Atropine, Sodium Cromoglycate, and Thymoxamine in PGF₂α-induced Bronchoconstriction in Extrinsic Asthma

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Summary

In six patients with extrinsic bronchial asthma the inhalation of prostaglandin (PG) F₂α in a small dosage produced significant bronchoconstriction, whereas PGE₂ produced bronchodilatation. In these patients cholinergic blockade with atropine partially inhibited the PGF₂α-induced bronchoconstriction, but the α-receptor-blocking drug thymoxamine and sodium cromoglycate did not. These results suggest that the effect of PGF₂α is mediated through cholinergic receptors in the airways, and this effect is grossly exaggerated in asthma. The failure to inhibit PGF₂α-induced bronchoconstriction with sodium cromoglycate and the observation of an inhibitory effect of sodium cromoglycate in both allergic and exercise asthma suggest that locally formed PGF₂α may not be the main factor in the pathogenesis of bronchial asthma.

Introduction

Human lung contains prostaglandins (PG) of both the E and F series, E₂ and F₂α being the most abundant.^{1,2} PGF₂α, a potent bronchoconstrictor to which patients with bronchial asthma are highly sensitive,³⁻⁵ is released from mammalian lungs during anaphylactic reactions⁶ and by various chemical and mechanical stimuli.⁷ Recently, a considerable increase in plasma levels of PGF₂α metabolites in asthmatic patients after allergen challenge was reported.⁸ Based on these observations it was postulated that locally formed PGF₂α may play an important part in the pathogenesis of bronchial asthma.^{5,8} PGE₂, on the other hand, causes bronchodilatation in man.^{3,5}

PGE₂ activates adenyl cyclase, now identified with β-receptor function,⁹⁻¹¹ and its bronchodilator effect is mediated by an increase in cyclic adenosine monophosphate (cyclic AMP). Conversely, PGF₂α has been reported to activate guanyl cyclase and lead to the formation of cyclic guanosine monophosphate (cyclic GMP).¹² Guanyl cyclase activity has been found in various tissues, including human lung.¹³ Cyclic GMP activates cholinergic responses,^{14,15} and guanyl cyclase may also be activated by α-stimulation.¹⁶ Cyclic GMP has been reported to have an opposing influence to cyclic AMP in regulating cell function, and according to Haddock *et al.*¹⁷ the relationship of cyclic AMP and cyclic GMP in the lung may influence bronchomotor tone. In patients with asthma the normal balanced relationship of PGE₂ and PGF₂α may be altered, giving rise to variability in the bronchomotor tone.¹² In the light of the biochemical observations on prostaglandins I have studied the effects of PGE₂ and PGF₂α on forced expiratory volume in one second (FEV₁) and specific airways conductance (SGaw) in six patients with extrinsic bronchial asthma and tested the effects of atropine, the α-receptor-blocking drug thymoxamine, and sodium cromoglycate on PGF₂α-induced bronchoconstriction in these patients.

Patients, Materials, and Methods

Six patients aged 15 to 37 years with extrinsic bronchial asthma and reversible airways obstruction were studied. All reacted to prick tests with inhalant allergens and had a blood eosinophilia of over 500 × 10⁶/l. Simple bronchodilators such as salbutamol and isoprenaline were stopped for at least 24 hours before the tests.

FEV₁ was recorded on a Garthur Vitalograph spirometer. Airways resistance (Raw) was measured in a constant-volume body plethysmograph at a flow rate of 0.5 l/s and a panting frequency of 2/s.¹⁸ Conductance (Gaw), the reciprocal of airways resistance, was divided by the thoracic gas volume at which Raw was measured to give SGaw (s⁻¹ kPa⁻¹). The mean of four recordings was calculated to give the SGaw for each step of the experiment.

Drugs.—A sterile aqueous solution of PGF₂α (as a tromethamine salt) 5 g/l was diluted with normal saline to give a concentration of 50 mg/l. Similarly, a stock solution of PGE₂ 1 g/l was diluted with normal saline to give a concentration of 50 mg/l. Other drugs used were atropine sulphate (600 mg/l; Antigen Ltd.), thymoxamine hydrochloride (15 g/l; Warner & Co. Ltd.), and sodium cromoglycate (20 mg in powder form dispensed in a Spincapsule; Fisons Ltd.).

