Effect of inhaled prostaglandin D_2 in normal and atopic subjects, and of pretreatment with leukotriene D_4

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Abstract

Background – Prostaglandin (PG) D_2 is a potent bronchoconstrictor mediator and is found, together with leukotriene (LT) D_4, in bronchoalveolar lavage fluid during the early response to allergen challenge in asthmatic subjects. The potency of PGD_2 has not been established in normal and atopic non-asthmatic subjects, nor has the contribution of cholinergic mechanisms to PGD_2 induced bronchoconstriction in normal subjects. Mediators released simultaneously may interact, so the effect of pre-inhalation of LTD_4 on PGD_2 responsiveness was investigated.

Methods – Six normal and six atopic non-asthmatic subjects performed histamine and PGD_2 challenges on separate occasions. Eight normal subjects performed PGD_2 challenges immediately before and 45 minutes after inhalation of 200 µg oxitropium bromide or placebo. Bronchial responsiveness to PGD_2 was established in six normal subjects immediately after pretreatment with saline or non-bronchoconstricting doses of methacholine or LTD_4 (challenge 1), and again at six hours (challenge 2). All studies were performed in a double blind, randomised, crossover fashion.

Results – PGD_2 was 25-fold and 18-fold more potent as a bronchoconstrictor than histamine in atopic non-asthmatic and normal subjects, respectively. Responsiveness ($PC_{35}sGaw$) to histamine and PGD_2 correlated significantly ($r=0.917$, $n=12$, $p<0.001$). Oxitropium bromide in a dose of 200 µg inhibited PGD_2 induced bronchoconstriction by 37.5%, although in two of these subjects no inhibition was seen. Pre-inhalation of LTD_4 and methacholine shifted the dose-response curve of PGD_2 to the left by 4.6-fold and 2.4-fold, respectively.

Conclusions – PGD_2 is a potent bronchoconstrictor in normal subjects, which is partly mediated by cholinergic mechanisms in some subjects. No significant interaction was found between LTD_4 and PGD_2 in six normal subjects.

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Asthma is characterised by reversible airways obstruction, chronic bronchial mucosal inflammation, and bronchial hyperresponsiveness. The airways obstruction is due to smooth muscle contraction, and to airway oedema and mucus hypersecretion caused by inflammation. Inflammatory mediators including prostaglandin D_2 (PGD_2) have been implicated in these processes.[1]

Prostaglandin D_2 is synthesised by alveolar macrophages, platelets and mast cells, and is the most potent bronchoconstricting prostanoid known. The immediate bronchoconstriction induced by allergen is largely due to the release of inflammatory mediators from mast cells,[2] and PGD_2 is the principal cyclooxygenase product of human lung mast cells following immunological or ionophore stimulation.[3 4] Increased levels of PGD_2 are found in bronchoalveolar lavage fluid during the early response to allergen challenge[5] and in the bronchoalveolar lavage fluid of mild asthmatics.[6] Other actions of PGD_2 relevant to asthma, although relatively weak, include augmented capillary permeability,[7 8] increased mucus secretion,[9] and induction of neutrophil infiltration into skin.[8]

Hardy *et al*[10] have shown that PGD_2 is 30 times more potent than histamine as a bronchoconstricting agent in asthmatic subjects, with a longer duration of action. Although several groups have subsequently confirmed the potency of PGD_2 in asthmatic subjects,[11–13] none has given a concentration of inhaled PGD_2 to normal subjects sufficiently high to measure the concentration provoking a 35% fall in specific airways conductance ($PC_{35}sGaw$) and therefore to assess potency. Pretreatment with ipratropium bromide inhibits PGD_2 induced bronchoconstriction by up to 79% in asthmatic subjects,[14] suggesting that bronchoconstriction is due to a combination of direct and cholinergically mediated mechanisms. However, the effect of cholinergic antagonists on PGD_2 induced bronchoconstriction has not been established in normal subjects.

