# Effect of inhaled prostaglandin $E_2$ on bronchial reactivity to sodium metabisulphite and methacholine in patients with asthma

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**Abstract**

**Inhaled frusemide protects against the bronchoconstrictor response to a wide range of stimuli that cause bronchoconstriction by indirect mechanisms. One possible explanation for this protection relates to the known ability of frusemide to enhance synthesis of prostaglandin $E_2$ ($PGE_2$). Studies in vitro suggest that $PGE_2$ might protect against indirectly acting bronchoconstrictor challenges rather than those that act directly on airway smooth muscle, though little is known about the effects of $PGE_2$ in vivo. The effect of inhaled $PGE_2$ on the bronchoconstrictor response to inhaled sodium metabisulphite (a stimulus with an indirect action) and methacholine (which acts directly on airway smooth muscle) was studied in nine patients with asthma. Subjects were studied on four days, inhaling $PGE_2$ (100 μg) or placebo in a double blind fashion followed immediately by a cumulative dose challenge with sodium metabisulphite or methacholine. The response to the constrictor stimuli was measured as the provocative dose causing a 20% fall in $FEV_1$ ($PD_{20}$). There was no significant change in $FEV_1$ after inhaled $PGE_2$ compared with placebo, nor any significant change in the response to methacholine; the geometric mean methacholine $PD_{20}$ was 0·9 μmol after $PGE_2$ and 0·56 μmol after placebo (mean difference 0·7 (95% confidence limits −0·1, 1·5) doubling doses). $PGE_2$, however, protected against sodium metabisulphite, the geometric mean sodium metabisulphite $PD_{20}$ being 11·8 μmol after $PGE_2$ and 1·8 μmol after placebo (mean difference 2·5 (95% CL 1·9, 3·1) doubling doses). $PGE_2$ conferred significantly greater protection against sodium metabisulphite than methacholine (mean difference 1·8 (95% CL 0·8, 2·8) doubling doses). This suggests that $PGE_2$, like frusemide, has an inhibitory effect on pathways relevant to the bronchoconstriction induced by sodium metabisulphite, with little or no effect on those relevant to methacholine.**

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The recent finding that inhaled frusemide protects subjects with asthma against the bronchoconstrictor response to stimuli that act indirectly but not directly on airway smooth muscle has aroused much interest.[1-5] The effects of frusemide in asthma include protection against stimuli that are thought to cause bronchoconstriction primarily through mast cell mediator release (the early response to allergen,[2] adenosine,[3] and osmolar challenges[4]) and through neural pathways (sodium metabisulphite[5]). In addition, frusemide protects against the late response to allergen,[2] which is thought to be related to inflammatory events. Any potential explanation for the effects of frusemide must take into account this wide range of action. One possible explanation relates to the known ability of frusemide to stimulate production of prostaglandin $E_2$ ($PGE_2$).[6 7] This hypothesis assumes that $PGE_2$ is produced in the airway in response to frusemide and that $PGE_2$ will protect against bronchoconstrictor stimuli that act indirectly but not directly on airway smooth muscle.

There is some circumstantial support for the first assumption. Frusemide has been shown to stimulate release of $PGE_2$ from renal tubular epithelium[6] and $PGE_2$ is a cyclo-oxygenase metabolite of human airway epithelium,[8] smooth muscle,[9] alveolar macrophages,[10] and eosinophils.[11] Studies in vitro support our second assumption. Although $PGE_2$ under most circumstances acts as a weak contractile agonist of human airway smooth muscle[9 12 13] and has no effect on histamine induced contraction,[9] its effects on other cells are largely inhibitory. These include inhibition of mast cell mediator release,[14] neurally induced airway smooth muscle contraction,[15] and inflammatory cell activation.[16 17] Thus any protective role $PGE_2$ may serve in the airway in vivo would be likely to be against indirectly acting bronchoconstrictor challenges rather than directly acting airway smooth muscle spasmogens. Studies in vivo are limited, however. There is indirect evidence that endogenous inhibitory prostaglandin production is responsible for the refractory period commonly observed after exercise[18] and osmolar challenge,[19] and inhaled $PGE_2$ has been shown to inhibit the bronchoconstrictor response to exercise, allergen, and ultrasonically distilled water in a few subjects with asthma.[20 21] To test our hypothesis that $PGE_2$ protects against constrictor stimuli that act indirectly but not those acting directly we have compared the ability of inhaled $PGE_2$ to protect against methacholine, which acts directly on airway smooth muscle, and sodium

metabisulphite, which is thought to cause bronchoconstriction indirectly via an effect on neural pathways.

