

Effects of prostaglandins E₁, E₂, and F_{2α} on mucin secretion from human bronchi in vitro

B RICH, AC PEATFIELD, IP WILLIAMS, PS RICHARDSON

From the Department of Physiology, St George's Hospital Medical School, London

ABSTRACT The effects of three prostaglandins on the output of ³⁵S labelled mucus glycoproteins (mucins), from explants of human bronchial tissue suspended in Ussing chambers, have been investigated. Prostaglandin F_{2α}, added to the Krebs-Henseleit solution bathing both luminal and submucosal sides of the tissue, significantly increased mucin output at concentrations of 0.1 and 1.0 μg/ml (0.28 and 2.8 μM), 11 preparations being used for each dose. Since prostaglandin F_{2α} has been shown to be released from human airways challenged by antigen, it may be important in the regulation of mucus secretion in these circumstances. Prostaglandins E₁ and E₂ had no significant effects on mucin output even at the highest concentration (1.0 μg/ml), though in half the tissues tested PGE₂ stimulated secretion consistently. These results contrast with the findings in a previous study, which showed an inhibition of mucin output by PGE₂.

Bronchial hypersensitivity to inhaled irritants and allergens (for example, in asthmatics) has two major components, bronchomotor and secretory. Human lung tissue when challenged by antigen in vitro releases various prostaglandins into the medium, particularly prostaglandins E₂ (PGE₂) PGF_{2α}, and PGD₂.¹⁻³ When applied to explants of human bronchi, PGE₂ usually causes them to relax,^{4,5} while PGF_{2α} causes contraction.⁴⁻⁶ Prostaglandins have therefore been assumed to mediate part of the bronchomotor effect of antigen challenge; might they also take part in the bronchosecretory response? Lopez-Vidriero *et al*⁷ showed that inhalation of PGF_{2α} aerosol by normal human subjects increased the amount of sputum expectorated. Close arterial injection of PGF_{2α} into the bronchi of the dog also elicited secretion.⁸ Furthermore, prostaglandins A₂, E₁, E₂, and F_{2α} have been shown to increase the output of radiolabelled mucus glycoproteins (mucins) from an isolated segment of cat trachea in vivo.⁹ In a more recent study, Marom *et al*¹⁰ showed that the output of radiolabelled macromolecules from human airways in vitro was increased by addition of PGF_{2α} and PGD₂ but inhibited by PGE₂. Their evidence suggested that those prostaglandins

which cause bronchoconstriction also increase the rate of secretion; and that PGE₂, which generally relaxes the bronchi, inhibits secretion. In view of the earlier finding that PGE₂ stimulated mucus secretion in the cat,⁹ we decided to compare the effects of three prostaglandins—PGE₁, PGE₂ and PGF_{2α}—on the output of radiolabelled macromolecules from human bronchi in vitro. A preliminary account of some of these results has been published.¹¹

Methods

The method we used has been described in detail.¹² A total of 50 pieces of mainstem, lobar, or segmental bronchus removed from 28 patients with bronchial carcinoma were used for this study. So far as could be ascertained macroscopically none of the tissues was invaded by carcinoma. After removal of superfluous connective tissue the pieces of airway were cut longitudinally, opened out flat, and mounted between the two halves of a modified Ussing chamber (internal diameter 6 or 12 mm). Each half chamber was filled with 15 ml Krebs-Henseleit solution containing 1 mg/ml glucose (K-H), circulated by bubbling with 5% carbon dioxide in oxygen, and maintained at 37°C. At the beginning of each experiment 74 MBq of sodium sulphate labelled with sulphur-35 (Amersham PLC) was added to the fluid bathing the submucosal side of each tissue. Samples were then collected from the luminal side of the tissue at timed intervals and

Address for reprint requests: Dr PS Richardson, Department of Physiology, St George's Hospital Medical School, London SW17 ORE.