There is increasing evidence that inhalation of one mediator can potentiate the bronchoconstriction or cause increased responsiveness to another, and such interactions within a complex network of mediators may be important in asthma. Prostaglandin $F_{2\alpha}$ increases histamine responsiveness in normal subjects,[15 16] and in asthmatic subjects histamine responsiveness is augmented by prior inhalation of PGD_2.[11] In normal subjects leukotriene (LT) D_4 potentiates $PGF_{2\alpha}$ responsiveness by approximately sevenfold,[17] and a broncho-

constricting dose of LTD_4 increases methacholine responsiveness for up to two weeks.[18] Inhaled LTE_4 increases histamine responsiveness in asthmatics for up to one week,[19] and LTC_4 enhances the immediate bronchoconstriction induced by PGD_2 and histamine in asthmatics,[20] although the effects seen are small. The mechanisms of these interactions have not been fully elucidated.

In this study we wished to investigate the potency of PGD_2 as a bronchoconstrictor in normal subjects and to examine the contribution of cholinergic mechanisms to this. We also aimed to discover whether atopic non-asthmatic subjects showed any differences in response from non-atopic subjects, and to examine the effect of pretreatment with LTD_4 on airway responsiveness to PGD_2. We therefore investigated the bronchoconstrictor potency of PGD_2 in six atopic non-asthmatic and six non-atopic normal subjects, the effect of an anticholinergic agent, oxitropium bromide, on PGD_2 induced bronchoconstriction in eight subjects, and the interaction of LTD_4 with PGD_2 responsiveness in six normal subjects.

Methods

SUBJECTS

All subjects were non-smokers with no history of respiratory disease and were not studied within six weeks of a respiratory tract infection. Atopic non-asthmatic subjects had a positive skin prick test ($>3\,mm$) to two or more of a battery of nine common allergens. Caffeine- or theophylline-containing food and drinks were withheld for 12 hours before each study day. Informed consent was obtained from all subjects, and the studies were approved by King's College Hospital ethics committee.

EFFECTS OF INHALED PGD_2 IN NON-ATOPIC NORMAL AND ATOPIC NON-ASTHMATIC SUBJECTS

Six normal subjects (two men) of mean (SE) age 25.7 (2.4) years and six atopic non-asthmatic subjects (three men) of mean (SE) age 23.8 (1.7) years took part in the study. Bronchial responsiveness to histamine and to PGD_2 was measured on two occasions at least two weeks apart in a double blind, randomised, crossover fashion. The potency ratio for PGD_2 relative to histamine was calculated as the geometric mean of individually determined ratios of $PC_{35}sGaw$ histamine to $PC_{35}sGaw$ PGD_2. The duration of PGD_2 induced bronchoconstriction was defined as the time taken for specific airways conductance (sGaw) to return to within 10% of control values. The repeatability of histamine and PGD_2 challenges was assessed on two occasions at least two weeks apart in two groups of five subjects.

CHOLINERGIC MEDIATION OF PGD_2 INDUCED BRONCHOCONSTRICTION

Eight non-atopic normal subjects (two men) of mean (SE) age 25.3 (2.1) years participated in the study. Subjects attended the laboratory on two occasions separated by at least two

weeks. Bronchial responsiveness to PGD_2 was determined 45 minutes after inhalation of either placebo or $200\,\mu g$ oxitropium bromide (Boehringer Ingelheim Ltd, Bracknell, UK) as two puffs from a metered dose inhaler in a randomised, double blind, crossover fashion. The percentage inhibition of PGD_2 induced bronchoconstriction by oxitropium bromide was calculated at the highest concentration of PGD_2 given on the placebo day, as the difference between the maximum falls in sGaw after oxitropium and placebo (measured as % change from baseline), divided by the maximum fall after placebo (%).

INTERACTION OF INHALED LTD_4 ON PGD_2 RESPONSIVENESS

Six normal subjects (four men) of mean (SE) age 25.2 (1.7) years were recruited. Subjects initially attended the laboratory on two occasions for measurement of bronchial responsiveness to methacholine and LTD_4. They returned for three further visits, separated by at least two weeks, when bronchial responsiveness to PGD_2 was measured after pretreatment with either 0.9% saline or non-bronchoconstricting concentrations of methacholine or LTD_4, and again six hours after pretreatment. Pretreatments were given in a randomised, double blind, crossover fashion. Repeat non-bronchoconstricting doses of LTD_4, methacholine, or saline alone were given to three separate subjects to assess cumulative effects.

