

Time-dependent effect of prostaglandin E₂ inhalation on airway responses to bronchoconstrictor agents in normal subjects

EH WALTERS, CAROL BEVAN, RW PARRISH, BH DAVIES, AP SMITH

From the Asthma Research Unit, Sully Hospital, Penarth, S Glam, and Medical Research Council Pneumoconiosis Unit and Thoracic Medical Unit, Llandough Hospital, Cardiff

ABSTRACT Studies were performed to investigate whether hyperresponsiveness of the airways could be induced in normal subjects by inhalation of prostaglandin E₂ (PGE₂). During the initial bronchodilator phase of PGE₂ action the bronchoconstrictor effect of inhaled histamine was significantly antagonised. When bronchoconstrictor challenges were started shortly after the end of the bronchodilator response to PGE₂, however, significant enhancement of the effects of both inhaled histamine and methacholine occurred. It was predominantly sensitivity to these agents that was increased, with a parallel shift of the dose-response curves towards increased bronchoconstriction. Thus PGE₂ may be protective in the acute phase of a bronchoconstrictor challenge, but in a chronic inflammatory condition its net effect may be a balance between this beneficial action and a non-specific potentiation of the activity of bronchoconstrictor agents.

Inflammatory conditions of the airways are associated with increased responsiveness to bronchoconstrictor agents such as histamine and methacholine. This occurs classically in asthma^{1,2} but also in atopic subjects, particularly after exposure to specific allergen,³ and in normal subjects shortly after an upper respiratory tract infection⁴⁻⁶ or inhalation of noxious fumes.⁷ Prostaglandin F_{2α} (PGF_{2α}) and its metabolites, which are associated with such allergic and inflammatory responses, induce hyperresponsiveness in bronchial smooth muscle in vitro to several bronchoconstrictor stimuli.⁸⁻¹⁰ We have recently shown in vivo that normal human subjects will develop hyperresponsiveness to inhaled histamine after inhaling small doses of PGF_{2α} which themselves have no demonstrable effect on airways calibre.¹¹ An increase in responsiveness of bronchial smooth muscle has been shown in vitro after brief contact with PGE₂ metabolites.¹² In this study we have investigated whether previous inhalation of the bronchodilator PGE₂, another product of inflammation, also induces a change in the bronchoconstrictor response to subsequent inhalation of histamine and methacholine.

Methods

In each part of the study groups of eight young adult non-asthmatic, non-atopic volunteers took part after informed consent had been obtained. Drugs were administered via a Wright's nebuliser driven by compressed air at 20 lb/sq in to give a flow of 10 l/min. A standard inhalation procedure consisting of 10 slow tidal breaths from functional residual capacity (FRC) was used throughout. For construction of histamine dose-response curves serial aqueous dilutions of histamine diphosphate from 1 mg/ml to 25 μg/ml in five increments were used, doses being given every 3 minutes. For construction of methacholine dose-response curves serial dilutions of acetyl-β-methylcholine chloride from 0.5 to 500 μg/ml in seven increments were used, doses also being given at 3-minute intervals.

Airway responses, measured using a constant volume body plethysmograph, were expressed as changes in specific airways conductance (sGaw) from a baseline defined as the mean of two sets of readings obtained 5 minutes apart before the first inhalation represented on the dose-response curve. sGaw was determined at FRC at a flow rate of less than 0.5 l/s and each recorded value represented the mean of at least six technically satisfactory meas-

urements. Measurement of individual angles was performed with an electronic resolver, the actual numerical reading being kept out of sight of the operator until the procedure was completed to prevent observer bias. During the dose-response studies sGaw was subsequently measured 1½–3 minutes after each dose.

In the first part of the study the subjects took a standard inhalation of either a 0.3% solution of PGE₂ in saline containing 3% ethanol (produced by saline dilution of a 10 mg/ml solution of Prostin E₂, Upjohn) or a 3% ethanol in saline placebo solution in a random, double-blind fashion after baseline measurements of sGaw. Preliminary studies had indicated that the dose of PGE₂ used (about 60 µg nebulised) causes a briefly sustained bronchodilatation beginning at 5 minutes, being maximal at about 15 minutes and returning to baseline by about 30 minutes. A histamine dose-response curve was therefore constructed starting at 5 minutes after PGE₂ or placebo inhalation, the baseline for this curve being taken as the initial preinhalation value of sGaw.