Procedure.—After establishing baseline values for FEV₁ and SGaw each patient inhaled about 0.5 ml of a PGF₂α solution through a Wright nebulizer using compressed air at a flow rate of 8 l/min. FEV₁ and SGaw were measured two minutes after inhalation and thereafter at five-minute intervals for 25 minutes. This test was repeated three times in all patients. Ten minutes before inhaling PGF₂α each patient inhaled 1.2 mg atropine sulphate or 40 mg sodium cromoglycate or 15 mg thymoxamine hydrochloride. Atropine sulphate and thymoxamine were inhaled through a Wright nebulizer, and sodium cromoglycate was inhaled using a Spinhaler. In all six patients the test was repeated after inhaling 0.5 ml of a PGE₂ solution through a Wright nebulizer. Placebo inhalations with normal saline were performed on each experimental day and the test procedure was carried out only when no significant change in FEV₁ and SGaw with normal saline was observed.

Results

Inhalation of PGF₂α produced maximum falls in the mean FEV₁ and SGaw of 27% and 53% respectively (tables I and II), which occurred five minutes after inhalation. These falls were highly significant ($P < 0.001$ and $P < 0.01$). Over the next 25 minutes the values gradually returned towards the baseline (figs. 1 and 2).

Inhalation of atropine sulphate increased the mean FEV₁ and SGaw by 9% and 68% respectively, the bronchodilatation produced being significant ($P < 0.025$) (tables I and II). PGF₂α inhalation 10 minutes later then reduced the mean FEV₁ and SGaw by 10% and 20% respectively. The maximum falls in FEV₁ and SGaw produced by PGF₂α differed significantly from those produced by PGF₂α after pretreatment with atropine sulphate ($P < 0.05$). This suggests that prior inhalation of atropine sulphate partially inhibited the bronchoconstriction induced by PGF₂α.

Sodium cromoglycate inhalation did not produce a significant change in the mean FEV₁ or SGaw, and PGF₂α inhalation 10 minutes later produced maximum falls of 32% and 55% respectively. There was no significant difference between the maximum falls in FEV₁ and SGaw produced by PGF₂α alone and those produced after pretreatment with sodium cromoglycate ($P > 0.10$). This suggests that sodium cromoglycate had no effect on PGF₂α-induced bronchoconstriction (tables I, II; figs. 1, 2).

Inhalation of thymoxamine produced no significant change in the mean FEV₁ or SGaw, and PGF₂α inhalation 10 minutes later produced maximum falls of 26% and 55% respectively. There was no significant difference between the maximum falls in FEV₁ and SGaw produced by PGF₂α alone and those produced by PGF₂α after pretreatment with thymoxamine ($P > 0.10$). This suggests that thymoxamine had no effect on PGF₂α-induced bronchoconstriction

TABLE I—Effect of Atropine, Sodium Cromoglycate (SCG) and Thymoxamine on $\text{PGF}_2\alpha$ -induced Fall in FEV_1 (l) in Six Patients with Extrinsic Bronchial Asthma