Accepted 27 January 1984

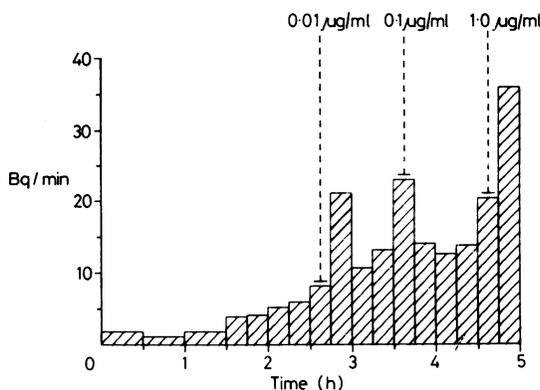
replaced with fresh K-H solution. The samples were dialysed exhaustively against distilled water to remove unbound radiolabel and against 6 mol/l urea to dissolve the mucus. The macromolecularly bound ³⁵S in each sample was then assayed by a liquid scintillation counter. A correction was made for quenching by an external standard channels ratio method, and the output rate of ³⁵S attached to macromolecules was expressed as Becquerels per minute of the collection period.

The prostaglandins used in this study were PGE₁, PGE₂, and PGF_{2α} (Prostins E1, E2, and F2α, Upjohn Ltd). Each was given three times during an experiment, for 15 minute periods at hourly intervals starting 2.5 hours after addition of the radioactivity. The prostaglandins were added to both the submucosal and the luminal sides of the tissue to give final concentrations of 0.01, 0.1, and 1.0 μg/ml (0.028, 0.28, and 2.8 μM). At the end of each stimulation period the fluid was drained from the submucosal half chamber and replaced with K-H, and for the first two concentrations a fresh supply of sodium ³⁵S-sulphate was also added. PGE₁ and PGF_{2α} were supplied as aqueous solutions but PGE₂ was dissolved in ethanol. On 22 occasions tissue was used for control experiments in which either K-H or appropriate concentrations of ethanol (ranging from 10 nl to 1 μl ethanol per ml K-H) were added in place of the drug.

Changes in radiolabelled mucin output were calculated by comparing the output during the period of drug administration with that during the preceding control period. Results are expressed as percentage changes, the median and the range being given. The Mann-Whitney U test was used to test for differences between grouped results and values of p less than 0.05 were considered statistically significant.

Results

As the radioactive precursor of mucins was present



Output of mucins labelled with sulphur-35 plotted against time in one experiment in which prostaglandin F_{2α} was administered at three concentrations, 0.01, 0.1, and 1.0 μg/ml.

on the submucosal side of the tissue throughout each experiment, there was a rising baseline in output of radiolabelled macromolecules collected from the luminal side. Thus after 2.5, 3.5, and 4.5 hours there were small increases (though not significant ones) in median output from the tissues given K-H as control. When dilute ethanol was used as a control the median changes at the same times in the experiment were not significantly different (table).

Prostaglandin E₁ had no effect on the release of radiolabelled mucins even at the highest concentration (table). In six out of 12 tissues PGE₂ appeared to cause an increase in secretory rate at both of the two higher doses; but the variability of the results was such that, although the median increases with PGE₂ at the two higher doses were greater than those in the appropriate ethanol controls, the differences were not significant (table). Prostaglandin F_{2α}, at the two higher concentrations, significantly increased mucin output and the stimulatory action of the drug often lasted into the following control period (fig).

Percentage changes in output of mucins labelled with sulphur-35 elicited by three prostaglandins given in increasing doses at hourly intervals, compared with equivalent volumes of control solutions

	Prostaglandin dose											
	0.01 μg/ml (2.5 h)				0.1 μg/ml (3.5 h)				1.0 μg/ml (4.5 h)			
	n	Median	Range	p	n	Median	Range	p	n	Median	Range	p
Control K-H	12	+18	-28 to +39		13	+1	-44 to +40		13	+5	-70 to +53	
Control ethanol	8	+25	+7 to +111	NS*	8	+13	-26 to +44	NS*	8	-13	-67 to +50	NS*
PGE ₁	12	+9	-32 to +39	NS*	12	+3	-19 to +41	NS*	11	+4	-27 to +21	NS*
PGE ₂	12	+19	-25 to +100	NS†	12	+27	-18 to +86	NS†	10	+34	-25 to +87	NS†
PGF _{2α}	10	+16	-8 to +37	NS*	11	+27	-42 to +75	<0.025*	11	+39	-16 to +466	<0.01*

*Compared with K-H control.

