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Effect of inhaled budesonide on bronchial reactivity to histamine, exercise, and eucapnic dry air hyperventilation in patients with asthma

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Abstract

Background **It has been suggested that inhaled corticosteroids may provide greater protection against constrictor stimuli that act indirectly such as exercise than those that act directly such as histamine.**

Methods **The effects of six weeks treatment with inhaled budesonide (800 μg twice daily) on bronchial reactivity to histamine, exercise, and eucapnic voluntary hyperventilation of dry air were compared in a double blind, placebo controlled, non-crossover study in 40 subjects with asthma. Change in bronchial reactivity to histamine and eucapnic hyperventilation over the six weeks was measured as change in the provocative dose of histamine or dry air causing a 20% fall in FEV_1 (PD_{20} histamine and PV_{20} eucapnic hyperventilation (EVH) of dry air); this was not possible for exercise because of the development of refractoriness. To enable the change in response to all three stimuli to be compared, the response (percent fall in FEV_1) to a fixed dose was measured for all three challenge tests.**

Results **After budesonide there was an increase in PD_{20} histamine from 0·48 to 2·81 μmol and in PV_{20} EVH from 364 to 639 litres, and a significant correlation between the changes in PD_{20} histamine and PV_{20} EVH ($r = 0·63$). The median percentage fall in FEV_1 in response to eucapnic hyperventilation, exercise, and histamine was similar before budesonide (25·5%, 26·6%, and 24·5%); the reduction in the percentage fall in FEV_1 with budesonide was also similar for the three challenges (18·9%, 17·5%, and 16·6%), and all differed significantly from the changes following placebo. There was a significant correlation between change in percentage fall in FEV_1 after budesonide with the three stimuli (histamine *v* exercise: $r = 0·48$; histamine *v* eucapnic hyperventilation: $r = 0·46$; exercise *v* eucapnic hyperventilation: $r = 0·63$).**

Conclusion **The similar magnitude of change in bronchial reactivity to all three stimuli after budesonide and the within subject correlation obtained between these changes suggest that corticosteroids act by a common mechanism to protect against eucapnic hyperventilation, exercise, and histamine.**

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Patients with asthma show increased airway reactivity to a wide range of stimuli, some of which appear to act directly on airway smooth muscle and some indirectly through mast cell mediator release or sensory nerve stimulation. Drugs such as $beta_2$ agonists reduce bronchial reactivity irrespective of the stimulus, whereas others, such as sodium cromoglycate and inhaled frusemide, reduce the airway response to challenges that act indirectly, such as exercise,[1 2] metabisulphite,[3 4] and cold air hyperventilation,[5] with little or no effect on directly acting stimuli such as histamine and methacholine.[6 7]

Corticosteroids when given regularly have been shown to inhibit the response to most bronchoconstrictor stimuli,[8–19] but whether they give similar protection against different forms of challenges is not clear. It has been suggested that they may have a greater effect on exercise induced bronchoconstriction than that induced by histamine,[13 15] and this could explain why inhaled corticosteroids are more effective in clinical practice than might be expected from their relatively modest effect on histamine reactivity. The greatest change in the provocative dose of histamine causing a 20% fall in FEV_1 (PD_{20}) with inhaled corticosteroids in placebo controlled studies has been only 1·2 doubling doses,[8–10] though recent uncontrolled studies have shown larger changes[16 19] and a change of two doubling doses of methacholine was seen in a recent placebo controlled study.[18]

We have therefore compared the effect of six weeks' treatment with budesonide 800 μg twice daily on the airway response to histamine, exercise, and eucapnic voluntary hyperventilation in 40 patients with asthma. A detailed analysis of our findings on the time course of change in airway reactivity to histamine before and after the end of treatment has been published.[20]

Certain problems had to be addressed in assessing the relative effect of corticosteroids on histamine and exercise induced bronchoconstriction as different methods are normally used to assess the response to the two stimuli. The effect of the drug on the response to histamine is usually measured as the change in PD_{20}, a measure of the shift in the dose-response curve after drug administration[21 22]; the effect on exercise has usually been measured as the change in response to a single "dose" of exercise because of the problem of refractoriness with repeated challenges.[23 24] We have tried to circumvent this problem by measuring the response in two ways. For

histamine and eucapnic hyperventilation we measured the response to budesonide conventionally as the change in the provocation dose of histamine or ventilation causing a 20% fall in FEV_1 (PD_{20} histamine or PV_{20} EVH). In addition, we have measured the response to budesonide from a fixed dose-response measurement for all three challenges, using the highest dose of stimulus that was common to the measurements before and after budesonide.

