

Dual effect of prostaglandin E₂ on normal airways smooth muscle in vivo

EH WALTERS, BH DAVIES

From the Asthma Research Unit, Sully Hospital, Cardiff

ABSTRACT Inhalation of prostaglandin E₂ by normal subjects caused initial bronchoconstriction followed by the predominant effect of bronchodilatation, which was maximal by 15 minutes. The degree of bronchoconstriction seen in the early phase appeared to be related to the initial tone of airways smooth muscle and was exaggerated after bronchodilatation with a large dose of ipratropium bromide. Bronchoconstriction induced by prostaglandin E₂ is a direct effect on muscle and not secondary to a vagal reflex initiated by airways irritant receptors.

Prostaglandin E₂ (PGE₂) is generally thought of as a bronchodilator agent.¹ In vitro, however, it has been shown to have contractile as well as relaxant effects on airways smooth muscle.² The balance between these antagonistic actions of PGE₂ may be influenced by the pre-existing tone of the muscle.³ In asthmatic patients a bronchoconstrictor action of inhaled PGE₂ has also been described,^{4,5} which cannot be blocked by atropine.⁶ There is little information in published reports on the time course of the action of inhaled PGE₂ in normal human airways. In patients with asthma a small dose of PGE₂ caused variable initial bronchoconstriction five minutes after inhalation, bronchodilatation occurring subsequently and being maximal at about 15 minutes.⁷

The aim of this study was to analyse in detail the actions of PGE₂ on airways calibre in normal subjects and to determine the influence on this of airways smooth muscle tone.

Methods

PRELIMINARY STUDY

Six normal men with a mean age of 25 years (range 19-30 years) took part in the preliminary study. As in all these studies, the subjects had fasted and avoided caffeinated drinks on the day of the investigation. None had a personal or family history of atopy or asthma and all were negative on skin prick testing to common allergens. None was a smoker.

After 15 minutes' rest in the laboratory each patient had baseline measurements of specific airways conductance (sGaw) in a constant-volume whole-

body plethysmograph.⁸ At five-minute intervals sets of seven readings of sGaw were made. The last six from each set were taken for calculation of the mean baseline sGaw. Resistance and volume angles were measured with an electronic resolver. The numerical values of the angles were kept out of sight of the operator until the procedure was completed in an attempt to prevent subjective bias in measurement.

A 3% ethanol-in-saline solution was then inhaled for two minutes from a Wright's nebuliser driven by compressed air at 20 lb/in² (138 kPa). Breathing pattern was standardised by means of a respiratory metronome set at 18 breaths/min. sGaw (mean of the last six of seven readings) was again measured at 5 and 10 minutes from the start of inhalation. At 15 minutes the same solution but now containing 1.5 mg of free PGE₂ per 5 ml (Prostin E₂, Upjohn Ltd) was inhaled for 2 minutes. About 240 µg of PGE₂ were delivered. sGaw was subsequently measured at 5, 10, 15, 20, 30, 45, and 60 minutes.

Before each measurement of sGaw each subject's pulse rate over 30 seconds and blood pressure were recorded.

DETAILED STUDY OF INITIAL TIME COURSE

Ten normal subjects took part, seven men and three women with a mean age of 27 years (range 20-34 years). Only one had taken part in the preliminary investigation.

In the first part of this study a baseline measurement of sGaw was followed by one minute of inhalation of the 3% ethanol-in-saline solution. This was followed by measurement of sGaw at 1, 2, 5, 10, and 14 minutes from the end of inhalation. The solution containing 1.5 mg PGE₂ per 5 ml was then inhaled for one minute (delivering about 120 µg PGE₂) and sGaw

Address for reprint requests: Dr EH Walters, Asthma Research Unit, Sully Hospital, Barry, S Glam.

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was again measured at 1, 2, 5, 10, 15, and 20 minutes from the end of the inhalation.

The second part of the study was performed on two other occasions. On each of these a baseline recording of sGaw was made. The subject then inhaled completely 2 ml of a 0.1% solution of ipratropium bromide diluted with 2 ml of normal saline. This was completed over six minutes and about 2 mg of ipratropium bromide was delivered. Seventy-five minutes later another recording of sGaw was made and repeated at 10-minute intervals until the means of two sets of readings were the same. sGaw was invariably stable after one and three quarter hours. At this point the subject inhaled for one minute either the 3% ethanol-in-saline solution as placebo or the same solution containing 1.5 mg PGE_2 per 5 ml. These were given in random order and so far as possible double blind. Both solutions were somewhat irritant but an experienced subject would probably have been able to recognise a difference between them. More importantly, the plethysmograph operator carried out the measurements completely blind.

sGaw was measured at 1, 2, 5, 10, 15, and 20 minutes from the end of inhalation. On both occasions the subject then took 10 breaths of a 20 mg/ml solution of methacholine and sGaw was measured 1½ and 5 minutes later.