†Compared with ethanol control.

Discussion

The finding that $\text{PGF}_{2\alpha}$ stimulated the release of mucins is in agreement with previous studies in the cat⁹ and man.^{7,10} Our finding that PGE_1 had no significant effect on mucin output confirms the study of Marom *et al*¹⁰ but contrasts with its apparent effectiveness in the cat trachea.⁹ Marom *et al* found that PGE_2 at doses of $0.01 \mu\text{M}$ ($0.0035 \mu\text{g/ml}$) and $1 \mu\text{M}$ ($0.35 \mu\text{g/ml}$) significantly inhibited radiolabelled mucin output from human bronchi but we were unable to confirm this finding: $0.1 \mu\text{g/ml}$ ($0.28 \mu\text{M}$) PGE_2 had no significant effect on mucin output. At none of the concentrations tested was there any evidence that PGE_2 inhibited secretion. Our results indicate that it may even stimulate mucin secretion from some tissues but the variability of results is too great to permit any firm conclusion. Marom *et al*,¹⁰ curiously, discovered no significant inhibition at the highest doses they used (10 and $100 \mu\text{M}$), a fact for which they offered no explanation.

The apparent discrepancy with regard to PGE_2 between our results and those of Marom *et al* may be explained by differences in experimental methods or analysis of results. Firstly, the numbers of experiments in their study were generally smaller than in ours, and their results may have been swayed by idiosyncratic responses. They presented no evidence to show that their data were normally distributed so the parametric statistical tests they used may have been inappropriate. Secondly, their tissues were incubated for at least 32 hours before prostaglandins were tested for the first time and tests continued until tissues had been in culture for up to seven days. During that time the condition of the tissues may have deteriorated. Prostaglandins have been shown to inhibit antigen induced degranulation of mast cells.¹³ During the prolonged incubation mast cells may have released mediators which raised the baseline output of radiolabelled mucins; so addition of PGE_2 , by preventing mediator release, could have reversed this effect. Unfortunately, the baseline data given by Marom *et al*¹⁰ are difficult to interpret. Thirdly, in their study the prostaglandin was added for four hours compared with 15 minutes in the present study. If the onset of inhibition of secretion was slow the shorter stimulus time might have prevented its detection. One further possibility is that PGE_2 has a biphasic action consisting of a brief stimulation followed by a prolonged inhibition. This hypothesis would explain the apparent contradiction between the results discussed here, the brief stimulation causing excitation and the longer stimulation resulting in inhibition.

Collier and Gardiner¹⁴ have proposed that there are two types of prostaglandin receptors on mam-

malian tracheobronchial muscle: the χ receptor, activation of which causes muscle contraction, and the ψ receptor, which has the opposite action. $\text{PGF}_{2\alpha}$ stimulates mainly the χ receptors; PGE_2 stimulates χ receptors but also ψ receptors; while PGE_1 acts almost entirely on ψ receptors. Our results could be explained if the secretory cells of human bronchi have χ receptors augmenting secretion (activated by $\text{PG}_{2\alpha}$ and to a lesser extent by PGE_2), but lack ψ receptors (hence PGE_1 was inactive). The inhibition by PGE_2 described by Marom *et al*¹⁰ could be explained if PGE_2 stimulates ψ receptors and if the number of these receptors on secretory cells increases during the culture period (up to seven days in their experiments). One would then expect PGE_1 to have inhibited mucin output, but it did not.

The production of prostaglandins is intimately linked with that of leukotrienes since both groups of substances are derived from arachidonic acid. Leukotrienes also stimulate mucus secretion,¹⁵⁻¹⁷ so products of both pathways of arachidonic acid metabolism, the cyclo-oxygenase and the lipoxygenase, stimulate mucin output from the bronchi of man, and both probably play a part in the regulation of secretion during antigen challenge.

We would like to thank Mr Norman Wright for supplying the human tissue and the pathology departments of St Helier and St George's Hospitals for their assistance in making them available to us. This work was funded by the Medical Research Council.

