

# Interactions between response to inhaled prostaglandin E<sub>2</sub> and chronic beta-adrenergic agonist treatment

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**ABSTRACT** Cumulative inhalation dose-response curves for the response to prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) have been constructed in normal subjects and patients with mild, stable asthma. In normal subjects cumulative inhalation dose-response curves were also constructed for salbutamol. In normal subjects dose-related bronchodilatation occurred in response to both PGE<sub>2</sub> and salbutamol, although both the within-subject and the between-subject variation was significantly greater with salbutamol. Most asthmatic subjects gave a biphasic response to PGE<sub>2</sub>, on at least one occasion, PGE<sub>2</sub> being a bronchoconstrictor above a certain level of specific airways conductance (sGaw) and a bronchodilator below. Chronic treatment with inhaled salbutamol (400 µg four times a day) had no effect on the normal subjects' response to salbutamol but there was a significant shift of the PGE<sub>2</sub> dose-response curve to the left, indicating increased bronchodilatation ( $p < 0.02$ ). Stabilisation of the asthmatics' dose-response curve in the direction of bronchodilatation also occurred and was more pronounced ( $p < 0.005$ ). In the normal subjects PGE<sub>2</sub> may be concerned in the control of airway smooth-muscle tone and in limiting bronchoconstriction induced by mediators such as histamine, and chronic salbutamol treatment may be important in enhancing these effects of PGE<sub>2</sub>. 80 mg oral propranolol given one and a half hours before had no effect on PGE<sub>2</sub>-induced bronchodilatation; but the question whether chronic treatment with beta-blockers has any effect needs investigation.

The prostaglandins are 20-carbon aliphatic monocarboxylic acids with a five-membered ring and one or more unsaturated double bonds. Human lungs are a particularly rich source, bronchial tissue containing predominantly prostaglandin E (PGE).<sup>1</sup>

Human bronchial muscle strips *in vitro* are contracted by PGF<sub>2α</sub> and relaxed by PGE<sub>1</sub> and PGE<sub>2</sub>; these effects are not blocked by α- or β-adrenergic antagonists, serotonin antagonists, or cholinergic blockade.<sup>3-5</sup> Furthermore, PGE<sub>2</sub> is released from human bronchial muscle when contracted by histamine, methacholine, or specific antigen after sensitisation<sup>6,7</sup> and it may be important in local control of airway smooth muscle.

*In vivo* studies in man have been largely confined to investigating the effects on the airways of single inhaled doses of prostaglandins and have in general confirmed *in vitro* findings. Inhaled PGF<sub>2α</sub> is a

bronchoconstrictor while PGE<sub>1</sub> and PGE<sub>2</sub> have bronchodilator activity, at least in normal subjects, although the effect may be more variable in asthmatic patients.<sup>8-11</sup> In the present study we have constructed cumulative dose-response curves for the response to inhaled PGE<sub>2</sub> to investigate more fully the effect of this prostaglandin in both normal human volunteers and asthmatic patients.

In normal subjects regular treatment with an inhaled β-adrenergic agonist has been reported to result in the development of hyporesponsiveness (or "resistance") of airway smooth muscle to the effect of such drugs.<sup>12</sup> The plasma cyclic AMP response to inhaled β-adrenergic agents was found, however, to be unaffected by chronic treatment,<sup>13</sup> suggesting that the site of change was intracellular and distal to cyclic AMP generation. The bronchodilator effect of PGE<sub>2</sub> is thought also to be mediated by adenylate cyclase activation and cyclic AMP generation, although via an independent receptor. To elucidate

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the site of any change in airway responses to bronchodilator agonists caused by regular  $\beta$ -adrenergic stimulation we have investigated whether the response to PGE<sub>2</sub> is altered by regular inhalation of salbutamol. If PGE<sub>2</sub> is indeed important in the normal homeostasis of the airways such changes would have important therapeutic implications.

## Methods

### STUDIES ON NORMAL VOLUNTEERS

For each section of the study six normal non-atopic male volunteers, age range 19–31 years, were investigated after informed consent had been obtained. All subjects were asked to fast and avoid caffeinated drinks on the days of the studies, which were performed at the same time of day in each subject.