In the second part of the study each subject was given an inhalation of either the PGE₂ solution or ethanol-saline placebo in a double-blind, random manner. sGaw was subsequently measured at 30 minutes and the measurement was repeated at 5-minute intervals until two consecutive recordings were the same. This value was taken as the baseline for the histamine dose-response curve that was then constructed.

The third part of the study was similar to the second except that dose-response curves were constructed for methacholine instead of histamine. In both the latter studies the dose-response curves were always started by 45 minutes from the inhalation of PGE₂ or placebo.

STATISTICAL ANALYSIS

Any overall shift in the dose-response curves in each part of the study was determined by analysis of the mean differences in sGaw response per dose of agonist between each set of dose-response curves. For each subject's pair of curves (one representing premedication with PGE₂, the other with placebo) the difference in response was calculated for each dose; any minor difference in individual baseline sGaw on the two occasions was taken into account by calculating all changes from the mean baseline value. The sum of these differences in response at each dose was then divided by the number of doses tolerated by that subject on both occasions to give the mean difference per dose between the two curves. The level of significance for any overall change was calculated by performing a Student's *t*

test on these values obtained from all eight subjects in each group. A Student's *t* test for paired values was also performed on the values for baseline sGaw and those obtained after each dose represented on the response curves.

A log cumulative dose-response regression was computed for each individual dose-response study, and from this the dose that caused a 20% decrease from baseline sGaw (D20%) and the slope of the regression line were calculated. For each study a Student's *t* test was performed on these values of D20% (or their logarithmic transformation) and slope.

Results

PGE₂/placebo inhalation followed by histamine challenge at 5 minutes There was no statistical difference in the mean baseline values of sGaw on the two occasions, the mean values being $3.0 \pm (\text{SE}) 0.18$ kPa⁻¹ s⁻¹ on the PGE₂ inhalation day and 2.9 ± 0.12 kPa⁻¹ s⁻¹ on the placebo day. Analysis of the mean differences per dose between the curves indicated a significant shift of the histamine dose-response curve to the right when constructed 5 minutes after PGE₂ inhalation ($p < 0.001$), indicating decreased response to histamine. Significant differences were present after all six histamine doses. The values of D20% were significantly increased after PGE₂ inhalation—from 5.3 ± 0.9 mg/ml to 40.0 ± 7.9 mg/ml ($p < 0.0005$). There was also a small but significant decrease in slope from 0.36 ± 0.03 kPa⁻¹ s⁻¹ to 0.28 ± 0.04 kPa⁻¹ s⁻¹ ($p < 0.05$). These changes are illustrated in figure 1.

PGE₂/placebo inhalation followed by histamine challenge at least 35 minutes later There was no significant difference in baseline sGaw values on the two occasions, the mean values being 3.6 ± 0.32 kPa⁻¹ s⁻¹ after PGE₂ inhalation and 3.5 ± 0.3 kPa⁻¹ s⁻¹ after placebo. There was a significant overall shift in the histamine dose-response curve to the left ($p < 0.001$) after PGE₂ inhalation, indicating increased responsiveness (fig 2), and this was significant for all doses of histamine. There was a significant decrease in D20% after PGE₂ from a mean of 15.3 ± 5.3 mg/ml to 4.6 ± 1.2 mg/ml ($p < 0.001$). There was no significant change in slopes of the log cumulative dose-response curves.