Results

PRELIMINARY STUDY

Most subjects experienced some initial discomfort on inhaling both solutions, particularly the one con-

taining PGE_2 . This took the form of retrosternal and pharyngeal irritation and a desire to cough. The subjects soon became tolerant, however, so that the effects were present only for the first few breaths and then rapidly subsided. PGE_2 also seemed to increase bronchial secretions since after inhalation the subjects felt a need to "clear the throat" repeatedly.

The results of this study are summarised in figure 1. The mean baseline sGaw for this group was relatively high at $3.8 \pm \text{SEM } 0.05 \text{ kPa}^{-1}\text{s}^{-1}$. The ethanol-saline solution alone caused no significant change in sGaw. The first change observed after PGE_2 inhalation was a bronchoconstriction at 5 minutes with a mean fall in sGaw of almost 16% from baseline ($0.6 \pm \text{SEM } 0.03 \text{ kPa}^{-1}\text{s}^{-1}$) ($p < 0.02$ by Student's t test for paired values).

Bronchodilatation then rapidly ensued, reaching a maximum at 15 and 20 minutes with a 47% mean increase in sGaw from baseline (an increase of $0.18 \pm \text{SEM } 0.04 \text{ kPa}^{-1}\text{s}^{-1}$) ($p < 0.01$). There was then a gradual decline but even by 1 hour the mean value of sGaw was still significantly greater than baseline and not significantly less than at 15 or 20 minutes.

In this study there was no significant correlation between initial baseline sGaw and the decrease in sGaw at 5 minutes ($r = 0.47$), but the only subject who did not show any bronchoconstriction was also the one with the lowest initial sGaw. There was no consistent or significant change in either pulse rate or blood pressure at any time after PGE_2 inhalation.

DETAILED STUDY OF INITIAL TIME COURSE Without pretreatment with ipratropium bromide

The results of this part of the study are summarised in figure 2 and table 1.

The inhalation of 3% ethanol-in-saline alone caused no significant change in sGaw from a mean baseline of $2.9 \pm \text{SEM } 0.10 \text{ kPa}^{-1}\text{s}^{-1}$. After PGE_2 inhalation there was initially a slight mean bronchodilatation of 7% from baseline, which was not quite significant at the 0.05 level. A significant degree of bronchoconstriction was seen at 5 minutes, with a mean fall of 13% ($0.4 \pm \text{SEM } 0.13 \text{ kPa}^{-1}\text{s}^{-1}$) ($p < 0.02$). Thereafter bronchodilatation once more developed, reaching a maximum at 20 minutes with a mean increase of 46% over baseline sGaw (a change of $1.3 \pm \text{SEM } 0.11 \text{ kPa}^{-1}\text{s}^{-1}$) ($p < 0.0001$).

The absolute fall in sGaw at 5 minutes just significantly correlated with the initial individual baseline sGaw value ($r = 0.63$; $p = 0.05$).

With pretreatment with ipratropium bromide

The results are summarised in figure 3 and table 2.

Inhaled ipratropium bromide 2 mg caused appreciable bronchodilatation in all subjects. The initial

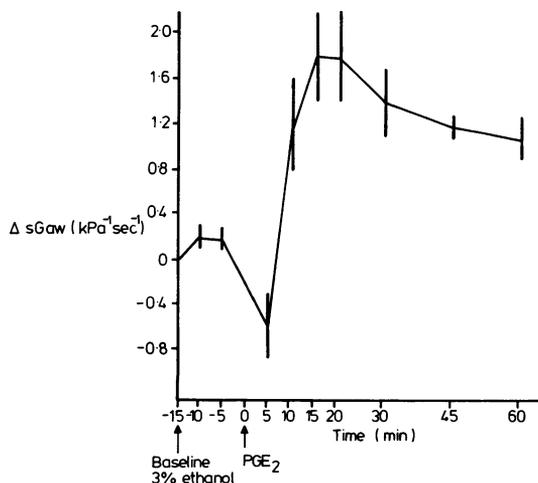


Fig 1 Mean time course of the action of prostaglandin E_2 inhalation on the airways of six normal subjects after previous inhalation of 3% ethanol in saline (SEM shown at each time point).