LUNG FUNCTION MEASUREMENT

Baseline lung function assessment was performed in all subjects. The forced expiratory volume in one second (FEV_1) was measured (mean of three values) using a dry wedge bellows spirometer (Vitalograph Ltd, Bucks,UK). Specific airways conductance (sGaw) (mean of 16 values) was measured in an automatic pressure-flow plethysmograph (Gould 2800 Autobox, Sensormedics, Salford, UK). Flow at 30% of vital capacity above residual volume ($\dot{V}max_{30}$) (mean of five values) was obtained from partial expiratory flow volume manoeuvres using a rolling seal spirometer (P K Morgan Ltd, Gillingham, UK) attached to a differentiator (P K Morgan) and analysed by computer (Amstrad PC2086) using software developed by Mr K Allen (Medical Physics Department, King's College Hospital).

BRONCHIAL CHALLENGE TESTING

Bronchial responsiveness was measured using a modification of a standard protocol.[21] Responsiveness was defined as the provocation concentration (PC) of histamine, PGD_2, methacholine, or LTD_4 causing a fall of 35% in sGaw ($PC_{35}sGaw$) and a 30% fall in $\dot{V}max_{30}$ ($PC_{30}\dot{V}max_{30}$) from control values. Half log dilutions of histamine acid phosphate from 1 to $100\,mM$ were prepared by King's College Hospital Pharmacy using 0.9% saline as diluent, with 0.5% chlorbutol BP, and stored at

Table 1   Geometric mean provocation concentration (PC) and related potencies of inhaled histamine and PGD₂ for airways conductance (sGaw) and flow at 30% of vital capacity above residual volume (V̇max₃₀)

	$PC_{35}sGaw$ (mM)	Potency†	$PC_{30}\dot{V}max_{30}$ (mM)	Potency†
Histamine				
Non-atopic	40.3	1	49.7	1
Atopic	13.4	1	16.5	1
PGD₂				
Non-atopic	2.26*	17.8	>10**	<5
Atopic	0.53*	25.4	3.43	7.0

* $p<0.05$ compared with histamine.
** $p<0.05$ compared with $PC_{35}sGaw$.
† Potency with respect to histamine.

Figure 1   Correlation between airways responsiveness to PGD₂ and histamine in normal (●) and atopic non-asthmatic (○) subjects.

4°C until required. Half log dilutions of methacholine (0.02–6.32 mM) were prepared and stored in an identical manner. Prostaglandin D₂ was supplied dissolved in ethanol (Cascade Biochem Ltd, Reading, UK), and stored at −80°C. Immediately before a challenge the ethanol was evaporated to dryness under nitrogen and the PGD₂ was resuspended in methanol:saline (1:25) to produce a concentration of 10 mM. Serial log dilutions to 1 μM were made using 0.9% sodium chloride. Leukotriene D₄ (Cascade Biochem Ltd) was supplied dissolved in ethanol. Immediately before each challenge, LTD₄ was diluted with 0.9% sodium chloride to give serial half log dilutions from 0.2 μM to 200 μM. Methanol concentration in the inhaled solutions did not exceed 4% in any study. Subjects initially inhaled diluent by tidal breathing from a Wright nebuliser, driven by air at 8 l/min for two minutes. The calibre of the airways was monitored by measurements of sGaw (mean of eight values) at one and six minutes and V̇max₃₀ (mean of two values) at three and eight minutes after inhalation to provide control measurements. Increasing concentrations of agonist were inhaled in the same manner as diluent and the response expressed as a percentage of the mean control values for sGaw and V̇max₃₀. Log₁₀ dose-response curves were constructed and $PC_{35}sGaw$ and V̇max₃₀ obtained by linear interpolation. If V̇max₃₀ fell by less than 30%, $PC_{30}\dot{V}max_{30}$ was obtained by extrapolation provided that $PC_{30}\dot{V}max_{30}$ was reached within the next doubling dilution, and $PC_{35}sGaw$ was taken as the maximum concentration of PGD₂ if sGaw had not fallen by 35% at this point.