## Methods

### SUBJECTS

We studied nine men, aged 18–52 years, with mild asthma requiring only inhaled drugs. Six were taking regular inhaled corticosteroids (beclomethasone 200–1500 μg daily) and all used an inhaled $beta_2$ agonist as required (table 1). Eight subjects were atopic and one was a current smoker; all had a forced expiratory volume in one second ($FEV_1$) above 70% predicted (mean 91%). Bronchodilator medication was withheld for at least six hours before each visit. Subjects gave signed consent to participation in the study, which was approved by the City Hospital ethics committee.

### MEASUREMENTS

$FEV_1$ was measured on a dry bellows spirometer (Vitalograph, Buckingham) and the higher of two successive readings within 100 ml was recorded. Sodium metabisulphite challenge was performed by a method based on that described by Nichol *et al.*[22] Serial dilutions, over the range 0·6–160 mg/ml, were made up in normal saline each day. Aerosols were delivered from a nebuliser attached to a breath actuated dosimeter (MEFAR, Brescia, Italy); the nebuliser was set to nebulise for one second with a pause of six seconds at a pressure of 22 $lb/in^2$ (152 kPa) and delivered 6·5 μl/puff. Subjects inhaled doubling doses (0·03–64 μmol) of sodium metabisulphite by inspiring rapidly from functional residual capacity to total lung capacity, holding their breath for three seconds and exhaling slowly for three seconds. $FEV_1$ was measured two minutes after each inhalation. The challenge was discontinued when the $FEV_1$ had fallen by 20% or more, or when subjects had inhaled the highest cumulative dose of sodium metabisulphite (128 μmol). After completion of the challenge subjects were asked to score the irritancy of the sodium metabisulphite challenge on a nine point scale from 1 (not irritant) to 9 (severely irritant).

Methacholine challenge was performed by a similar method. Serial dilutions of methacholine (Sigma, Poole) were made up in normal saline over the range 0·39–25 mg/ml. Doubling doses (0·02–5·12 μmol) were administered via the breath actuated dosimeter every two minutes as in the metabisulphite challenge, except that the output was 10 μl per puff. $FEV_1$ was measured two minutes after each inhalation. In the main study the starting dose of methacholine and sodium metabisulphite was four doubling doses below the provocative dose causing a 20% fall in $FEV_1$ ($PD_{20}$) at an initial assessment visit.

### PROTOCOL

Subjects attended on four separate occasions at the same time of day. $PGE_2$ 100 μg (a dose that causes near maximum bronchodilatation in normal subjects[23]) was made up from a concentrated stock solution of Prostin $E_2$ (UpJohn) diluted to 2 mg/ml in ethanol and further diluted in 4·95 ml normal saline on the day of the challenge. The placebo was 0·05 ml ethanol in 4·95 ml normal saline. Drugs were administered in random order and double blind via a Medix ultrasonic nebuliser (output 1 ml/minute), the subjects inhaling through a face mask at tidal volume until the nebuliser was dry. $FEV_1$ was measured before and immediately after inhalation. Because cough may occur during inhalation of $PGE_2$, drugs were administered by a second investigator in a room separate from the challenge laboratory; this investigator also asked the subjects after inhalation about side effects. The sodium metabisulphite or methacholine challenges proceeded immediately after inhalation of $PGE_2$ or placebo, with the $FEV_1$ value obtained after $PGE_2$ or placebo inhalation used as the baseline for the challenge study.

### ANALYSIS

$FEV_1$ before and after inhalation of $PGE_2$ or placebo and change in $FEV_1$ after $PGE_2$ and placebo were compared within subjects by the paired *t* test.

Sodium metabisulphite and methacholine $PD_{20}$ values were calculated by linear interpolation of the log dose-response curve. When the fall in $FEV_1$ was less than 20% with the maximum cumulative dose of sodium metabisulphite (128 μmol) this value was assigned as the $PD_{20}$. The $PD_{20}$ values were log transformed for analysis and expressed as geometric