For construction of PGE<sub>2</sub> dose-response curves a 10 mg/ml solution of free PGE<sub>2</sub> in ethanol (Prostin E<sub>2</sub>, Upjohn Ltd) was freshly diluted with normal saline to give the required concentrations for inhalation. Ethanol was added so that each 5-ml aliquot contained 3% ethanol. Four concentrations, containing from 0.0625 mg to 1.5 mg PGE<sub>2</sub> in 5 ml, were administered by a Wright's nebuliser driven by compressed air at 20 lb/sq in to give a flow of 10 l/min. Each solution was breathed for exactly 2 minutes at a rate of 18 breaths a minute set by a respiratory metronome. The nebuliser was weighed before and after each inhalation and the amount of PGE<sub>2</sub> lost was calculated, but standardisation depended on breathing in the set manner for an exact length of time.

Specific airways conductance (sGaw) was measured in a constant-volume whole-body plethysmograph according to the method of Dubois *et al.*<sup>14</sup> Measurement of angles was performed with an electronic resolver, the actual numerical readings being kept out of sight of the operator until the procedure was complete in an attempt to prevent observer bias. The first reading was always disregarded and the mean taken of at least six subsequent technically satisfactory recordings. Changes in sGaw were calculated from a baseline value measured after the subject had been sitting quietly for 15 minutes in the laboratory. Preliminary investigations had indicated that inhaled PGE<sub>2</sub> caused maximum bronchodilatation at about 15 minutes. Doses of PGE<sub>2</sub> were therefore given at 15-minute intervals with measurement of sGaw between 13 and 15 minutes after each dose.

In the first part of the experiment PGE<sub>2</sub> dose-response curves were constructed on two separate days at least 72 hours apart and on a third occasion placebo solutions in the form of 3% ethanol in saline were breathed instead in identical fashion. These

tests were performed double blind and in random order.

Subsequently two salbutamol dose-response curves were constructed for each subject on separate occasions at least 72 hours apart and at least 72 hours after the last of the PGE<sub>2</sub> studies. For construction of salbutamol dose-response curves subjects inhaled cumulative doses of the drug ranging from 25 to 400  $\mu$ g, administered as single inhalations in the conventional way from specially prepared metered aerosols (provided by Allen and Hanburys Ltd) after measurement of baseline sGaw. Doses were administered at 15-minute intervals, with measurement of sGaw immediately before drug administration and subsequently 13–15 minutes after each dose.

Each subject was then started on regular inhaled salbutamol from a commercially available pressurised aerosol in a dose of 400  $\mu$ g (four puffs) four times a day. After three weeks on this treatment the salbutamol dose-response study was repeated, no treatment being taken on that day. Regular salbutamol treatment was then continued for about a week more, after which the PGE<sub>2</sub> dose-response study was repeated, again without salbutamol on that day and with an interval of at least 10 hours from the last salbutamol dose. Treatment was then stopped and a further PGE<sub>2</sub> dose-response curve was constructed a minimum of six weeks later.

In a separate study the effect of oral propranolol (a  $\beta$ -adrenergic antagonist) on PGE<sub>2</sub>-induced bronchodilatation was investigated. On two occasions at least 72 hours apart either 80 mg propranolol or identical placebo tablets were ingested by each subject and PGE<sub>2</sub> inhalation dose-response curves were constructed after one and a half hours.

### STUDIES ON ASTHMATIC PATIENTS

Eight patients with mild, stable asthma (all men; mean age 32 years, range 28–45 years) took part in the study after informed consent had been obtained. All had previously used salbutamol during exacerbations but were currently having no regular treatment. All had at least one positive reaction (weal over 3 mm) to prick skin testing with a range of common allergens; had a history of at least two episodes of wheezing in the previous year; and had a documented change of at least 15% in one-second forced expiratory volume (FEV<sub>1</sub>) or peak flow rate (PFR) either spontaneously, after exercise, or after inhalation of a  $\beta$ -adrenergic agonist drug (200  $\mu$ g salbutamol). PGE<sub>2</sub> dose-response curves were constructed in the same way as for the normal subjects. Two curves were constructed at least 72 hours apart at the same time of day and after the subject had been sitting quietly in the laboratory for at least

30 minutes.

Regular inhaled salbutamol treatment (400  $\mu\text{g}$  four times a day) was then started and at the end of four weeks the PGE<sub>2</sub> inhalation study was repeated, no salbutamol being taken on that day. Once more an interval of at least 10 hours was allowed from the last dose of salbutamol.