PRETREATMENT WITH SALINE, METHACHOLINE OR LTD₄

From initial dose-response curves to methacholine and LTD₄ the highest concentration of each agonist causing a fall in sGaw of less than 10% from control was established. On subsequent study days the initial PGD₂ challenge was performed, with each concentration of PGD₂ being immediately preceded by a two minute inhalation of either saline or the previously determined non-bronchoconstricting concentration of methacholine or LTD₄.

DATA ANALYSIS

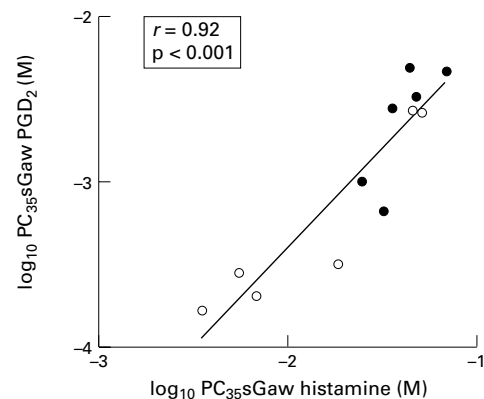Provocation concentrations were log transformed before analysis by Mann-Whitney or Wilcoxon signed rank tests. Correlations were assessed by linear regression analysis. All other data were analysed by appropriate non-parametric tests. All analyses were performed using the Minitab statistical software package.

Results

INHALED PGD₂ IN NORMAL AND ATOPIC NON-ASTHMATIC SUBJECTS

Baseline lung function tests were not significantly different (p>0.05) between study days in either the normal or atopic non-asthmatic subjects. PGD₂ was tolerated well, although initial concentrations caused a significant cough in all subjects. Inhaled PGD₂ caused bronchoconstriction in both normal and atopic non-asthmatic subjects. In normal subjects bronchoconstriction was more clearly apparent as falls in sGaw than in V̇max₃₀, which remained above 70% of control values in all subjects, and thus $PC_{30}\dot{V}max_{30}$ could not be calculated. Compared with normal subjects, atopic non-asthmatic subjects were, on average, 4.29-fold (95% CI 0.78 to 11.59) more responsive to PGD₂ (p=0.031); however, the 3.01-fold (95% CI 1.11 to 16.56) greater histamine responsiveness seen in the atopic non-asthmatic group was not significantly different from that in the normal subjects (p=0.174). Both normal and atopic non-asthmatic subjects were more responsive to PGD₂ than to histamine (table 1), with PGD₂ being 17.8 (95% CI 9.62 to 38.9) and 25.4 (95% CI 15.5 to 41.8) times, respectively, more potent (geometric mean) than histamine when $PC_{35}sGaw$ was measured. There was no significant difference in potency compared with histamine between the two groups (p=0.295).

The correlation between airway responsiveness to inhaled histamine with that to PGD₂ in normal and atopic non-asthmatic subjects is shown in fig 1. The combined correlation coefficient for $PC_{35}sGaw$ histamine with $PC_{35}sGaw$ PGD₂ in normal and atopic non-asthmatic subjects was highly significant (r= 0.917, p<0.001, n=12). Atopic non-asthmatic subjects (n=5) took significantly longer to recover from PGD₂ induced bronchoconstriction
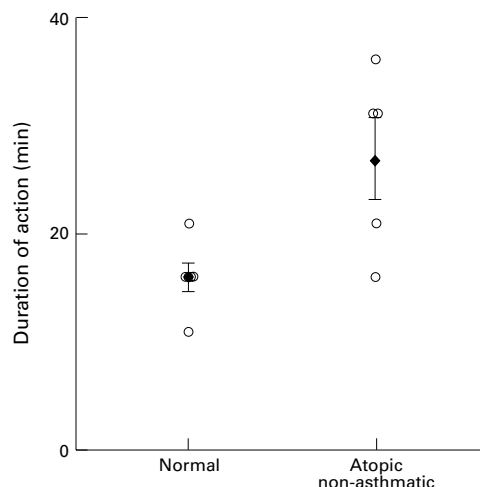
Figure 2 Duration of action of PGD₂ in six normal and five atopic non-asthmatic subjects. ◆ represents mean and bars represent SE.

