PAPERS AND ORIGINALS

Bronchial Hyperreactivity to Prostaglandin $F_{2\alpha}$ and Histamine in Patients with Asthma

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Summary

The influence on airway conductance of inhaled aerosols of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), histamine, and prostaglandin E_2 (PGE_2) was studied in 10 patients with spirometrically reversible bronchial asthma and in 10 healthy subjects with no history of lung disorder. Both groups responded with bronchoconstriction after inhalation of $PGF_{2\alpha}$ but the asthmatic patients were about 8,000 times more sensitive to the compound than were the healthy controls. In the patients, but not in the controls, $PGF_{2\alpha}$ often caused a long-standing decrease in airway conductance with symptoms resembling allergen-provoked asthmatic attacks. On the other hand, the patients showed less than a 10-fold increase in sensitivity to histamine, and the ratio of histamine: $PGF_{2\alpha}$ doses causing a 50% decrease of airway conductance was 2.6:1 and 2,400:1 in controls and patients respectively. Inhalation of PGE_2 while moderately but consistently increasing airway conductance in controls, had a variable—occasionally slight bronchoconstrictive—effect in patients. The decrease in airway conductance by a given dose of $PGF_{2\alpha}$ was little modified by the simultaneous inhalation of a 100-times higher PGE_2 dose. It is suggested that endogenous, locally formed $PGF_{2\alpha}$ may play an important part in the pathogenesis of bronchial asthma.

Introduction

Antigen-antibody reaction plays an important part in bronchoconstriction in asthma, though the exact mechanisms involved are still unknown. Chemical mediators such as histamine, slow-reacting substance, kinins, and 5-hydroxytryptamine may be important—but their precise role is not clear (Brocklehurst, 1962; Douglas, 1970a, 1970b; Austen, 1965)—as may be other substances such as prostaglandins (Horton, 1969).

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is present in normal lung tissue in many animal species and in man (Änggård, 1965; Karim *et al.*, 1967). It is released from the lungs of guinea-pigs and rats during anaphylactic reaction and by various chemical and mechanical stimuli (Edmonds *et al.*, 1969; Piper and Vane, 1969; Alabaster and Bakhle, 1970; Lindsey and Wyllie, 1970; Palmer *et al.*, 1970). In cats, guinea-pigs, and dogs $PGF_{2\alpha}$ produces effects consistent with bronchoconstriction (Änggård and Bergström, 1963; Berry and Collier, 1964; James, 1969) and in man it contracts bronchial smooth muscle in vitro and in vivo (Sweatman and Collier, 1968; Hedqvist *et al.*, 1971; Mathé *et al.*, 1971).

The present investigation was done to study the influence of inhalation of $PGF_{2\alpha}$ on airway resistance in healthy subjects and in patients with bronchial asthma, and to compare its bronchoconstrictive effect with that of histamine. Furthermore, prostaglandin E_2 (PGE_2), also a normal constituent of lung tissue (Änggård, 1965; Karim *et al.*, 1967), which has been reported to cause both bronchodilation and, less frequently, constriction (Main, 1964; Sweatman and Collier, 1968; James, 1969; Piper and Vane, 1969; Cuthbert, 1971; Mathé *et al.*, 1971; Rosenthale *et al.*, 1971), was given in the same way to some patients to study the possible interaction between the two prostaglandins.

Patients and Methods

Ten patients (two men and eight women, aged 19-52 years) with bronchial asthma of two to 20 years' duration were included in the study (table I). All patients showed clinical and spirometric reversibility of the bronchoconstriction on inhalation of isoprenaline. The patients were studied in asthma-free periods and had had no symptomatic medication for at least four hours beforehand.