Case No.	Age (Years)	Sex	$\text{PGF}_2\alpha$		Atropine + $\text{PGF}_2\alpha$			SCG + $\text{PGF}_2\alpha$			Thymoxamine + $\text{PGF}_2\alpha$		
			Baseline	$\text{PGF}_2\alpha$	Baseline		$\text{PGF}_2\alpha$	Baseline		$\text{PGF}_2\alpha$	Baseline		$\text{PGF}_2\alpha$
					B	A		B	A		B	A	
1	15	M.	3.20	2.30	2.90	3.45	3.22	3.30	3.30	2.00	3.27	3.35	2.75
2	29	M.	3.42	2.00	2.45	3.60	3.25	3.25	3.30	2.35	3.37	3.25	2.60
3	23	F.	3.00	2.55	2.55	3.62	2.25	3.15	3.20	2.30	3.15	3.00	2.00
4	37	M.	2.80	1.62	2.25	2.20	2.00	2.40	2.60	1.55	2.90	3.00	1.70
5	29	M.	2.60	2.00	4.50	5.07	4.96	2.70	2.65	1.87	2.50	2.50	2.20
6	24	M.	4.80	4.00	2.40	2.60	2.00	4.85	4.90	3.60	4.75	4.40	3.15
Mean			3.30	2.41	3.00	3.26	2.95	3.28	3.33	2.28	3.32	3.25	2.40
S.E. of Mean			0.32	0.34	0.35	0.42	0.47	0.35	0.34	0.29	0.31	0.26	0.22
P				<0.001		<0.025	<0.05		N.S.	N.S.		N.S.	N.S.

B = Before inhalation of atropine, thymoxamine, or sodium cromoglycate.

A = After inhalation of atropine, thymoxamine, or sodium cromoglycate.

N.S. = Not significant.

TABLE II—Effect of Atropine, Sodium Cromoglycate (SCG), and Thymoxamine on $\text{PGF}_2\alpha$ -induced Fall in SGaw ($\text{s}^{-1} \text{kPa}^{-1}$) in Six Patients with Extrinsic Bronchial Asthma

Case No.	$\text{PGF}_2\alpha$		Atropine + $\text{PGF}_2\alpha$			SCG + $\text{PGF}_2\alpha$			Thymoxamine + $\text{PGF}_2\alpha$		
	Baseline	$\text{PGF}_2\alpha$	Baseline		$\text{PGF}_2\alpha$	Baseline		$\text{PGF}_2\alpha$	Baseline		$\text{PGF}_2\alpha$
			B	A		B	A		B	A	
1	1.34	1.09	0.72	1.87	1.95	1.37	1.44	0.93	1.94	1.54	0.72
2	0.68	0.23	1.20	1.42	0.72	0.74	0.78	0.38	0.71	0.81	0.40
3	2.12	1.16	1.53	2.00	1.08	2.42	2.46	0.99	2.46	2.31	0.90
4	1.28	0.53	1.22	1.70	1.07	1.21	1.26	0.56	1.30	1.38	0.48
5	0.84	0.33	0.56	0.78	0.77	0.83	0.81	0.36	0.78	0.72	0.56
6	2.82	0.91	1.04	2.79	2.86	2.89	2.85	1.08	1.62	1.66	0.74
Mean	1.51	0.71	1.05	1.76	1.41	1.58	1.60	0.72	1.47	1.40	0.63
S.E. of mean	0.33	0.16	0.15	0.27	0.34	0.36	0.35	0.13	0.28	0.24	0.08
P		<0.01		<0.025	<0.05		N.S.	N.S.		N.S.	N.S.

B = Before inhalation of atropine, thymoxamine, or sodium cromoglycate.

A = After inhalation of atropine, thymoxamine, or sodium cromoglycate.

FIG. 1—Effect of Atropine (ATR), sodium cromoglycate (SCG), and thymoxamine (THY) on $\text{PGF}_2\alpha$ -induced fall in mean FEV_1 in six patients with extrinsic bronchial asthma. FIG. 2—Effect of atropine (ATR), sodium cromoglycate (SCG), and thymoxamine (THY) on $\text{PGF}_2\alpha$ -induced fall in mean SGaw in six patients with extrinsic bronchial asthma.

(tables I, II; figs. 1, 2). In some patients $\text{PGF}_2\alpha$ inhalation produced coughing and mucous secretion. These side effects, however, were minor and the patients were not unduly distressed.

Inhalation of PGE_2 increased the mean FEV_1 and SGaw in the six patients. The maximum increase in mean FEV_1 was 10% and the maximum increase in mean SGaw 81%. The bronchodilatation produced by PGE_2 was highly significant ($P < 0.01$). This effect, however, started to wear off by 20 minutes, and by 30 minutes the mean FEV_1 was 10% and the mean SGaw 49% above the baseline (table III; fig. 3).