PGE₂/placebo inhalation followed by methacholine challenge at least 35 minutes later Baseline values of sGaw were again similar and not significantly different on the two occasions: 3.3 ± 0.17 kPa⁻¹ s⁻¹ after PGE₂ and 3.25 ± 0.14 kPa⁻¹ s⁻¹ after placebo. There was a significant overall left-

ward shift of the methacholine dose-response curve towards increased responsiveness after initial PGE_2 inhalation ($p < 0.025$) (fig 3), although differences were significant only for individual doses

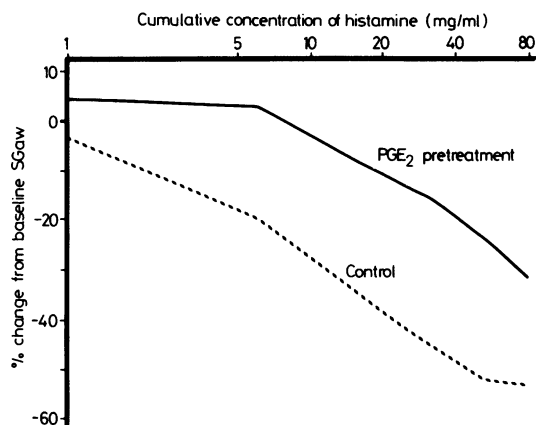


Fig 1 Mean cumulative dose-response curves to inhaled histamine constructed from 5 minutes after inhalation of either PGE_2 (about $60 \mu\text{g}$) or placebo. Airway responses are given as percentage changes from the baseline specific airways conductance (sGaw) and concentrations of histamine are plotted on a logarithmic scale. Preinhalation of PGE_2 caused a parallel shift of the mean curve to the right ($p < 0.001$), indicating decreased sensitivity to histamine. The baseline value of sGaw used was the one obtained before PGE_2 or placebo inhalation; there was no significant difference between the mean values of these on the two occasions.

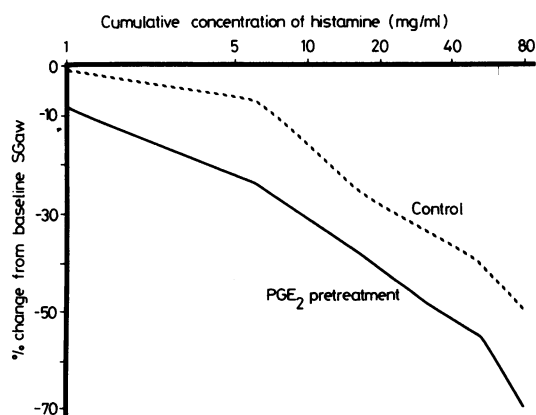


Fig 2 Mean cumulative dose-response curves for inhaled histamine constructed at least 35 minutes after inhalation of either PGE_2 or placebo. Airway responses are given as percentage changes from the baseline specific airways conductance (sGaw) and concentrations of histamine are plotted on a logarithmic scale. Preinhalation of PGE_2 caused a parallel shift of the mean curve to the left ($p < 0.001$), indicating increased sensitivity to histamine.

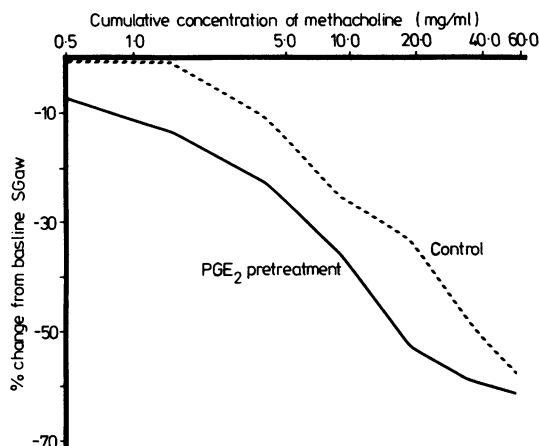


Fig 3 Mean cumulative dose-response curves for inhaled methacholine at least 35 minutes after inhalation of either PGE_2 or placebo. Airway responses are given as percentage changes from the baseline specific airways conductance (sGaw) and concentrations of methacholine are plotted on a logarithmic scale. Preinhalation of PGE_2 caused a parallel shift of the mean curve to the left ($p < 0.025$), indicating increased sensitivity to methacholine.

up to 9 mg/ml. Values for D20% were significantly decreased after PGE_2 —from 6.9 ± 1.8 mg/ml after placebo to 2.2 ± 0.5 mg/ml ($p < 0.015$). There was no significant change in the slope of the log cumulative dose-response regression lines.