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TABLE 1—Summary of Clinical Features in 10 Patients with Bronchial Asthma

Case No.	Sex and Age	Duration of Asthma in Years	Asthmatic Symptoms Provoked by		Steroids	Baseline (SGaw, l./cm H ₂ O/sec)	FEV ₁ (% of Predicted)	FEV ₁ (% of Predicted)	Remarks
			Specific Allergens	Exercise					
1	F. 23	3	+	—	—	0.08	102	108	Asthma as a child. Recurrence since 1968. Decreasing during hyposensitization Heavy smoker with allergic asthma since 1968. Decreasing during hyposensitization Asthma since 1967, accentuated 1970. Diet and hyposensitization without results Acute nephritis and allergic rhinitis 1959. Onset of asthma during pregnancy, 1969. Decreasing during hyposensitization Allergic eczema and rhinitis since childhood. Asthma since 1956. Decreased after hyposensitization Allergic asthma. Also symptoms while exposed to various chemicals Asthma since childhood. Hyposensitization 1965 with temporary decrease of symptoms Lung eosinophil infiltrates 1966/7. Asthma since 1967. Hyposensitization with decreasing symptoms Allergic rhinitis and asthma since 1952 Goiter operation, 1958. Sarcoidosis I-II 1960. Asthma since 1958. Exacerbation with upper respiratory infections
2	F. 35	3	+	—	—	0.06	62	59	
3	M. 38	4	+	+	+	0.18	92	54	
4	F. 29	2	+	—	—	0.11	78	70	
5	F. 19	15	+	+	—	0.16	72	45	
6	M. 30	5	+	+	+	0.10	77	79	
7	F. 28	20	+	+	+	0.16	85	86	
8	F. 23	5	+	+	—	0.12	101	86	
9	F. 21	19	+	+	—	0.08	68	49	
10	F. 52	13	+	+	+	0.09	40	19	

Ten healthy young men and women without history of allergic manifestations or lung disease and with normal chest radiographs and static and dynamic spirometry served as controls. The first subjects tested were two of the authors of this report. Subsequently, informed consent for the study was obtained from both groups.

Airway Conductance (Gaw).—Airway resistance was measured in a whole body plethysmograph (Dubois *et al.*, 1956) at functional residual capacity, at gas flow of 0.5 l./sec, and at rapid breathing 2 c.p.s. It was converted to its reciprocal, Gaw, and expressed per litre thoracic gas volume (SGaw, l./cm H₂O/sec). Five determinations of both Gaw and thoracic gas volume were made at each step and the mean was calculated.

Drugs.—A stock solution of PGF₂α containing 5 mg/ml saline had its pH adjusted to 7.0 with tromethamine. Appropriate dilutions were made in isotonic NaCl. The doses used were 0.56 × 10⁻² – 2.8 × 10³ nmol, and the administered dose was contained in 0.5 ml of solution. PGF₂α was also administered as free acid. PGE₂ in a stock solution 50 μg/ml saline was prepared. Dilutions were made in isotonic NaCl and the doses given were 17.75, 35.5, and 71.0 nmol. Histamine chloride, dissolved in the same vehicle, was administered in doses of 5.4–10.8 × 10³ nmol free base.

Procedure.—All subjects were tested on at least two experimental sittings and some on four to six occasions. They were blind to the exact nature of the substance to be inhaled in order to minimize the possible effects of suggestion on the airway conductance (Luparello *et al.*, 1970). Physiologic saline was inhaled on each experimental day, whereas other substances were given only on a particular day. The administration was performed with an ultrasonic nebulizer which generated a microaerosol with a median particle size of 2 μm (LKB-Medical,

Stockholm, Sweden). The subject inhaled through the nebulizing chamber 0.5 ml of solution each time and exhaled into the room air. Before each experiment the control SGaw was obtained as the mean of 10 measurements at functional residual capacity. The subject then inhaled solvent only and SGaw was recorded after 1, 5, and 10 minutes. If it was not significantly changed, inhalation of prostaglandin solution or the histamine chloride was started and measurements were continued in the same way. The nebulized dose was doubled with each successive administration, and inhalations were continued at 30 to 45-minute intervals until 50% or greater reduction of SGaw was obtained.