#### STATISTICAL ANALYSIS

For each set of dose-response curves the difference in sGaw response was determined at each dose, account being taken of any difference in baselines by calculation of all changes from the mean baseline value. The sum of these differences gave a figure  $d$  for that subject's set of curves. A Student's  $t$  test was then performed on the values of  $d$  or their logarithmic transformations obtained from all the subjects. Paired Student's  $t$  tests were also performed on the sGaw changes obtained for each dose.

For each study the response to treatment on each occasion was computed by trapezoidal integration of the area under the dose-response curve, all changes being calculated from the mean baseline for that set of curves. The logarithmic transformations of these values were then compared by using Student's  $t$  test.

### Results

#### NORMAL SUBJECTS

##### PGE<sub>2</sub> dose-response studies

The mean cumulative doses of PGE<sub>2</sub> nebulised during the dose-response studies were about 10, 40, 160 and 400  $\mu\text{g}$  respectively and for convenience these numbers have been used in construction of the PGE<sub>2</sub> dose-response curves.

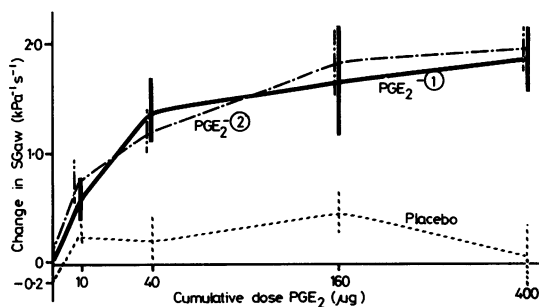


Fig 1 Normal subjects: mean dose-response curves for inhaled PGE<sub>2</sub> obtained on two separate occasions (1 and 2) and mean response to repeated inhalation of placebo (SEM shown at each point). The cumulative dose of PGE<sub>2</sub> is given on a logarithmic scale.

The mean inhalation dose-response curves for PGE<sub>2</sub> on two separate occasions and the 3% ethanol placebo solution are shown in fig 1. There was no significant difference between the two mean PGE<sub>2</sub> dose-response curves. Analysis of variance of the mean response on each occasion computed by trapezoidal integration of the area under each curve gave a within-subject coefficient of variation in mean response of 26.5%, with a between-subject coefficient of variation of 41.7%. Responses after PGE<sub>2</sub> inhalation on each occasion were significantly greater than after placebo ( $p < 0.01$ ). At the lowest dose of PGE<sub>2</sub> (10  $\mu\text{g}$ ) the difference from the placebo response determined by a paired Student's  $t$  test reached only borderline significance at the 0.05 level but was significant at all higher doses ( $p < 0.025$  at the 40- $\mu\text{g}$  dose and  $< 0.0025$  at the higher doses).

Comparison of the response to PGE<sub>2</sub> at each dose on each occasion showed the 40- $\mu\text{g}$  dose to cause significantly more bronchodilatation than the 10- $\mu\text{g}$  dose ( $p < 0.005$  by Student's  $t$  test); the 160- $\mu\text{g}$  dose was significantly more effective than the 40- $\mu\text{g}$  dose ( $p < 0.025$ ), as was the 400- $\mu\text{g}$  dose ( $p < 0.005$ ). There was no significant difference between the 400- $\mu\text{g}$  and 160- $\mu\text{g}$  doses. In nine of the 12 curves the 400- $\mu\text{g}$  dose caused the greatest bronchodilatation and in only one subject on one occasion was there a greater than 5% fall in sGaw from the 160- $\mu\text{g}$  to the 400- $\mu\text{g}$  dose, in contrast to the results later obtained in asthma patients (see below).

##### Salbutamol dose-response studies

The mean cumulative dose-response curves for the group of subjects as a whole on two separate occasions are shown in fig 2. There was no significant

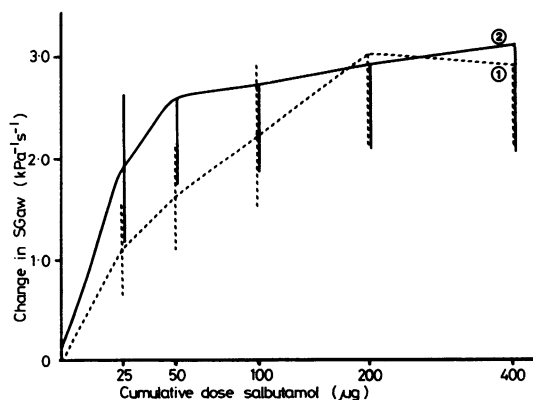


Fig 2 Normal subjects: mean dose-response curves for inhaled salbutamol obtained on two separate occasions (1 and 2) (SEM shown at each point). Cumulative doses are given on a logarithmic scale.