*Table 1 Details of the subjects*

| | | | | *Metabisulphite $PD_{20}$ (μmol)* | | *Methacholine $PD_{20}$ (μmol)* | |
|---|---|---|---|---|---|---|---|
| *Subject No* | *Age (y)* | *$FEV_1$ (% pred)* | *Treatment* | *$PGE_2$* | *Placebo* | *$PGE_2$* | *Placebo* |
| 1 | 52 | 93 | S | >128·00 | 16·00 | 1·28 | 0·76 |
| 2 | 35 | 90 | T, B | 46·85 | 6·96 | 1·99 | 1·28 |
| 3 | 37 | 98 | S | 5·96 | 0·75 | 3·71 | 1·69 |
| 4 | 18 | 89 | S, B | 8·39 | 1·91 | 0·91 | 0·83 |
| 5 | 34 | 83 | S, B | 2·32 | 0·72 | 0·32 | 0·96 |
| 6 | 23 | 110 | S, B | 4·36 | 0·97 | 0·81 | 0·23 |
| 7 | 50 | 70 | S, B | 4·00 | 1·13 | 0·32 | 0·10 |
| 8 | 30 | 83 | S, B | 2·95 | 0·79 | 0·55 | 0·22 |
| 9 | 29 | 105 | S | >128·00 | 7·76 | 0·98 | 0·86 |
| Mean | 34 | 91 | | | | | |
| Geometric mean | | | | 11·84 | 1·78 | 0·90 | 0·56 |

S—salbutamol; T—terbutaline; B—beclomethasone.

*Table 2 Mean forced expiratory volume in one second ($FEV_1$) for sodium metabisulphite and methacholine challenges before and after inhalation of prostaglandin $E_2$ ($PGE_2$) or placebo: mean (95% confidence limits) within subject differences*

| Challenge | Inhalation | Mean $FEV_1$ Before | Mean $FEV_1$ After | Mean difference (l) (95% CL) | Mean difference placebo v $PGE_2$ (l) (95% CL) |
|---|---|---|---|---|---|
| Sodium metabisulphite | $PGE_2$ | 3·25 | 3·34 | 0·09 (−0·05, 0·24) | 0·13 (−0·08, 0·34) p = 0·18 |
| | Placebo | 3·21 | 3·17 | −0·04 (−0·11, 0·04) | |
| Methacholine | $PGE_2$ | 3·14 | 3·27 | 0·13 (−0·02, 0·28) | 0·14 (−0·01, 0·29) p = 0·06 |
| | Placebo | 3·19 | 3·18 | −0·01 (−0·08, 0·06) | |

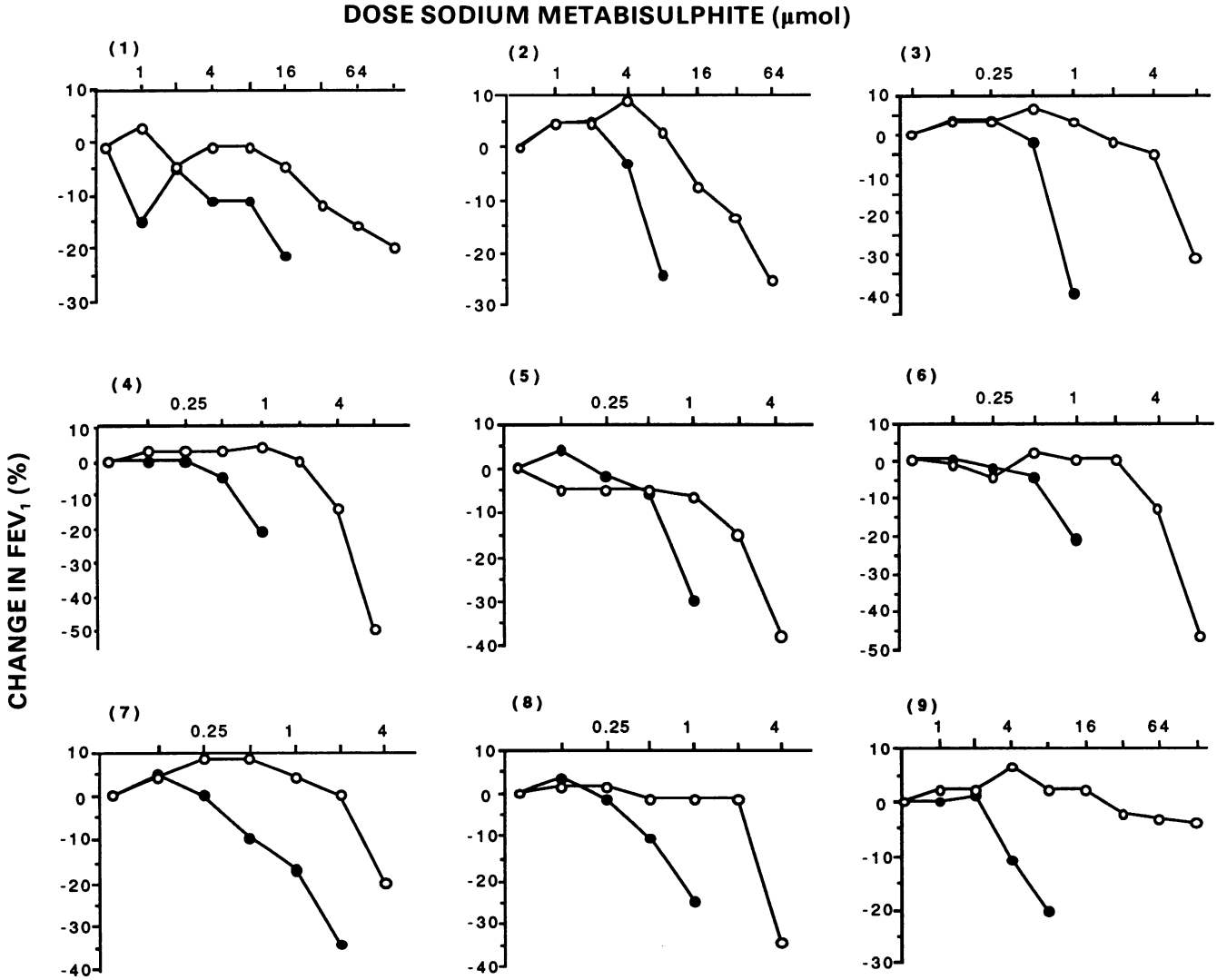
mean values; the differences in $PD_{20}$ between $PGE_2$ and placebo for sodium metabisulphite and methacholine were expressed as doubling doses with 95% confidence limits (CL). $PD_{20}$, difference in $PD_{20}$, and irritancy scores for the sodium metabisulphite challenge were compared within subjects by the paired *t* test.