Results

Aerosols of PGF₂α, histamine, and PGE₂ were given to 10 healthy subjects and to 10 patients with spirometrically reversible bronchial asthma. Baseline value for SGaw was 0.163 ± 0.020 l./cm H₂O/sec (mean ± S.E. of mean) for the controls, which is higher than the corresponding value for the asthmatics (0.129 ± 0.013). Inhalation of isotonic NaCl caused inconsistent and minor changes in airway conductance in 10 healthy controls and 10 asthmatics. However, two subjects, one in each group, showed significant reduction and were excluded from the investigation (McFadden *et al.*, 1969; Luparello *et al.*, 1970). Irrespective of the form of PGF₂α (free acid or tromethamine) it caused no irritation or only a minimal irritation of the upper respiratory tract and there were no differences in the effect on the airway conductance. Histamine had no or only a slight irritating effect, whereas one control and two patients felt considerable irritation after PGE₂.

TABLE 2—Inhaled Doses of PGF₂α and Histamine Eliciting an Approximate 50% Decrease of Specific Airway Conductance (SGaw) in 10 patients with Reversible Bronchial Asthma and 10 Healthy Subjects. For Comparison of Bronchoreactivity the Histamine Dose Causing the Closest Similar Effect on SGaw as PGF₂α was Chosen.

Asthmatic Patients				Healthy Controls			
PGF ₂ α (nmol)	Decrease in SGaw (%)	Histamine (nmol)	Decrease in SGaw (%)	PGF ₂ α (nmol)	Decrease in SGaw (%)	Histamine (nmol)	Decrease in SGaw (%)
0.71	70	6.8 × 10 ³	84	1.4 × 10 ³	47	10.8 × 10 ³	40
0.23 × 10 ⁻¹	57	4.3 × 10 ¹	50	2.8 × 10 ³	50	5.4 × 10 ³	55
0.18	89	5.4 × 10 ³	50	2.8 × 10 ³	55	5.4 × 10 ³	50
0.71	46	6.8 × 10 ³	73	2.8 × 10 ³	74	5.4 × 10 ³	56
0.71	50	2.7 × 10 ³	81	0.7 × 10 ³	50	1.3 × 10 ³	57
0.56 × 10 ⁻³	75	1.4 × 10 ³	70	2.8 × 10 ³	46	2.7 × 10 ³	57
0.45 × 10 ⁻¹	75	6.8 × 10 ³	68	1.4 × 10 ³	55	2.7 × 10 ³	50
0.18	50	1.74 × 10 ³	66	2.8 × 10 ³	18	10.8 × 10 ³	19
0.56 × 10 ⁻²	62	8.7 × 10 ¹	53	1.4 × 10 ³	27	10.8 × 10 ³	50
0.18	50	1.74 × 10 ³	56	2.8 × 10 ³	20	2.7 × 10 ³	58
Mean ± S.E. of mean { 0.275 ± 0.097	62 ± 4.5	6.61 × 10 ³ ± 2.6 × 10 ³	65 ± 8.9	2.2 × 10 ³ ± 0.27 × 10 ³	44 ± 5.5	5.9 × 10 ³ ± 1.2 × 10 ³	49 ± 4.0

HEALTHY SUBJECTS

Inhalation of $\text{PGF}_2\alpha$.— $\text{PGF}_2\alpha$ aerosol was inhaled in metered doses ranging from 5.6 to 2.8×10^3 nmol. It produced a decrease of SGaw, which was monitored 1, 5, and 10 minutes after the inhalation, or until control values were reached. For seven of the normal subjects SGaw was reduced by 50% or more with a $\text{PGF}_2\alpha$ dose ranging from 0.7×10^3 to 2.8×10^3 nmol. Three subjects showed a significant reduction but did not reach the 50% level after 2.8×10^3 nmol, which was the highest dose used (table II). The onset of the effect was always rapid, usually within a minute, was maintained for about 2 to 3 minutes, and returned to near control levels 10–15 minutes after the inhalation (see chart). Doses lower than 0.35×10^3 nmol produced inconsistent effects, varying from moderate reduction to, less frequently, slight increase of SGaw.