difference between the two curves. The within-subject coefficient of variation in mean response was 35%, with a 75.5% between-subject coefficient of variation. An  $F$  test indicated that the variations in response to salbutamol were significantly greater than to  $\text{PGE}_2$  ( $p < 0.05$  for the within-subject variation and  $p < 0.015$  for the between-subject variation in response).

Although the mean maximum bronchodilatation achieved with salbutamol (78% increase from baseline sGaw) was higher than that achieved with  $\text{PGE}_2$  (50% increase in sGaw) this difference was not statistically significant, nor was there any significant difference between the areas under the salbutamol and  $\text{PGE}_2$  dose-response curves.

#### Effect of chronic salbutamol

There was an increase in mean baseline sGaw of 10% but this was not statistically significant. There was no significant difference between the mean dose-response curve for salbutamol after three weeks of regular administration of salbutamol when this mean was compared with the mean of the two pretreatment curves (fig 3). In no subject was there any suggestion of development of hyporesponsiveness to salbutamol.

After four weeks of regular salbutamol treatment the mean cumulative dose-response curve for  $\text{PGE}_2$  was shifted upwards and to the left of the mean pretreatment curve. The overall mean change in sGaw was significant ( $p < 0.02$ ), as was the change in the areas under the curves ( $p < 0.0275$ ). Differences between the responses at each dose analysed by Student's  $t$  test were significantly increased, how-

ever, only for the lowest dose ( $p < 0.025$ ). The final dose-response curve constructed after at least six weeks without regular treatment had returned to control levels (fig 4).

#### Effect of propranolol

Oral propranolol 80 mg given one and a half hours before had no consistent effect either on baseline values of sGaw or on the response to  $\text{PGE}_2$  (fig 5).

#### ASTHMATIC PATIENTS

##### $\text{PGE}_2$ dose-response studies

The mean baseline sGaw was 39% lower in the patients with asthma than in the normal subjects. The mean maximum increase in sGaw after  $\text{PGE}_2$  was 61%. There were some striking differences between the response to inhaled  $\text{PGE}_2$  in the asthmatic patients and in the normal subjects. Six of the eight subjects on at least one occasion had biphasic dose-response curves, usually bronchodilatation followed by bronchoconstriction, with the final sGaw value at least 10% lower than the maximum achieved (fig 6). In one subject (No 5) there was a 20% initial fall in sGaw followed by bronchodilatation. In two subjects with large differences in their baseline values of sGaw (Nos 1 and 3: 60% and 30% respectively) there were pronounced differences in the shapes of the  $\text{PGE}_2$  dose-response curves: when the baseline sGaw was low (high initial resistance) dose-related bronchodilatation only was seen, whereas when the baseline sGaw was high (lower resistance) initial bronchodilatation followed by bronchoconstriction occurred. The final values of

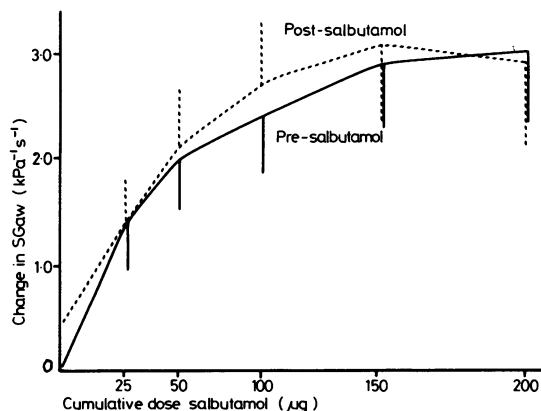


Fig 3 Normal subjects: mean dose-response curves for inhaled salbutamol before and three weeks after regular salbutamol treatment (SEM shown at each point). Cumulative doses are given on a logarithmic scale.

