potency of inhaled $9\alpha,11\beta$-PGF_2 and PGD_2, when compared with histamine was of similar magnitude irrespective of whether sGaw, FEV_1, or $\dot{V}max_{30}$ were used to measure airway caliber. This may indicate that both prostanoids have similar contractile activities on central and peripheral airways, or that the physiological measurements are insensitive in detecting small regional differences in airway dimensions (19). The high degree of correlation observed between the PC values of $9\alpha,11\beta$-PGF_2 with those of both histamine and PGD_2, and the less marked effect in provoking bronchoconstriction in normal subjects, indicates that the airway responsiveness to $9\alpha,11\beta$-PGF_2 is related to the degree of nonspecific bronchial reactivity.

Narumiya and Toda (20) have recently described three operationally distinct receptor mechanisms for the pharmacologic effects of PGD_2, with the contractile activity on airway and cerebral artery smooth muscle being distinct from that linked to adenylate cyclase mediating the platelet and mast cell effects and also different from that inhibiting tumor cell growth.

Table II. Geometric Mean PCs and Relative Potency Ratios of Inhaled Histamine, PGD_2, and $9\alpha,11\beta$-PGF_2 for Different Measurements of Airway Caliber in Asthmatic Subjects

	PC_{20} FEV_1		PC_{35} sGaw		PC_{30} $Vmax_{30}$	
Agonist	Geometric mean ($\times 10^{-3}$ M)	Potency ratio (range)	Geometric mean ($\times 10^{-3}$ M)	Potency ratio (range)	Geometric mean ($\times 10^{-3}$ M)	Potency ratio (range)
Histamine	2.36	1.0	2.27	1.0	2.02	1.0
PGD_2	0.15‡	25.7 (7.2–42.5)	0.11‡	29.0 (1.6–74.9)	0.10*	54.3 (0.76–198)
$9\alpha,11\beta$-PGF_2	0.17‡	29.7 (1.6–60.3)	0.14‡	28.2 (2.4–92.5)	0.10‡	32.3 (1.8–96.8)

Significance of differences from histamine (rank Wilcoxon test). * $P < 0.05$. ‡ $P < 0.01$.

Table III. Correlation between PCs of 9α,11β-PGF_2 and Those of Histamine and PGD_2, for the Three Different Measurements of Airway Caliber in Asthmatic Subjects

Agonist	PC_{20} FEV_1 ($\times 10^{-3}$ M)	PC_{35} sGaw ($\times 10^{-3}$ M)	PC_{50} $Vmax_{30}$ ($\times 10^{-3}$ M)
	r	r	r
Histamine	0.78*	0.61‡	0.77*
PGD_2	0.91§	0.72‖	0.95§

Mean least-square linear regression.
* $P < 0.02$.
‡ NS.
‖ $P < 0.05$.
§ $P < 0.001$.

Whereas in the present study a potency difference was observed between 9α,11β-PGF_2 and PGD_2 on human airway in vitro, no such difference was observed in vivo or in guinea pig trachea in vitro. We are therefore unable to comment on whether the bronchoconstrictor effects of the two prostanoids are mediated through the same receptor as might be suggested from their similar activities on human coronary artery strips and platelet aggregation (21).

We have recently demonstrated that histamine may contribute up to 50% of the immediate allergen-induced asthmatic response in vivo (22). After immunologic activation, the human pulmonary mast cell releases between 40 and 50 times more histamine than PGD_2 (23). Our observation that PGD_2 and 9α,11β-PGF_2 are between 26 and 54 times more potent than histamine as bronchoconstrictor agonists when inhaled in vivo, suggests that both these PGs have a physiologic effect comparable to that of histamine in producing bronchoconstriction after allergen challenge. This would indicate that PGD_2 and its initial metabolite 9α,11β-PGF_2 may contribute significantly to the immediate allergen-induced asthmatic response.

In contrast to PGD_2 and 9α,11β-PGF_2, the concentration response relationship with $PGF_{2\alpha}$ was complex. Four of the eight asthmatic subjects tested had a single concentration response curve, while the remaining subjects demonstrated a bi- or triphasic response to increasing concentrations of inhaled $PGF_{2\alpha}$. Although this precluded direct measurement of its bronchoconstrictor potency in vivo, we were able to obtain a rank assessment of activity of $PGF_{2\alpha}$ relative to 9α,11β-PGF_2 from the time course and in vitro studies, both of which suggested that 9α,11β-PGF_2 was more potent than $PGF_{2\alpha}$. The mechanisms causing the multiphasic responses to $PGF_{2\alpha}$ may involve a complex interaction between vagal stimulation (24), tachyphylaxis (25), and release of a bronchodilator substance.

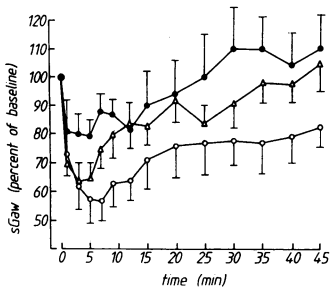

Figure 4. Time course of bronchoconstriction induced by a single inhalation of PGD_2 (*open triangles*), 9α,11β-PGF_2 (*open circles*), of $PGF_{2\alpha}$ (*filled circles*) in asthmatic subjects. Each point represents the mean (±SEM) percent of baseline for sGaw in six subjects. The concentration of each prostanoid inhaled was 0.71×10^{-3} M.

We conclude that 9α,11β-PGF_2, a metabolite of PGD_2 found in substantial quantities during mast cell activation in man (10, 12), is also a potent bronchoconstrictor agonist. In view of the recent observations that PGD_2 is preferentially metabolized by human lung to 9α,11β-PGF_2 (26), our studies suggest that at least some of the bronchoconstrictor activity of PGD_2 could be achieved after its local biotransformation to 9α,11β-PGF_2. Thus, 9α,11β-PGF_2 is a novel bronchoconstrictor prostanoid whose activity may contribute to the pathogenesis of airflow obstruction in patients with allergic asthma.

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