Time course of $\text{PFG}_2\alpha$ -induced bronchoconstriction in 10 healthy subjects and 10 asthmatic patients. Specific airway conductance (SGaw, expressed as percentage of control values) was measured 1, 5, 10, or 15, 30, and 60 minutes after inhalation of $\text{PFG}_2\alpha$ that caused SGaw to decrease about 50%.

Inhalation of Histamine.—For comparison of bronchoreactivity histamine aerosol was administered to the same control subjects using an identical routine as with $\text{PFG}_2\alpha$. Nine of the subjects responded with a 50% reduction of SGaw after inhalation of histamine doses ranging from 1.3×10^3 to 10.8×10^3 nmol (mean $5.9 \times 10^3 \pm 1.2 \times 10^3$, table II). One subject did not reach this level after 10.8×10^3 nmol but had headaches, palpitations, and was flushed. The histamine-induced reduction of SGaw lasted much the same as after $\text{PFG}_2\alpha$ —that is, the effect was relatively short and normal values were obtained 10–15 minutes after the inhalation.

Inhalation of PGE_2 .—Six of the 10 control subjects were given PGE_2 aerosol in three metered doses of 17.75, 35.5, and 71.0 nmol contained in 0.5 ml of solvent. A consistent though modest increase of SGaw with little variation among the different doses was obtained (table III). The effect occurred rapidly and was maintained for at least 15 minutes.

TABLE III—Effect of Metered PGE_2 Doses on Specific Airway Conductance (SGaw) in Six Healthy Subjects 1, 5, and 15 Minutes after Inhalation. Results expressed as Mean $\pm$ S.E. of Mean percentage increase of SGaw

PGE_2 (nmol)	Percentage increase of SGaw		
	1 min	5 min	15 min
17.8	16 $\pm$ 4	15 $\pm$ 9	16 $\pm$ 4
35.5	22 $\pm$ 6	20 $\pm$ 8	21 $\pm$ 6
71.0	21 $\pm$ 7	22 $\pm$ 7	24 $\pm$ 11

ASTHMATIC PATIENTS

Inhalation of $\text{PFG}_2\alpha$.—Very low doses of $\text{PFG}_2\alpha$ caused a severe bronchoconstriction in the patients. In two, airway conductance was reduced by more than 50% after inhalation

of 0.56×10^{-2} nmol of $\text{PFG}_2\alpha$, and in none of the cases were doses higher than 0.71 nmol necessary to produce this effect (table II). The dose of $\text{PFG}_2\alpha$ to reduce SGaw by 50% or more calculated for all 10 patients was 0.275 ± 0.097 nmol (mean $\pm$ S.E. of mean). The asthmatic patients thus showed a near 10^4 -fold higher sensitivity to $\text{PFG}_2\alpha$ than did the controls. This remarkable difference is further emphasized when it is considered that three of the 10 healthy subjects did not reach the 50% decrease level and that the reduction of SGaw was more pronounced in the patients. The time course of $\text{PFG}_2\alpha$ -induced bronchoconstriction differed significantly from that in the controls (see chart). In only two cases was maximal effect seen 1 minute after inhalation of $\text{PFG}_2\alpha$. In eight out of 10 patients SGaw, though markedly reduced after 1 minute, progressively decreased to a maximal effect 5 to 15 minutes after the inhalation. The effect of $\text{PFG}_2\alpha$ in the patients was also longer lasting and the recovery gradual. Thus in five asthmatics SGaw remained more than 50% below the baseline even 60 minutes after the inhalation. These patients also had wheezing, rhonchi, dyspnoea, and the usual sensation of an asthmatic attack. Inhalation of isoprenaline or intravenous injection of aminophylline, or both, relieved the distress and increased SGaw considerably in these subjects. On the other hand, premedication with isoprenaline did not prevent $\text{PFG}_2\alpha$ from causing a noticeable reduction of SGaw in one studied subject.