## Results

Inhalation of $PGE_2$ caused initial transient cough and retrosternal soreness in most subjects, though symptoms rapidly subsided as the inhalation proceeded. $PGE_2$ was otherwise well tolerated.

There was no significant difference in mean $FEV_1$ before and after inhaled $PGE_2$ and placebo on either the sodium metabisulphite or methacholine challenge days, nor did the mean change in $FEV_1$ after inhaled $PGE_2$ and placebo differ significantly on the two days (table 2).

The dose-response curve for sodium meta-




*Individual cumulative dose-response curves for inhaled sodium metabisulphite after inhalation of placebo (closed circles) and prostaglandin $E_2$ ($PGE_2$; open circles).*

bisulphite induced bronchoconstriction was displaced to the right in all subjects after inhaled $PGE_2$ by comparison with placebo. The difference in $PD_{20}$ ranged from 1·7 to 4 doubling doses (figure, table 1). The geometric mean $PD_{20}$ sodium metabisulphite was 1·8 μmol after placebo inhalation and 11·8 μmol after inhaled $PGE_2$ (mean difference 2·5 (95% CL 1·9, 3·1) doubling doses; $p < 0·001$). The irritancy score after completion of the metabisulphite challenge was similar after inhaled $PGE_2$ (mean 4·9) and placebo (mean 4·6).

The dose-response curve for methacholine was displaced to the right in eight of the nine subjects after inhaled $PGE_2$ (from 0·14 to 1·8 doubling doses); the mean change was not, however, significant. The geometric mean methacholine $PD_{20}$ was 0·56 μmol after placebo and 0·9 μmol after $PGE_2$, a mean difference of 0·7 (95% CL −0·1, 1·5) doubling doses; $p = 0·08$)—see table 1.

Inhaled $PGE_2$ conferred significantly greater protection against sodium metabisulphite than against methacholine challenge. The mean difference in $PD_{20}$ after $PGE_2$ and placebo was 1·8 (95% CL 0·8, 2·8) doubling doses greater for sodium metabisulphite than for methacholine ($p < 0·005$).

## Discussion

$PGE_2$ provided considerable protection against sodium metabisulphite induced bronchoconstriction in these subjects with mild asthma and this protection was significantly greater than that afforded against methacholine challenge. There was a trend towards bronchodilatation and protection against methacholine after inhalation of $PGE_2$ in our subjects but neither change was significant. Possibly $PGE_2$ has a small effect against methacholine that would require more subjects to confirm it, but any effect was very much less than the effect seen against sodium metabisulphite.

$PGE_2$ is often regarded as a bronchodilator and has caused bronchodilatation consistently when inhaled by normal subjects.[23–26] The bronchodilatation may be preceded by transient bronchoconstriction, which has been attributed to a direct contractile effect of $PGE_2$ on airway smooth muscle,[24] as is seen in vitro.[9 12] The effect of inhaled $PGE_2$ in asthmatic subjects has been more variable. Smith *et al* [25] showed bronchodilatation after 55 μg inhaled $PGE_2$ in the four subjects they studied. Mathe and Hedqvist,[26] however, showed no change in specific airway conductance over 15 minutes in eight subjects given inhaled $PGE_2$ in doses (6·25–100 μg) that caused dose related bronchodilatation in normal subjects. A delayed bronchodilator response to $PGE_2$ could have occurred in our subjects had they not had a constrictor challenge. Sodium metabisulphite and methacholine were, however, given at the same time after $PGE_2$ inhalation and both challenges were of similar duration, so any effect of bronchodilatation as such would have affected the two challenges in a similar way.