Discussion

In this study we have shown a time-dependent effect of PGE_2 inhalation on the subsequent response of the airways to inhaled bronchoconstrictor agonists in normal subjects. PGE_2 is a bronchodilator in normal subjects.¹³ Preliminary studies indicated that the dose of PGE_2 used in the three parts of our study (about $60 \mu\text{g}$ nebulised) causes an increase in sGaw of about 20%, maximal at about 15 minutes with decline to baseline values of sGaw by about 30 minutes. When a dose-response study of inhaled histamine was performed from 5 and 25 minutes after PGE_2 inhalation the bronchoconstrictor effect was appreciably antagonised. When, however, the histamine dose-response study was started 35–45 minutes after PGE_2 inhalation, when values of sGaw were stable, the opposite effect was seen, with an enhancement of the bronchoconstrictor effect and a parallel shift to the left of the dose-response curve. The same phenomenon was observed when we repeated the study in another group of subjects using methacholine for the dose-response curve. It was the sensitivity to these agents, represented by

D20%,¹⁴ that was predominantly affected rather than reactivity, represented by the slope of the dose-response regression line,¹⁴ which was not significantly altered. This is the same qualitative change that occurred with histamine challenge when premedication with inhaled PGF_{2α} was used.¹¹

There are several anatomical sites at which the change resulting in clinically evident hypersensitivity to histamine could be induced by prostaglandins or their metabolites. These include vagal irritant receptors, which could possibly undergo a modification resulting in greater neurological activity after stimulation, and synapses in vagally mediated reflex arcs, which could be altered to increase transmission of impulses. Many studies, however, have shown that prostaglandins can modify autonomic neurotransmission both by changing mediator release at the level of smooth muscle and also by changing the response of the muscle to these mediators under conditions in which there is no direct action on muscle tone by the prostaglandins.¹⁵⁻¹⁷

The fact that the response to a direct cholinergic challenge, which does not activate vagal irritant receptors,^{18,19} was also altered after PGE₂ inhalation would favour a change at the level of smooth muscle. This would also be consistent with the results of *in vitro* studies,⁸⁻¹⁰ which have shown a direct effect of prostaglandins and their metabolites on airway smooth muscle preparations in terms of subsequently enhanced responses to bronchoconstrictor agents. A recent *in vivo* study using atopic human volunteers, in which the ganglionic blocker hexamethonium was used to dissociate the preganglionic and postganglionic limbs of the vagally mediated reflex arc in the lung, emphasised that the hyperresponsiveness of the airways in these individuals was also probably at the level of the smooth muscle fibre.²⁰

The mechanism by which the response of muscle to stimulation is changed is not yet fully understood. A change in the number or affinity (or both) of smooth muscle agonist receptors could be responsible or partial depolarisation of the smooth muscle cell membrane associated with partial influx of calcium ions may occur, thus decreasing the threshold for contraction.²¹ The predominant change in sensitivity rather than reactivity would suggest that the smooth muscle fibre has not been altered more distally.²²

There are already several recognised interacting components in the activity of prostaglandins in inflammatory conditions in the lung. For example, after immunological challenge of sensitised lung tissue several prostaglandin-like substances derived from arachidonic acid are generated,²³ probably as a secondary event after the release of mast cell

mediators.²⁴⁻²⁷ These prostanoids have direct effects on respiratory smooth muscle—mostly bronchoconstrictor, but predominantly bronchodilator in the case of PGE₂.²³ They also have feedback effects on further release of mediators from the mast cell,^{23,28-31} and thromboxane B₂ has been shown to be chemotactic.³² Bronchial tissue contains predominantly PGE₂,^{26,33} and has a slower metabolic turnover of prostaglandins than lung tissue has.³³ When contracted by bronchoconstrictor agents or specific antigen bronchial tissue releases PGE₂,^{8,27,34,35} and this may be important as a homeostatic mechanism in limiting the acute bronchoconstriction.³⁶

We have now identified another possible element in this complex series of prostaglandin interreactions. Thus prostaglandins (or their metabolites) not only have direct agonist activity on bronchial smooth muscle but may, in addition and separately from this direct effect, modify the response of the muscle to other agonists. This would be particularly paradoxical for PGE₂: in the acute phase it may be protective but in a chronic inflammatory condition its net effect may be a balance between its own specific beneficial action and a non-specific potentiation of the activity of bronchoconstrictor agents.

