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Time-dependent effect of prostaglandin E_2 inhalation on airway responses to bronchoconstrictor agents in normal subjects

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ABSTRACT Studies were performed to investigate whether hyperresponsiveness of the airways could be induced in normal subjects by inhalation of prostaglandin E_2 (PGE_2). During the initial bronchodilator phase of PGE_2 action the bronchoconstrictor effect of inhaled histamine was significantly antagonised. When bronchoconstrictor challenges were started shortly after the end of the bronchodilator response to PGE_2, 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 PGE_2 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 infection[4–6] or inhalation of noxious fumes.[7] Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 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 $PGF_{2\alpha}$ 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 PGE_2 metabolites.[12] In this study we have investigated whether previous inhalation of the bronchodilator PGE_2, another product of inflammation, also induces a change in the bronchoconstrictor response to subsequent inhalation of histamine and methacholine.

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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 mg/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 50 mg/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 PGE_2 in saline containing 3% ethanol (produced by saline dilution of a 10 mg/ml solution of Prostin E_2, 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 PGE_2 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 PGE_2 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 PGE_2 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 PGE_2 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 PGE_2, 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

PGE_2/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 ± (SE) 0·18 $kPa^{-1}\,s^{-1}$ on the PGE_2 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 PGE_2 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 PGE_2 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.

PGE_2/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 PGE_2 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 PGE_2 inhalation, indicating increased responsiveness (fig 2), and this was significant for all doses of histamine. There was a significant decrease in D20% after PGE_2 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.

PGE_2/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 PGE_2 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

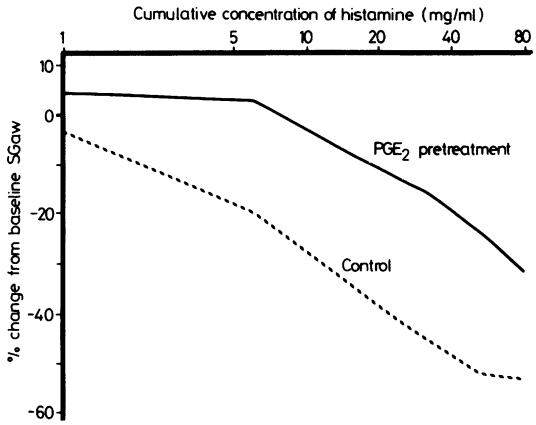

Fig 1 *Mean cumulative dose-response curves to inhaled histamine constructed from 5 minutes after inhalation of either PGE_2 (about 60 μg) or placebo. Airway responses are given as percentage changes from the baseline specific airways conductance (sGaw) and concentrations of histamine are plotted on 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.*

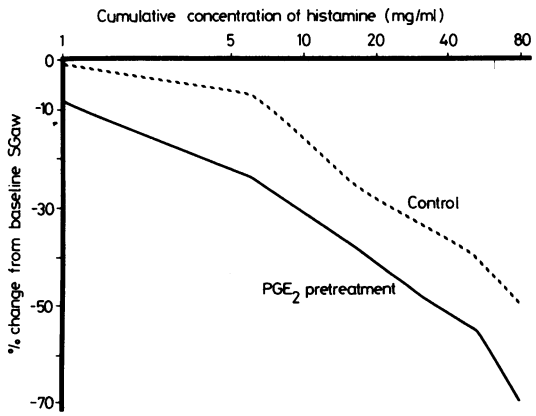

Fig 2 *Mean cumulative dose-response curves for inhaled histamine constructed at least 35 minutes after inhalation of either PGE_2 or placebo. Airway responses are given as percentage changes from the baseline specific airways conductance (sGaw) and concentrations of histamine are plotted on a logarithmic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

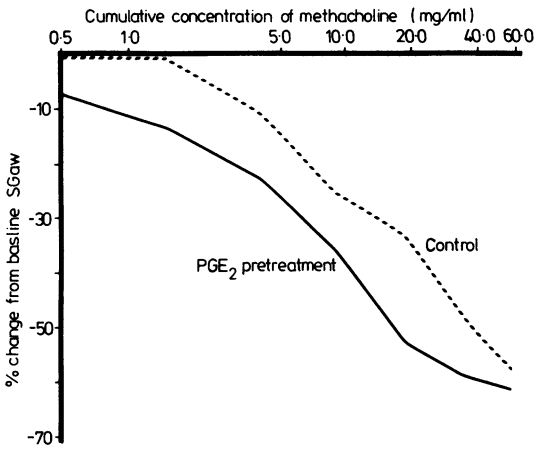

Fig 3 *Mean cumulative dose-response curves for inhaled methacholine at least 35 minutes after inhalation of either PGE_2 or placebo. Airway responses are given as percentage changes from the baseline specific airways conductance (sGaw) and concentrations of methacholine are plotted on a logarithmic 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 μg 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 $PGF_{2\alpha}$ 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 PGE_2 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 interreacting 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 PGE_2.[23] They also have feedback effects on further release of mediators from the mast cell,[23 28-31] and thromboxane B_2 has been shown to be chemotactic.[32] Bronchial tissue contains predominantly PGE_2[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 PGE_2[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 PGE_2: 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.

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