The protection by $PGE_2$ against sodium metabisulphite is similar to that seen with salbutamol 200 μg[27] and considerably more than the protection recorded by others after inhaled sodium cromoglycate,[28] frusemide,[5] or antimuscarinic agents.[22] The greater protection against sodium metabisulphite (a stimulus that acts indirectly) than against methacholine (a stimulus that acts directly on airway smooth muscle) resembles the pattern of protection seen with sodium cromoglycate and frusemide. It appears to differ from that seen with $beta_2$ receptor agonists, where conventional doses have displaced the dose-response curves for methacholine and sodium metabisulphite to a similar degree, albeit in different studies.[27 29] The difference in response to the two stimuli supports the suggestion that the effects of $PGE_2$ are indirect and not due to airway smooth muscle relaxation.

An inhibitory effect of $PGE_2$ on neural pathways is the most likely explanation for our findings. Sodium metabisulphite solutions appear to cause bronchoconstriction through release of sulphur dioxide, because this is released from sodium metabisulphite solutions in a dose dependent manner[30] and the response to the two agents is similar in time course and in the way it can be modified by drugs.[22 30] Bronchoconstriction is thought to be neurally mediated,[22 30 31] though inhaled antimuscarinic agents have only a weak protective effect, suggesting a role for non-adrenergic, non-cholinergic excitatory nerve pathways in addition to cholinergic pathways.[22] The protection afforded by $PGE_2$ could be due to inhibition of the afferent or efferent limb of these neural pathways.

Irritancy scores after sodium metabisulphite inhalation were similar with inhaled $PGE_2$ and placebo, despite the larger inhaled dose of sodium metabisulphite on the $PGE_2$ day. This suggests that $PGE_2$ may have an inhibitory effect on sensory nerve endings. This is perhaps surprising given that $PGE_2$ causes cough when inhaled and potentiates cough induced by capsaicin.[32] The cough response to inhaled $PGE_2$ becomes refractory with repeat doses,[32] so cross refractoriness might occur between $PGE_2$ and sodium metabisulphite. An inhibitory effect of $PGE_2$ on efferent neural activity is suggested by studies showing that low concentrations of $PGE_2$ inhibit cholinergic contractions of airway smooth muscle stimulated by an electric field in vitro.[15 33] A similar effect in vivo in man (which might also affect non-adrenergic, non-cholinergic pathways) would provide an attractive explanation for the protection afforded by $PGE_2$ against sodium metabisulphite induced bronchoconstriction. It would also provide a plausible explanation for the bronchodilatation observed after inhalation of $PGE_2$ in normal subjects and in some subjects with asthma.[23–26] The more variable effects of inhaled $PGE_2$ on airway tone in subjects with asthma may be due to an exaggerated direct contractile effect of $PGE_2$ on airway smooth muscle.

$PGE_2$, like prostacyclin and $PGE_1$, is a vasodilator. Oral misoprostol ($PGE_1$)[34] and inhaled prostacyclin[35] have been shown to provide a small degree of protection (less than one doubling dose change in $PD_{20}$) against

bronchoconstriction induced by methacholine without altering airway tone. The vasodilatation produced by all three prostaglandins would be expected to increase bronchial blood flow and may increase clearance of inhaled spasmogens.[35] This could explain the small effect of the prostaglandins on methacholine induced bronchoconstriction but would not explain the difference in protection against the two stimuli seen in our study.

Thus our finding that inhaled $PGE_2$ confers considerably greater protection against the bronchoconstrictor response to inhaled sodium metabisulphite than against the airway smooth muscle spasmogen methacholine is consistent with $PGE_2$ having an indirect effect against neural pathways relevant to sodium metabisulphite induced bronchoconstriction. These data, together with those from earlier studies showing that inhaled $PGE_2$ protects subjects with asthma against the bronchoconstrictor response to exercise, ultrasonically nebulised distilled water, and allergen,[20,21] suggest that $PGE_2$ is capable of modulating asthma induced by a wide range of stimuli that act indirectly. The data also support our hypothesis that the effects of frusemide in asthma are due to stimulation of endogenous production of $PGE_2$. The role of endogenously produced $PGE_2$ in modulating the response to indirect challenges and the importance of possible defects in this mechanism in asthma deserve further study.

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