Inhalation of Histamine.—The asthmatic patients responded with bronchoconstriction to lower doses of histamine than did healthy subjects, confirming previous observations (Tiffeneau, 1958). The time course of the induced alteration of SGaw was similar to that seen in the healthy subjects. The maximal effect was obtained 1 to 2 minutes after the inhalation; thereafter it slowly subsided and baseline values were reached 15 to 30 minutes after the inhalation. Although histamine was administered in doses that caused about the same initial reduction of SGaw as did $\text{PFG}_2\alpha$, the protracted and noticeable reactions seen after inhalation of $\text{PFG}_2\alpha$ did not occur. The dose of histamine causing at least a 50% fall of SGaw was $6.61 \times 10^2 \pm 2.6 \times 10^2$ nmol (mean $\pm$ S.E. of mean). This implies an eight-fold increase in sensitivity to histamine in the patients compared with the controls (table II). The asthmatic patients were also hyperreactive to $\text{PFG}_2\alpha$. However, there seems to be a significant difference in the pattern of response to the two drugs. Thus the ratio of histamine to $\text{PFG}_2\alpha$ doses causing a 50% fall of SGaw was 2.6:1 in the controls and 2,400:1 in the asthmatic patients.

Inhalation of PGE_2 .—A modest increase of SGaw was obtained in two patients, no change in one case, and a slight decrease in another three patients after inhalation of 71 nmol of PGE_2 . The possible interaction of PGE_2 and $\text{PFG}_2\alpha$ was also studied in these six subjects. First $\text{PFG}_2\alpha$ was given in a dose producing about a 50% fall of SGaw. After control values had been re-established, the same dose of $\text{PFG}_2\alpha$ was given simultaneously with PGE_2 (71 nmol). Consistent with the action of PGE_2 when given alone, the effect of $\text{PFG}_2\alpha$ —in the presence of PGE_2 —was moderately reduced in two patients, was not changed in one case, and showed a more pronounced decrease of SGaw in the other three patients.

Discussion

The striking finding in this study is the extreme sensitivity of asthmatic patients to inhaled $\text{PFG}_2\alpha$. Picomole doses were sufficient to provoke severe and prolonged decrease in airway conductance with symptoms in many cases apparently indistinguishable from allergen-provoked asthmatic attacks. At this dose range no other naturally occurring substance in the body seems to elicit such profound effects on the bronchial tree.

Nonspecific "hyperreactivity" of the asthmatic bronchial tree has been shown previously to histamine, acetylcholine, and various mechanical stimuli (Tiffeneau, 1958; Parker *et al.*, 1965; Simonsson *et al.*, 1967). Conceivably, $\text{PFG}_2\alpha$ could belong to the same category. The remarkably low doses of $\text{PFG}_2\alpha$ used,

the time course of induced changes, and the asthma-like clinical picture appear to mediate against such an explanation. Moreover, when compared to the healthy controls, the asthmatic patients were 8,000 times more sensitive to $\text{PGF}_2\alpha$ but showed less than a 10-fold increase in sensitivity to histamine. Similarly, the ratio of molar doses of histamine to $\text{PGF}_2\alpha$ required to produce about a 50% fall of SGaw was only 2.6:1 in the healthy controls but 2,400:1 in the asthmatic patients. Furthermore, $\text{PGF}_2\alpha$ is released from guinea-pig and rat lungs during anaphylaxis and by chemical and mechanical treatment (Edmonds *et al.*, 1969; Piper and Vane, 1969; Alabaster and Bakhle, 1970; Lindsey and Wyllie, 1970; Palmer *et al.*, 1970). If the same conditions also apply for human lung tissue our results would suggest that the pulmonary response to $\text{PGF}_2\alpha$ may be specific, and imply a significant role for the substance in bronchial asthma.