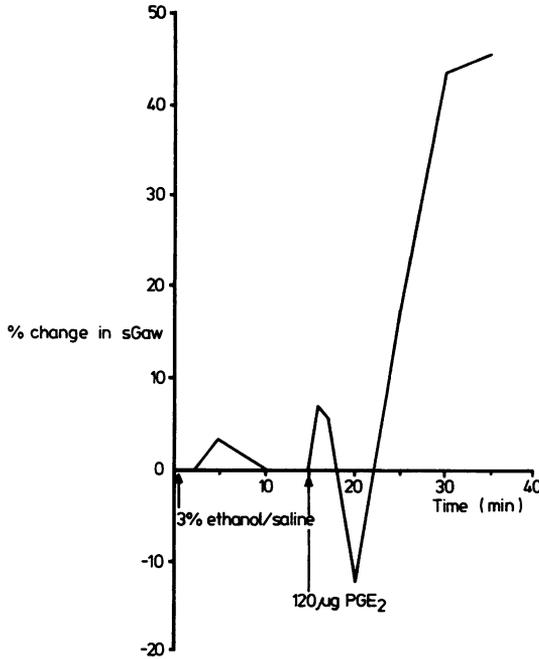


Fig 2 Mean response curve for early phase of PGE₂ action on the airways for 10 normal subjects (SEM at each time point omitted for clarity).

mean baseline on the day that PGE₂ was subsequently inhaled was $3.0 \pm \text{SEM } 0.12 \text{ kPa}^{-1}\text{s}^{-1}$ and this increased by 53% to $4.6 \pm \text{SEM } 0.22 \text{ kPa}^{-1}\text{s}^{-1}$. On the other study day the initial mean baseline was $3.1 \pm \text{SEM } 0.13 \text{ kPa}^{-1}\text{s}^{-1}$ and this increased after inhalation of ipratropium bromide by 45% to $4.5 \pm \text{SEM } 0.19 \text{ kPa}^{-1}\text{s}^{-1}$. Both bronchodilator effects

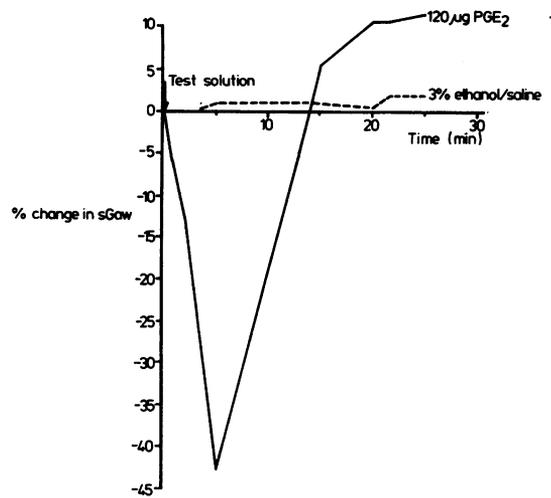


Fig 3 Mean response curves for inhaled PGE₂ and placebo for 10 normal subjects after previous inhalation of 2 mg ipratropium bromide (SEM at each time point omitted for clarity).

were highly significant ($p < 0.0001$). There was no significant difference between the baseline values of sGaw after inhalation of ipratropium bromide on the two study days.

When 3% ethanol in saline was inhaled after ipratropium bromide there was again no significant change in sGaw at any time. The inhalation of PGE₂, however, caused considerable changes. Bronchoconstriction was seen at 1 minute; by 2 minutes the change was significant, with a fall in sGaw of 13% ($0.6 \pm \text{SEM } 0.20 \text{ kPa}^{-1}\text{s}^{-1}$) ($p < 0.05$). Maximum bronchoconstriction occurred at 5 minutes, with a fall

Table 1 Details of the early phase of PGE₂ action on the airways

n = 10	Baseline	Minutes after 3% ethanol in saline placebo					Minutes after PGE ₂					
		1	2	5	10	14	1	2	5	10	15	20
Mean sGaw (kPa ⁻¹ s ⁻¹)	2.9	2.9	2.9	3.0	2.9	2.9	3.1	3.0	2.5	3.4	4.2	4.3
SEM (±)	0.10	0.10	0.10	0.10	0.10	0.11	0.12	0.16	0.10	0.15	0.18	0.37