TABLE III—Effect of PGE_2 Inhalation on FEV_1 and SGaw in Six Patients with Extrinsic Bronchial Asthma

Case No.	FEV_1 (l)		SGaw ($\text{s}^{-1} \text{kPa}^{-1}$)	
	Baseline	10 min	Baseline	10 min
1	3.30	3.45	0.91	1.55
2	2.65	3.25	0.33	0.96
3	2.30	2.47	1.19	1.88
4	3.15	3.65	1.21	1.55
5	2.55	2.65	0.61	1.42
6	4.90	5.20	1.15	2.43
Mean	3.14	3.45	0.90	1.63
S.E. of Mean	0.38	0.40	0.15	0.20
P		<0.01		<0.01

Discussion

In these patients the inhalation of $\text{PGF}_2\alpha$ in a small dosage produced significant bronchoconstriction. There was a close time relationship between the maximum falls in SGaw and FEV_1 , suggesting that the airways obstruction after $\text{PGF}_2\alpha$ inhalation occurs simultaneously in the central and peripheral airways. On the other hand, PGE_2 produced bronchodilatation in these patients. The bronchodilator effect of PGE_2 , however, is less potent and of shorter duration as compared with the effect observed with isoprenaline. Atropine sulphate given by inhalation produced significant bronchodilatation and also

FIG. 3—Effect of PGE_2 inhalation on mean FEV_1 and SGaw in six patients with extrinsic bronchial asthma.

inhibited $\text{PGF}_2\alpha$ -induced bronchoconstriction in these patients. In contrast, sodium cromoglycate and thymoxamine failed to change the mean FEV_1 and SGaw , and neither of these drugs had any effect on the $\text{PGF}_2\alpha$ -induced bronchoconstriction. $\text{PGF}_2\alpha$ in some patients caused symptoms of upper airway irritation—cough, substernal tightness, and increased mucous secretion—similar to those experienced after methacholine inhalation.

$\text{PGF}_2\alpha$ is released from guinea-pig and rat lungs during anaphylactic reactions⁶ and by various chemical and mechanical stimuli.⁷ The release of $\text{PGF}_2\alpha$ during type I allergic reactions and the observation of increased sensitivity of asthmatic patients to inhaled $\text{PGF}_2\alpha$ led Mathé *et al.*⁵ to postulate that endogenous, locally formed $\text{PGF}_2\alpha$ may play an important part in the pathogenesis of bronchial asthma. This view was supported by a report of about an eightfold increase in plasma levels of 15-keto-13,14-dihydroprostaglandin $\text{F}_{2\alpha}$, the main metabolite of $\text{PGF}_2\alpha$, in asthmatic patients after allergen challenge.⁸ Local $\text{PGF}_2\alpha$ release in the lung has been suggested as the mechanism of exercise-induced asthma,¹⁰ and the demonstration of this release in guinea-pig lungs in response to minor mechanical stimuli supports this view. The results of the present investigation, however, suggest that sodium cromoglycate does not inhibit $\text{PGF}_2\alpha$ -induced bronchoconstriction in asthmatic patients.

This effect of sodium cromoglycate on $\text{PGF}_2\alpha$ -induced bronchoconstriction together with the observations of its inhibitory effect in allergen-provoked²⁰ and exercise-provoked²¹ asthma suggest that the release of prostaglandins locally in the lung may not be the primary factor in the pathogenesis of asthma. In addition, indomethacin administration, which considerably reduces the total body production of $\text{PGF}_2\alpha$, does not completely inhibit allergen-provoked asthma in man or anaphylactic reactions in guinea-pigs.²²