Table 2 Concentration of PGD₂ provoking a fall in airways conductance of 35% (PC₃₅sGaw) measured 45 minutes after inhalation of placebo or oxitropium bromide and the percentage inhibition of PGD₂ induced bronchoconstriction by oxitropium bromide

Subject no.	$PC_{35}sGaw$ PGD_2 (mM)		% Inhibition
	Placebo	Oxitropium bromide	
1	1.5	6.32	42.5
2	6.32	6.32	45.5
3	3.0	6.32	20.9
4	1.2	6.32	38.1
5	1.4	6.32	97.9
6	6.32	5.4	−15.2
7	5.7	6.32	75.0
8	5.2	4.4	−5.1
Geometric mean	3.88	5.92	
Mean			37.5

(mean (95% CI) 27 (16.8 to 37.2) minutes) than normal subjects (16 (12.7 to 19.3) minutes; n = 6, p = 0.034; fig 2), although there was no difference in the degree of bronchoconstriction (measured as maximum fall in sGaw) induced by PGD₂ between the two groups (p = 0.10). Recovery time of one atopic non-asthmatic subject could not be recorded due to a technical problem with the body plethysmograph. Both histamine and PGD₂ challenges were reproducible in the five subjects tested, with the geometric mean (95% CI) $PC_{35}sGaw$ on the two test days being 38.3 mM (20.0 to 73.6) and 39.5 mM (27.3 to 57.1) for histamine, and 1.8 mM (0.8 to 4.1) and 2.8 mM (0.8 to 9.9) for PGD₂, although the coefficient of repeatability could not be calculated for this number of subjects.

CHOLINERGIC MEDIATION OF PGD₂ INDUCED BRONCHOCONSTRICTION

There was no significant difference (p > 0.05) in baseline lung function tests between study days. Inhaled oxitropium bromide (200 µg) produced significant bronchodilation, causing mean (95% CI) increases from baseline in FEV_1 (3.75% (1.09 to 6.41), p = 0.014), sGaw (82.1% (44.7 to 119.5), p = 0.014), and $\dot{V}max_{30}$ (34.8% (16.2 to 53.4), p = 0.014) at 45 minutes compared with pretreatment values. Due to the relative insensitivity of $\dot{V}max_{30}$ to PGD₂ induced bronchoconstriction in normal subjects, bronchial responsiveness was recorded as $PC_{35}sGaw$ only. Mean (95% CI) inhibition of PGD₂ induced bronchoconstriction by oxitropium bromide in eight subjects was 37.5% (95% CI 5.8 to 69.1), although in two cases no inhibition was seen (table 2).

INTERACTION OF INHALED LTD₄ WITH PGD₂ RESPONSIVENESS

Three subjects inhaled six non-bronchoconstricting concentrations of LTD₄, methacholine, and saline from a Wright's nebuliser

driven by air at 8 l/min. There was no evidence of a cumulative effect when concentrations were given at 12 minute intervals, which was the minimum time between pre-inhalations during the study. Mean sGaw remained within 10% of the baseline value at all time points.