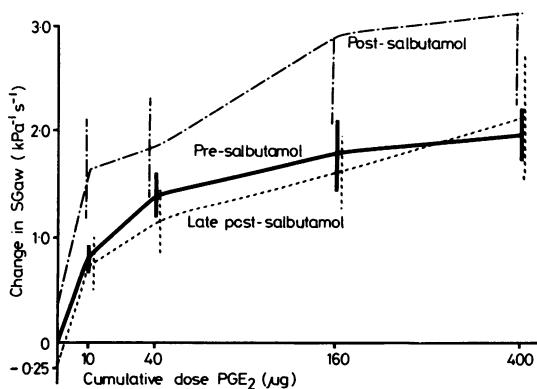


Fig 4 Normal subjects: mean dose-response curves for inhaled  $\text{PGE}_2$  before (pre) and four weeks after regular salbutamol treatment (post) and at least six weeks after treatment was stopped (late post) (SEM shown at each point). Cumulative doses are given on a logarithmic scale.

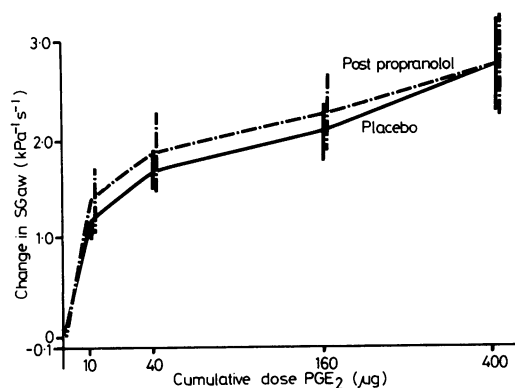


Fig 5 Normal subjects: mean dose-response curves for inhaled  $PGE_2$  after premedication with placebo and propranolol given one-and-a-half hours before (SEM shown at each point). Cumulative doses of  $PGE_2$  are given on a logarithmic scale.

sGaw achieved by most individuals, however, were very similar on the two occasions. In only two subjects did the final values of sGaw differ by 10% or greater.

Analysis of variance of the mean response gave a within-subject coefficient of variation of 24%, with a between-subject coefficient of variation of 97%. An  $F$  test showed that the difference in this between-subject variation in the asthmatics and the normal subjects reached borderline levels of significance ( $p < 0.075$ ). There was no significant difference between normal subjects and asthmatics in terms of maximum sGaw changes after  $PGE_2$  or the areas under the dose-response curves.

#### Effect of chronic salbutamol treatment

After four weeks of regular salbutamol inhalations there was a 12% increase in the mean baseline ( $p < 0.075$ ) and a pronounced increase in the bronchodilator response to inhaled  $PGE_2$ . The responses

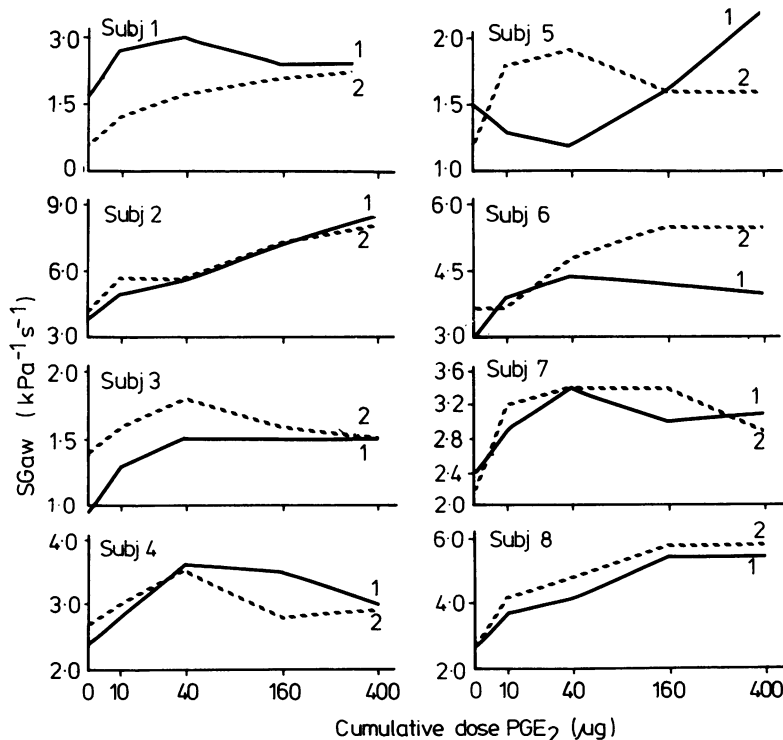


Fig 6 Patients with asthma: dose-response curves for inhaled  $PGE_2$  on two occasions in all eight subjects. Cumulative doses of  $PGE_2$  are given on a logarithmic scale.