Table 2 Details of responses to inhaled placebo and PGE₂ after previous inhalation of 2 mg ipratropium bromide

n = 10	Baseline	After ipratropium	Minutes after 3% ethanol in saline placebo or PGE ₂						Minutes after methacholine	
			1	2	5	10	15	20	1½	5
Placebo mean sGaw (kPa ⁻¹ s ⁻¹)	3.1	4.5	4.5	4.5	4.6	4.6	4.6	4.5	4.6	4.6
SEM (±)	0.13	0.19	0.21	0.22	0.20	0.20	0.20	0.20	0.19	0.19
PGE ₂ mean sGaw (kPa ⁻¹ s ⁻¹)	3.0	4.6	4.3	4.0	2.65	3.7	4.9	5.1	5.1	5.2
SEM (±)	0.12	0.22	0.23	0.18	0.19	0.41	0.33	0.31	0.29	0.32

of 43% in sGaw (a change of $1.95 \pm \text{SEM } 0.12 \text{ kPa}^{-1}\text{s}^{-1}$) ($p < 0.0001$). By 10 minutes bronchoconstriction was decreasing and by 15 minutes bronchodilatation had ensued. This became significant at 20 minutes with an increase of 11% above the stable baseline sGaw value after ipratropium (an increase of $0.5 \pm \text{SEM } 0.12 \text{ kPa}^{-1}\text{s}^{-1}$) ($p < 0.05$).

Ten breaths of a 20 mg/ml solution of methacholine caused no bronchoconstriction on either occasion.

Discussion

The preliminary study indicated that the time course of bronchodilatation after PGE₂ inhalation in normal subjects was similar to that reported for patients with asthma.⁷ The lack of cardiovascular changes suggested that bronchodilatation was not due to an autonomic reflex secondary to a fall in blood pressure.

The most unexpected finding was the initial bronchoconstriction, which occurred in five of the six subjects. The solution of PGE₂ had a pH of 4.6 and was, at least initially, irritant to inhale. Possibly therefore the bronchoconstriction was reflexly mediated by vagal irritant receptors, in the same way as after citric acid inhalation,⁹ rather than by a direct effect on airways smooth muscle. To investigate this possibility, detailed studies of the early changes in the airways after PGE₂ inhalation were performed.

These studies confirmed a bronchoconstrictor action of PGE₂ in normal subjects even under basal conditions, although the effect was slight. Bronchoconstriction was observed before bronchodilatation developed and seemed to be related to initial airways calibre. The bronchoconstrictor effect of PGE₂ inhalation became exaggerated when the airways were dilated by removing vagal tone with a very large dose of ipratropium bromide. This confirmed that in vivo as well as in vitro the response of airways smooth muscle to PGE₂ is influenced by its initial level of tone.

The results also showed that PGE₂-induced bronchoconstriction was probably a direct effect of PGE₂ and not related to its irritant effect with secondary constriction via vagal cholinergic reflexes. The time course of PGE₂-related bronchoconstriction was anyway unlike that reported after inhalation of irritant citric acid (pH 1.1), where bronchoconstriction was maximal at 30 seconds with return to baseline by 2–3 minutes.⁹

Ipratropium bromide is a synthetic cholinergic-blocking drug derived from atropine with a bronchodilating action that is maximal between one and two hours after inhalation.^{10 11} The dose of ipratropium bromide used in the present study was about 40 times larger than is required to produce maximal broncho-

dilatation¹² but may be necessary to block vagal reflexes completely.^{13 14} The efficacy of cholinergic blockade was attested to by the absence of bronchoconstriction after inhalation of a large dose of the cholinergic agonist methacholine. In previous studies the dose used caused considerable bronchoconstriction in normal subjects.¹⁵

PGE₂-induced bronchodilatation continued beyond the bronchodilatation achieved by ipratropium bromide alone. Thus PGE₂ caused bronchodilatation even in the absence of cholinergic tone. This action was not, therefore, simply due to an inhibition of basal cholinergic stimulation. Similarly, a previous study showed that the action of PGE₂ was not antagonised by β -adrenergic blockade.¹⁶

We may conclude that PGE₂ acts directly on airways smooth muscle. Both contractile and relaxant effects can be observed even in vivo but the latter is predominant with normal pre-existing levels of smooth muscle tone.

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