There was no difference in baseline lung function between the three study days or between first and second challenges (p > 0.05), except the FEV_1 at challenge 1 on the methacholine study day was significantly higher than at challenge 1 on the saline study day (p = 0.036). PGD₂ responsiveness was not altered by pretreatment with saline, with no significant difference between challenges 1 and 2 (p = 0.59; fig 3). Pre-inhalation of LTD₄ caused a mean (95% CI) increase in PGD₂ responsiveness of 4.6-fold (0.6 to 36.7) compared with saline, but this was not significant (p = 0.142). After six hours bronchial responsiveness had recovered, being significantly lower at challenge 2 than at challenge 1 (p = 0.036). However, pre-inhalation of methacholine also produced a non-significant 2.4 (0.7 to 8.1)-fold increase in responsiveness at challenge 1 (p = 0.142). At six hours there was no significant difference in PGD₂ responsiveness between the three treatment groups (p > 0.05).
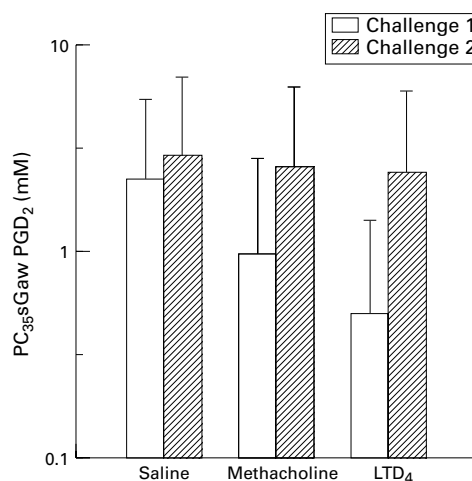

Figure 3 Effect of pretreatment with inhaled saline, methacholine, and LTD₄ on concentration of PGD₂ provoking a 35% fall in airways conductance (PC₃₅sGaw) immediately after pretreatment (challenge 1) and at six hours (challenge 2). Bars represent geometric mean (SE) values.

Discussion

The results show that PGD$_2$ is a potent bronchoconstrictor in atopic non-asthmatic and normal subjects, being 25 times and 18 times more potent than histamine, respectively. There was a positive linear correlation between histamine and PGD$_2$ responsiveness when atopic non-asthmatic and normal subjects were considered together, in agreement with previous studies which have shown that asthmatic subjects who are hyperresponsive to histamine and methacholine are also hyperresponsive to PGD$_2$.[12] Both PGD$_2$ and histamine challenges were reproducible in the five normal subjects tested. PGD$_2$ repeatability is reported as being less than that for histamine or LTD$_4$ in asthmatic subjects,[13] which may reflect the greater lability of bronchial responsiveness in asthmatic subjects. The potency of PGD$_2$ compared with histamine (18 times in normal subjects) is similar on a molar basis to that described for asthmatic subjects (17.8–32.4).[10–12]

Atopic non-asthmatic subjects were 3.01 times and 4.29 times more responsive to histamine and PGD$_2$, respectively, than normal subjects. Although none of the atopic non-asthmatic subjects had any history of respiratory symptoms, other allergic diseases were not excluded. The increased bronchial responsiveness seen in this group agrees with the increased bronchial responsiveness seen in atopic subjects from a random population[22] and in asthmatics who show bronchial hyperreactivity to various stimuli including histamine,[21] prostaglandins,[10] and leukotrienes.[23] Atopic subjects without asthma share, to a lesser extent, some of the features of bronchial mucosal inflammation observed in subjects with asthma, including mast cell degranulation, eosinophil activation, and collagen deposition in the basement membrane;[24] these subclinical features may result in a degree of bronchial responsiveness in atopic non-asthmatic subjects. Despite similar baseline lung function tests and a similar degree of bronchoconstriction following inhalation of PGD$_2$, atopic non-asthmatic subjects took significantly longer for sGaw to return to baseline. This may reflect the increased peripheral bronchoconstriction induced in this group as detected by changes in $\dot{V}max_{30}$.

PGD$_2$ causes bronchoconstriction of human airway smooth muscle both by direct mechanisms, acting via the thromboxane receptor,[25 26] and by cholinergically mediated mechanisms. Oxitropium bromide is an anticholinergic agent which acts as a potent bronchodilator in both normal and asthmatic subjects.[27 28] We showed a significant increase in all lung function parameters measured, with sGaw, FEV$_1$ and $\dot{V}max_{30}$ increasing by 82.1%, 3.75%, and 34.8%, respectively, following inhalation of 200 µg oxitropium. Oxitropium caused a mean 37.5% inhibition of PGD$_2$ induced bronchoconstriction, shifting the dose-response curve to the right in six out of eight subjects. It is not clear why in two of the subjects there was no inhibition, but these subjects did bronchodilate after inhaling oxitropium, suggesting that the drug had reached the airways. In asthmatic subjects a 79% inhibition of PGD$_2$ induced bronchoconstriction by ipratropium bromide has been reported.[14] Thus, in normal subjects cholinergic mediation of PGD$_2$ induced bronchoconstriction may play a relatively smaller part than in asthmatic subjects. It is unlikely that the reduction in bronchoconstriction was due to functional antagonism as it has been shown that bronchodilation produced by cholinergic agents does not affect responsiveness to inhaled bronchoconstrictors;[29] however, the data were standardised as percentage baseline to overcome the effect of bronchodilation.