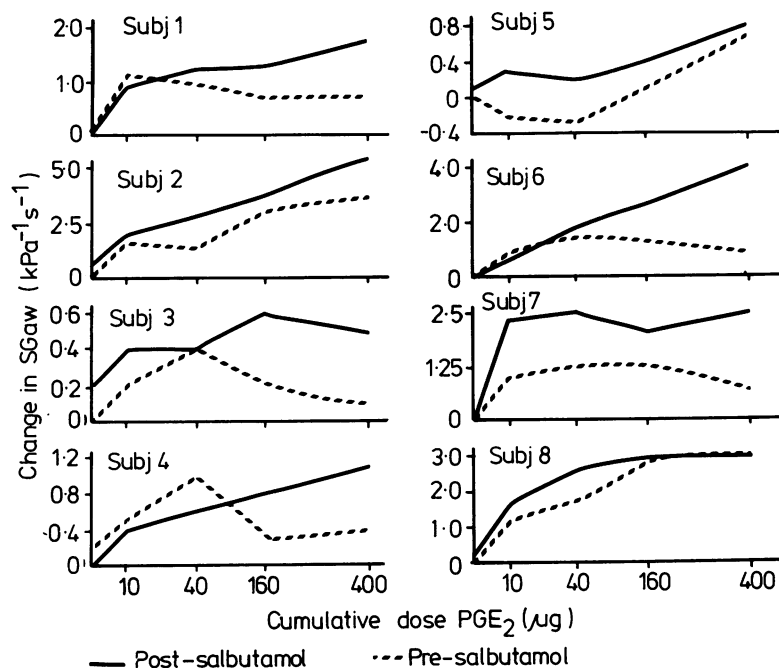


Fig 7 Patients with asthma: pre-salbutamol treatment dose-response curve for inhaled PGE<sub>2</sub> with the higher baseline specific airway conductance (sGaw) value in each subject and dose-response curve obtained after four weeks taking regular salbutamol. For subject 8 the pretreatment curve given is the mean of the two constructed. Cumulative doses of PGE<sub>2</sub> are shown on a logarithmic scale.

were also more consistent and the dose-response curves normal in shape; a 10% fall in sGaw on the final dose from the maximum achieved occurred in only one subject.

Analysis of the mean sGaw response after salbutamol treatment compared with the mean pretreatment responses showed a highly significant increase ( $p < 0.005$ ), evident for all doses (increasing from  $p < 0.02$  for the lowest dose to  $p < 0.005$  for the highest). If the post-salbutamol treatment PGE<sub>2</sub> curves are compared only with the pretreatment curves with the highest baselines (a non-significant 6% mean difference) the increase in mean response is still significant ( $p < 0.05$ ) (fig 7).

Analysis of the areas under the dose-response curves also showed a highly significant increase ( $p < 0.00025$  when compared to the mean pretreatment curves and  $p < 0.001$  when only the maximum pretreatment responses were compared).

## Discussion

One of the initial intentions of this study was to investigate whether hyporesponsiveness to sal-

butamol inhalation occurred in normal subjects on regular  $\beta$ -adrenergic treatment and whether the response to inhaled PGE<sub>2</sub> was altered at the same time. We were unable to show any fall-off in response to salbutamol in our group of normal subjects. This contrasts with the results of Holgate *et al.*,<sup>12</sup> who found a significant attenuation in response during regular treatment; but a more recent study<sup>15</sup> was also unable to confirm the development of hyporesponsiveness. The design of these two studies and ours were very similar, although we used a higher total dose of salbutamol in the chronic treatment phase.

In our two pretreatment dose-response studies with salbutamol the mean maximum response achieved on each occasion was very similar, but the variation in mean response calculated from the areas under the dose-response curves were quite large and significantly greater than the variation in response to PGE<sub>2</sub>. This probably reflects the greater difficulty in standardising the single-breath inhalation technique for salbutamol administration from metered aerosols, used in constructing the salbutamol dose-response curves, than the two minutes of tidal breath-

ing used for each PGE<sub>2</sub> dose. This may play some part in explaining the discrepancies in the studies mentioned.