The studies on cyclic nucleotide systems suggest that the effects of PGE_2 are mediated through β -adrenergic receptors, whereas $\text{PGF}_2\alpha$ may activate cholinergic receptors. The partial

inhibition of $\text{PGF}_2\alpha$ -induced bronchoconstriction by atropine as reported here is consistent with this hypothesis. α -Blockade with thymoxamine failed to inhibit the $\text{PGF}_2\alpha$ -induced bronchoconstriction in these patients with asthma, which suggests that $\text{PGF}_2\alpha$ does not stimulate α -receptors in the lung. In man, cholinergic or vagal stimulation causes bronchoconstriction, which is grossly exaggerated in asthma.²³ The evidence so far suggests that $\text{PGF}_2\alpha$ together with other chemical mediators such as histamine, bradykinin, serotonin, and SRS-A are released during the type I allergic reaction. $\text{PGF}_2\alpha$ is a potent bronchoconstrictor to which asthmatic patients are highly sensitive, and it may act by stimulating cholinergic receptors. Airways hyperreactivity to chemical mediators released in the type I allergic reaction is now well recognized in patients with asthma. Though Szentivayni²⁴ suggested that this hyperreactivity in asthma may result from an imbalance between the α - and β -adrenergic receptors in the lung, the evidence to support this theory is not conclusive.²⁵ It would be wrong to consider $\text{PGF}_2\alpha$ as the main factor in the pathogenesis of asthma until we further clarify the cause of airways hyperreactivity, which appears to be the primary defect in asthmatic patients.

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References

- Anggard, E., *Biochemical Pharmacology*, 1965, 14, 1507.
- Karim, S. M. M., Sandler, M., and Williams, E. D., *British Journal of Pharmacology*, 1967, 31, 340.
- Sweatman, W. J. F., and Collier, H. O. J., *Nature*, 1968, 217, 69.
- Hedqvist, P., Holmgren, A., and Mathé, A. A., *Acta Physiologica Scandinavica*, 1971, 82, 17A.
- Mathé, A. A., *et al.*, *British Medical Journal*, 1973, 1, 193.
- Edmonds, J. F., Berry, E., and Wyllie, J. H., *British Journal of Surgery*, 1969, 56, 622.
- Piper, P. J., and Vane, J. R., *Nature*, 1969, 223, 29.
- Green, K., Hedqvist, P., and Svanborg, N., *Lancet*, 1974, 2, 1419.
- Robison, G. A., Butcher, R. W., and Sutherland, E. W., *Annals of the New York Academy of Sciences*, 1967, 139, 703.
- Bourne, H. R., and Melmon, K. L., *Journal of Pharmacology and Experimental Therapeutics*, 1971, 178, 1.
- O'Donnell, E. R., *Journal of Biological Chemistry*, 1974, 249, 3615.
- Middleton, E., jun., and Coffey, R. G., in *Annual Reports on Medicinal Chemistry*, ed. R. V. Heinzelman, vol. 8, p. 274. New York, Academic Press, 1973.
- Goldberg, N. D., *et al.*, in *Proceedings of 5th International Congress on Pharmacology*, p. 146, Basle, Karger, 1973.
- Lewis, A. J., Douglas, J. S., and Bouhuys, A., *Journal of Pharmacy and Pharmacology*, 1973, 25, 1101.
- Eichhorn, J. H., Salzman, E. W., and Silen, W., *Nature*, 1974, 248, 238.
- Ball, J. H., *et al.*, *Journal of Clinical Investigations*, 1972, 51, 2124.
- Haddock, A., *et al.* In press.
- Dubois, A., Bothelho, S. Y., and Comroe, J. H., *Journal of Clinical Investigation*, 1956, 35, 327.
- Paterson, N. A. M., Ahmad, D., and Lefcoe, N. M., *British Journal of Diseases of the Chest*, 1973, 67, 197.
- Pepys, J., *et al.*, *Lancet*, 1968, 2, 134.
- Davies, S. E., *British Medical Journal*, 1968, 3, 164.
- Svanborg, M., Hamberg, M., and Hedqvist, P., *Acta Physiologica Scandinavica*, 1973, 89, Suppl. No. 396, p. 101.
- Tiffeneau, R., *Acta Allergologica*, 1958, 12, Suppl. No. 5, p. 187.
- Szentivayni, A., *Journal of Allergy*, 1968, 42, 203.
- Patel, K. R., Alston, W. C., and Kerr, J. W., *Clinical Allergy*, 1974, 4, 311.