Several mechanisms, separately or in combination, could be operative.

(1) Overproduction of $\text{PGF}_2\alpha$ at the expense of PGE_2 , as Horton (1969) speculated, could account for certain pathological changes associated with bronchoconstriction.

(2) Since asthmatic patients seem to be remarkably hyper-reactive to inhaled $\text{PGF}_2\alpha$, conceivably the bronchial smooth muscle has become sensitized to the substance—possibly as a consequence of a preceding antigen-antibody reaction.

(3) Altered metabolism of $\text{PGF}_2\alpha$ could also explain the findings in the present study. Lung tissue contains among the highest levels of 15-hydroxy prostaglandin dehydrogenase in the body (Ånggård *et al.*, 1971) thus enabling virtually total inactivation of prostaglandins during their passage through the lung (Ferreira and Vane, 1967). Increased sensitivity to $\text{PGF}_2\alpha$ or decreased 15-hydroxy prostaglandin dehydrogenase activity, or both, in the lung should inevitably exaggerate and prolong the bronchoconstrictor action of $\text{PGF}_2\alpha$.

Various stimuli can increase $\text{PGF}_2\alpha$ formation and release in anaphylactic as well as in seemingly normal guinea-pig lung tissue. Large doses of $\text{PGF}_2\alpha$ given intravenously to normal subjects produce only relatively mild symptoms (Bygdeman and Wijkvist, 1971; Karim, 1971). Human bronchial smooth muscle is usually relaxed by PGE_2 (Sweatman and Collier, 1968; Mathé *et al.*, 1971), and inhalation of PGE_2 may increase the forced expiratory volume in asthmatic patients (Cuthbert, 1971). We have shown that healthy controls were insensitive to inhaled $\text{PGF}_2\alpha$ when compared with asthmatic patients. Moreover, in the present series of patients with asthma the effect of PGE_2 per se was variable, and it only partly and irregularly counteracted the bronchoconstrictor action of $\text{PGF}_2\alpha$. Thus overproduction of $\text{PGF}_2\alpha$ is not likely to be the only or even the major cause of severe bronchoconstriction in asthmatic patients. However, coupled with hyperreactivity and altered metabolism increased formation of $\text{PGF}_2\alpha$ might be one factor in the pathogenesis of bronchial asthma.

(4) Complementary to and interacting with the antigen-antibody reaction a dysfunction of the autonomic nervous system—specifically, decreased availability of catecholamines locally in the lung (Mathé, 1971)—has been suggested as contributing to the pathogenesis of asthma. In this context the demonstrated inhibitory effect of catecholamines on the release of mediators of allergic reaction (Schild, 1936; Lichtenstein and Margolis, 1968; Assem and Schild, 1971) might be of paramount importance. In parallel, existence of a negative feedback mechanism whereby prostaglandins inhibit the release of catecholamines has been found in several organs (Hedqvist, 1970; Wennmalm, 1971). Such a phenomenon could conceivably exist in lungs, too. Thus inverse relation between both the action and release of catecholamines and prostaglandins, with predominance of the latter compounds, in asthma can be hypothesized. At present, available data do not permit a critical evaluation of such an assumption.

In conclusion, the natural occurrence of $\text{PGF}_2\alpha$ and its ready release from lung tissue, and the remarkable sensitivity of asthmatic patients to inhaled $\text{PGF}_2\alpha$ as shown in this study, suggest that endogenous $\text{PGF}_2\alpha$ may play a significant part in the pathogenesis of bronchial asthma. If our supposition is correct we may eventually understand why asthmatic attacks can be provoked by a wide variety of seemingly unrelated stimuli and conditions. The common underlying factor might be local formation of $\text{PGF}_2\alpha$ producing severe and prolonged bronchoconstriction as a consequence of increased sensitivity of bronchial smooth muscle to $\text{PGF}_2\alpha$ or decreased 15-hydroxy prostaglandin dehydrogenase activity, or both, in asthmatic lung tissue.

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