The response to PGE<sub>2</sub> was qualitatively different from normal in most of the asthmatics. Whereas normal subjects almost exclusively showed consistent dose-related bronchodilatation, the asthmatic patients frequently showed biphasic responses. In such patients there seemed to be a point of airway smooth muscle tone above which PGE<sub>2</sub> caused muscle relaxation and below which it caused contraction, the final point of tone being a balance between these two effects. That PGE<sub>2</sub> may have both constrictor and relaxant actions on bronchial smooth muscle has been shown in vitro,<sup>5 16 17</sup> and another recent in vitro study has emphasised that the balance of these dual effects may indeed depend on the initial tone of the preparation.<sup>18</sup> Our data would suggest that in normal subjects the point of muscle tone at which balance between the contractile and relaxant actions of PGE<sub>2</sub> is set is low and bronchoconstriction is not usually seen, at least in dose-response studies of the sort described. In most asthmatics, however, this point of balance may be abnormal, so that bronchoconstrictive effects are readily seen.

We were expecting either no change in the PGE<sub>2</sub> effect in normal subjects after regular salbutamol treatment or possibly a decrease in response if there had been a change in the common intracellular pathway leading to bronchodilatation. We found, however, a significant increase in bronchodilator response to PGE<sub>2</sub> in normal subjects, and the effect was even more pronounced in asthmatic patients. Apparently the balance of constrictor to dilator actions of PGE<sub>2</sub> is altered in the direction of dilatation and the point of tone at which bronchoconstriction supervenes is shifted in the asthmatic patients towards normal. PGE<sub>2</sub> may be concerned in both control of airway smooth-muscle tone and in limiting bronchoconstriction induced by mediators such as histamine,<sup>19-22</sup> so that development of hyperresponsiveness of the airways to the bronchodilator effects of PGE<sub>2</sub> after chronic treatment with the  $\beta$ -adrenergic agonist salbutamol may be an important aspect of such treatment in clinical practice.

Our study provided no information on a mechanism by which chronic salbutamol treatment could initiate such a change in drug response. Possibly, however, repeated stimulation of the adrenoreceptor causes a relative change in the number of antagonistic prostaglandin smooth muscle receptors. Alternatively, there may have been a change (positive or negative) in "co-operation" between occupied  $\beta$ -receptors and prostaglandin receptors, resulting in a change in the relative affinity of PGE<sub>2</sub> for its two smooth muscle receptors (contractile and

relaxant), without a change in receptor numbers. That interactions between receptor systems can occur has been shown in several sites—for example, between  $\alpha$ -adrenoreceptors and  $\beta$ -adrenoreceptors in rat aortic smooth muscle<sup>23</sup> and human lymphocytes,<sup>24</sup> PGF<sub>2 $\alpha$</sub>  and luteinising hormone receptors in ovine corpora lutea,<sup>25</sup> and PGE<sub>1</sub> and  $\beta$ -adrenoreceptors in human lymphocytes.<sup>26</sup>

Change could also have been induced intracellularly. Adenylate cyclase activity can be modified by divalent cations or guanine nucleotides or by changes in membrane phospholipids,<sup>27</sup> and phosphorylation of protein kinases by cyclic AMP can be increased in some circumstances.<sup>28</sup> Any such intracellular change could give the increase in response to PGE<sub>2</sub> that we have observed without an increase in prostaglandin receptor number or binding affinity. In such circumstances the response to salbutamol could remain unchanged even if there was a decrease in available  $\beta$ -adrenoreceptors. This would provide a homeostatic mechanism in bronchial smooth muscle cells to overcome any change in numbers of available  $\beta$ -adrenoreceptors that might occur after chronic  $\beta$ -adrenergic agonist treatment.

Eighty milligrams of oral propranolol, which has been shown significantly to antagonise salbutamol-induced bronchodilatation,<sup>29</sup> had no effect on the response to PGE<sub>2</sub>. This would suggest that the prostaglandin bronchodilator receptor and the  $\beta$ -adrenoreceptor are different and confirms in vitro results.<sup>3-5</sup> Whether chronic treatment with  $\beta$ -adrenergic blocking drugs, however, has any effect upon PGE<sub>2</sub>-induced bronchodilatation is not yet known. Such an effect would be potentially important in asthmatics and needs further investigation.

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