References

- Curry JJ. The action of histamine on the respiratory tract in normal and asthmatic subjects. *J Clin Invest* 1946;**25**:785-91.
- Benson MK. Bronchial hyper-reactivity. *Br J Dis Chest* 1975;**69**:227-39.
- Hargreave FE, Frith PA, Dolovich M, Morse JC, Newhouse MT. Influence of allergen deposition site on airway response. In: Sadoul P, Milic-Emile J, Simonsson BG, Clark TJH, eds. *Small airways in health and disease*. Amsterdam: Excerpta Medica, 1979:143-51.
- Laitinen LA, Elkin RB, Empey DW, Jacobs L, Mills J, Gold WM, Nadel JA. Changes in bronchial reactivity after administration of live attenuated influenza virus. *Am Rev Respir Dis* 1976;**113**:194.
- Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA. Mechanisms of bronchial hyper-reactivity in normal subjects after upper respiratory tract infection. *Am Rev Respir Dis* 1976;**113**:131-9.
- Laitinen LA, Kava T, Penttinen K, Riski H. Bronchial reactivity following uncomplicated influenza A infection in healthy subjects and in asthmatic patients. *Bull Eur Physiopathol Resp* 1978;**14**:195P.
- Golden JA, Nadel JA, Boushey HA. Bronchial hyper-irritability in healthy subjects after exposure to ozone. *Am Rev Respir Dis* 1978;**118**:287-94.
- Orehek J, Douglas JS, Bouhuys A. Contractile responses of the guinea-pig trachea *in vitro*: modification by prostaglandin synthesis inhibiting drugs. *J Pharmacol Exp Ther* 1975;**194**:554-64.
- Kitamura S, Ishihara Y, Yotsumoto H, Sasaki K, Kudoh S. Effect of prostaglandin F_{2α} on the contractile responses of guinea-pig tracheal tissues induced by various bronchoconstrictors. *Jpn J Thoracic Dis* 1978;**16**:315-9.
- Dawson W, Sweatman WJF. Probable role of prostaglandins in asthma. *Int Arch Allergy Appl Immunol* 1975;**49**:213-6.