There are methodological problems when investigating the interaction of inhaled mediators. Different investigators have chosen different techniques, making comparisons between studies difficult. We were unable to demonstrate any significant interaction of inhaled sub-bronchoconstricting doses of LTD$_4$ or methacholine on PGD$_2$ responsiveness in normal subjects. LTD$_4$ produced a 4.6-fold shift in the PGD$_2$ dose-response curve which failed to reach significance (p = 0.142) compared with saline pretreatment, but this is similar in magnitude to the significant sevenfold increase in PGF$_{2\alpha}$ responsiveness seen in asthmatic subjects pretreated with a non-bronchoconstricting dose of LTD$_4$.[17] However, the latter study established dose-response curves to PGF$_{2\alpha}$ over a period of days to avoid the multiphasic dose-response characteristics of this prostaglandin, and there may have been changes in the responsiveness of the asthmatic subjects during this period. Since mediators would be released simultaneously in vivo, we used a method whereby each concentration of PGD$_2$, as well as the diluent, was immediately preceded by inhalation of a non-bronchoconstricting concentration of the pretreatment. It is possible that this method results in increased smooth muscle tone undetectable by the relatively insensitive measurements of airways conductance. However, it has been shown that, while LTC$_4$ and methacholine do increase the baseline contractile state of isolated human airway, they do not, unlike other mediators, increase methacholine sensitivity of the tissue.[30] We controlled for possible physiological interactions by measuring the effect of non-bronchoconstricting concentrations of methacholine on PGD$_2$ responsiveness. While responsiveness was increased in comparison with saline pretreatment, the effect was less than that seen following pretreatment with LTD$_4$, which suggests that LTD$_4$ may have slightly increased PGD$_2$ responsiveness. These changes did not reach significance, possibly because of the relatively few subjects studied, and the possibility of a false negative result cannot be excluded.

The role of prostanoids such as PGD$_2$ in allergic asthma is unclear. Although mast cell derived prostanoids are generated during bronchoconstrictor responses to allergen, non-specific cyclo-oxygenase (COX) inhibitors lack significant therapeutic benefit, and may even exacerbate asthmatic symptoms,[31–33] probably due to concomitant inhibition of the synthesis

of PGE$_2$ which may have a protective role. PGE$_2$ is only a weak bronchodilator but it blocks allergen induced bronchoconstriction in man,[34] probably by reducing the synthesis of bronchoconstrictor mediators including PGD$_2$, PGF$_{2\alpha}$ and the cysteinyl leukotrienes in a number of cell types.[35] Our results reinforce understanding of the potency of inhaled PGD$_2$ in normal subjects and of the hyperresponsiveness shown to PGD$_2$ even in atopic non-asthmatic subjects, and support the development of specific COX-2 inhibitors, which may block the synthesis of bronchoconstrictor prostanoids from inflammatory cells, without eliminating constitutive PGE$_2$ protection.[36]

In conclusion, PGD$_2$ is a potent bronchoconstrictor in normal and atopic non-asthmatic subjects, and challenges are highly repeatable. Responsiveness to PGD$_2$ in these groups relative to histamine was similar to that described in asthmatics by other workers. Intriguingly, atopic non-asthmatic subjects were significantly more responsive to PGD$_2$ than normal subjects. Compared with previous studies in asthmatic subjects, a smaller proportion of PGD$_2$ induced bronchoconstriction in normal subjects is mediated by cholinergic mechanisms. We were unable to demonstrate any interaction between LTD$_4$ and PGD$_2$ in six normal subjects.

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