References

- Adkinson NF jun, Newball HH, Findlay S, Adams K, Lichtenstein LM. Anaphylactic release of prostaglandins from human lung *in vitro*. *Am Rev Respir Dis* 1980; **121**:911-20.
- Schulman ES, Newball HH, Demers LM, Fitzpatrick FA, Adkinson NF jun. Anaphylactic release of thromboxane A_2 , prostaglandin D_2 and prostacyclin from human lung parenchyma. *Am Rev Respir Dis* 1981; **124**:402-6.
- Holgate ST, Burns GB, Church MK. Anaphylactic release of prostaglandins from dispersed human lung cells. *Clin Sci* 1983; **64**:40P (abstract).
- Sweatman WJF, Collier HOJ. Effects of prostaglandins on human bronchial muscle. *Nature* 1968; **217**:69.
- Gardiner PJ. The effects of some natural prostaglandins on isolated human circular bronchial muscle. *Prostaglandins* 1975; **10**:607-16.
- Mathé AA, Hedqvist P. Effect of prostaglandins $\text{F}_{2\alpha}$ and E_2 on airway conductance in healthy subjects and asthmatic patients. *Am Rev Respir Dis* 1975; **111**:313-20.
- Lopez-Vidriero MT, Das I, Smith AP, Picot R, Reid L. Bronchial secretion from normal human airways after inhalation of prostaglandins $\text{F}_{2\alpha}$, acetylcholine, histamine, and citric acid. *Thorax* 1977; **32**:734-9.

- ⁸ Yamatake Y, Yanaura S. New method for evaluating bronchomotor and bronchosecretory activities: effects of prostaglandins and antigen. *Japan J Pharmacol* 1978;**28**:391-402.
- ⁹ Richardson PS, Phipps RJ, Balfre K, Hall RL. The roles of mediators, irritants and allergens in causing mucin secretion from the trachea. In: Porter R, Rivers J, O'Connor M, eds. *Respiratory tract mucus*. (Ciba Symposium No 54, new series.) Amsterdam: Elsevier, 1978:111-31.
- ¹⁰ Marom Z, Shelhamer JH, Kaliner M. Effects of arachidonic acid, monohydroxyeicosatetraenoic acid and prostaglandins on the release of mucous glycoproteins from human airways *in vitro*. *J Clin Invest* 1981;**67**:1695-702.
- ¹¹ Peatfield AC, Rich B, Richardson PS, Williams IP. The action of prostaglandins on secretion from human airways *in vitro*. *J Physiol* 1983;**340**:30-1P (abstract).
- ¹² Phipps RJ, Williams IP, Richardson PS, Pell J, Pack RJ, Wright N. Sympathomimetic drugs stimulate the output of secretory glycoproteins from human bronchi *in vitro*. *Clin Sci* 1982;**63**:23-8.
- ¹³ Moncada S, Flower RJ, Vane JR. Prostaglandins, Prostacyclin and thromboxane A_2 . In: Goodman LS, Gilman A, eds. *The pharmacological basis of therapeutics*. 6th ed. New York: Macmillan Publishing Co, 1980:677.
- ¹⁴ Collier HOJ, Gardiner PJ. Prostaglandin receptors in the airways. In: Porter R, Whelan J, eds. *Metabolic activities of the lung*. (Ciba Symposium No 78, new series.) Amsterdam: Elsevier, 1980:333-50.
- ¹⁵ Shelhamer JH, Marom Z, Sun F, Bach MK, Kaliner M. The effects of arachinoids and leukotrienes on the release of mucus from human airways. *Chest* 1982;**81**, suppl:36-7S (abstract).
- ¹⁶ Peatfield AC, Piper PJ, Richardson PS. The effect of leukotriene C₄ on mucin release into the cat trachea *in vivo* and *in vitro*. *Br J Pharmacol* 1982;**77**:391-3.
- ¹⁷ Johnson HG, Chinn RA, Morton DR, McNee ML, Miller MD, Nadel JA. Diphenhydramine blocks the leukotriene-C₄ enhanced mucus secretion in canine trachea *in vivo*. *Agents Actions* 1983;**13**:1-4.