- ¹¹ Walters EH, Parrish RW, Bevan C, Smith AP. Induction of bronchial hypersensitivity in normal subjects by prostaglandin $F_{2\alpha}$. *Thorax* 1981;**36**:571-4.
- ¹² Boot JR, Dawson W, Harvey J. Comparative biological activity of prostaglandin E_2 and its C_{20} metabolites on smooth muscle preparations. *Adv Prostaglandin Thromboxane Res* 1976;**2**:958.
- ¹³ Mathé AA, Hedqvist P, Holgren A, Svanborg N. Bronchial hyperreactivity to prostaglandin $F_{2\alpha}$ and histamine in patients with asthma. *Br Med J* 1973;**ii**:193-6.
- ¹⁴ Orehek J, Gayraud P, Smith AP, Grimaud C, Charpin J. Airway response to carbachol in normal and asthmatic subjects. *Am Rev Respir Dis* 1977;**115**:937-43.
- ¹⁵ Brody MJ, Kadowitz PJ. Prostaglandins as modulators of the autonomic nervous system. *Fed Proc* 1974;**33**:48-60.
- ¹⁶ Hedqvist P. Basic mechanisms of prostaglandin action on autonomic neurotransmission. *Ann Rev Pharmacol Toxicol* 1977;**17**:259-79.
- ¹⁷ Malik KU. Prostaglandins—modulators of adrenergic nervous system. *Fed Proc* 1978;**37**:203-7.
- ¹⁸ Vidruk EH, Hahn HL, Nadel JA, Sampson SR. Mechanisms by which histamine stimulates rapidly adapting receptors in dog lung. *J Appl Physiol* 1977;**43**:397-402.
- ¹⁹ Hahn HL, Wilson AG, Graf PD, Fischer SP, Nadel JA. Interaction between serotonin and efferent vagus nerves in dog lungs. *J Appl Physiol* 1978;**44**:144-9.
- ²⁰ Holtzman MJ, Sheller JR, Dimeo M, Nadel JA, Boushey HA. Effect of ganglionic blockade on bronchial reactivity in atopic subjects. *Am Rev Respir Dis* 1980;**122**:17-25.
- ²¹ Bouhuys A. Action and interaction of pharmacological agents on airway smooth muscle. In: Stephen NL, ed. *Biochemistry of smooth muscle*. Baltimore: University Park Press, 1977:703-22.
- ²² Boushey HA, Holtzman MJ, Sheller JR, Nadel JA. Bronchial hyper-reactivity. *Am Rev Respir Dis* 1980;**121**:389-413.
- ²³ Boot JR, Dawson W, Cockerill AF, Mallen DNB, Osborne DJ. The pharmacology of prostaglandin-like substances released from guinea-pig lungs during anaphylaxis. *Prostaglandins* 1977;**13**:927-32.
- ²⁴ Piper P, Vane J. The release of prostaglandins from lung and other tissues. *Ann NY Acad Sci* 1971;**180**:363-85.
- ²⁵ Piper PJ, Walker JL. The release of spasmogenic substances from human chopped lung tissue and its inhibition. *Br J Pharmacol* 1973;**47**:291-304.
- ²⁶ Yen SS, Mathé AA, Dugan JJ. Release of prostaglandins from healthy and sensitised guinea-pig lung and trachea by histamine. *Prostaglandins* 1976;**11**:227-39.
- ²⁷ Adkinson NF, 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.
- ²⁸ Kaliner M, Austen KF. Immunologic release of chemical mediators from human tissues. *Ann Rev Pharmacol* 1975;**15**:177-89.
- ²⁹ Kaliner M. The role of cyclic GMP as a modulator of the immunologically induced secretory process. *J Allergy Clin Immunol* 1977;**60**:204-11.
- ³⁰ Engineer DM, Niederhauser V, Piper PJ, Sirois P. Release of mediators of anaphylaxis: inhibition of prostaglandin synthesis and the modification of release of slow reacting substance of anaphylaxis and histamine. *Br J Pharmacol* 1978;**62**:61-6.
- ³¹ Mathé AA, Yen SS, Sohn R, Hedqvist P. Release of prostaglandins and histamine from sensitized and anaphylactic guinea-pig lungs—changes in cyclic AMP levels. *Biochem Pharmacol* 1977;**26**:181-8.
- ³² Boot JR, Dawson W, Kitchen EA. The chemotactic activity of thromboxane B_2 , a possible role in inflammation. *J Physiol* 1976;**257**:47-8P.
- ³³ Karim SMM, Sandler M, Williams ED. Distribution of prostaglandins in human tissues. *Br J Pharmacol Chemother* 1967;**31**:340-4.
- ³⁴ Gryglewski RJ, Dembinska-Kiec A, Grodzinska L, Panczenko B. Differential generation of substances with prostaglandin-like and thromboxane-like activities by guinea-pig trachea and lung strips. In: Bouhuys A, ed. *Lung cells in disease*. Elsevier/North-Holland Biomedical Press, 1976:289-307.
- ³⁵ Steel L, Platshon L, Kaliner M. Prostaglandin generation by human and guinea-pig lung tissue: comparison of parenchymal and airway responses. *J Allergy Clin Immunol* 1979;**64**:287-93.
- ³⁶ Grodzinska L, Panczenko B, Gryglewski RJ. Generation of prostaglandin E-like material by the guinea-pig trachea contracted by histamine. *J Pharm Pharmacol* 1975;**27**:88-91.