Methods

SUBJECTS

Forty subjects (28 male) aged 18–45 (median 28) years were entered into the study, having met the following inclusion criteria: a history of asthma for at least two years, a forced expiratory volume in one second (FEV_1) greater than 50% of the predicted value, a provocative dose of histamine that caused a 20% fall in FEV_1 (PD_{20}) of 4 μmol or less and a provocative dose of dry air that caused a 20% fall in FEV_1 (PV_{20}) of less than 640 l. Subjects had to be current non-smokers who had never smoked more than 10 cigarettes a day for two years, be having no treatment other than an inhaled $beta_2$ agonist, be considered to have stable asthma when they entered the study, and not to have had a chest infection for at least six weeks before entry into the study. Skin tests with grass pollen, cat fur, and house dust mite with saline and histamine controls were carried out; 37 of the 40 subjects had a positive response (weal of more than 2 mm) to at least one allergen.

MEASUREMENT OF BRONCHIAL REACTIVITY

FEV_1 was measured with a dry bellows spirometer (Vitalograph, Buckingham), the higher of two measurements within 100 ml being recorded.

We measured bronchial reactivity to histamine by the method of Yan *et al*,[25] with doubling dose increments from 0·03 to 32 μmol, using hand held No 40 De Vilbiss nebulisers with an output range of 2·5–3·5 μl per actuation. The test was stopped when the FEV_1 had fallen by 20% from the post-saline value.[20]

Measurement of bronchial reactivity to eucapnic hyperventilation was carried out according to the method described by Phillips *et al*.[26] The subjects, seated and with a noseclip fitted, inhaled dry air containing 5% carbon dioxide at room temperature from a 6 litre target reservoir bag through a Collins triple J valve. The bag was filled continuously from a cylinder containing the dry gas mixture via a rotameter, which was regulated to achieve the desired minute ventilation. After baseline measurement of FEV_1 the subject breathed the dry air mixture at 20 l/min for four minutes. FEV_1 was measured three and five minutes later. The sequence was repeated with further four minute periods of ventilation with flow rates of 20, 40, 80 and a second 80 l/min until a fall in FEV_1 of 20% from baseline was obtained. The flow rates used provided cumulative volumes of dry air of 80, 160, 320, 640, and 960 litres.

A six minute exercise challenge was carried out with continuous electrocardiographic monitoring on a treadmill at room temperature (maintained at 18–20°C), with the subject wearing a noseclip and breathing dry air through a mouthpiece from a cylinder via a Collins triple J valve and a 100 litre Douglas bag reservoir. A nomogram[27] was used to determine the level of exercise (running speed and incline) required to attain a heart rate of 90% predicted maximum for each patient. FEV_1 was measured before exercise and three, five, 10, 15, and 20 minutes after exercise and the maximum fall from the pre-exercise baseline determined. Two practice exercise tests were carried out on different days to determine the six minute workload for each patient that would cause a fall in FEV_1 of around 20%. The workload was then kept constant for subsequent tests.

STUDY DESIGN

The study was a double blind, non-crossover comparison in which subjects were randomly allocated to receive either inhaled budesonide 800 μg twice daily or placebo for six weeks. Bronchial reactivity to eucapnic hyperventilation, exercise, and histamine was measured (in that order) on three consecutive days at the same time of day (0830) at the beginning of the treatment period. Subjects took the first dose of budesonide 800 μg or placebo from a metered dose inhaler via a pear shaped 750 ml spacing device (Nebuhaler, Astra Pharmaceuticals) in the laboratory under supervision after the histamine challenge test (day 1 of treatment). They were instructed to take the same dose of budesonide via the spacing device twice daily at 0900 and 2200, starting on day 2 at 0900 and stopping on day 41 at 2200.[20] Subjects were asked not to take their beta agonist inhaler for at least eight hours before each challenge and to refrain from taking any drug apart from the trial inhaler or inhaled $beta_2$ agonist, as required, throughout the study. We repeated the three challenges at the end of the treatment period on consecutive mornings at 0830 in the same order as at the beginning of the study, starting with the eucapnic hyperventilation challenge test on day 40.

ANALYSIS

Of the 40 subjects entered into the study one (budesonide group) withdrew because of change of employment and five (four placebo, one budesonide) required treatment with prednisolone for an exacerbation of their asthma. Because exclusion of the results of these six subjects would have introduced bias, non-parametric methods were used to compare budesonide and placebo to enable all subjects recruited into the study to be included in the analysis. Subjects who failed to complete the study were assumed to have deteriorated and were allocated the lowest values for the two groups from the time they withdrew.[28] Parametric methods were used for within subject comparisons.

The log cumulative dose of histamine and volume of dry air were plotted against FEV_1 to obtain a PD_{20} histamine and a PV_{20} EVH value by linear interpolation. PD_{20} histamine and PV_{20} EVH values were log_2 transformed before analysis. A PD_{20} value of 32 μmol (one subject) and a PV_{20} of 960 litres (11 subjects) was assigned for subjects who did not achieve a 20% fall in FEV_1 with the highest dose of stimulus at the end of the treatment period. Shift in the dose-response curves for histamine and eucapnic hyperventilation after budesonide treatment has been expressed in doubling doses (log_2 units), as described previously.[22]

For exercise the maximum percentage fall from pre-exercise baseline FEV_1 (exercise % fall) was recorded. To allow a more direct comparison with exercise we also determined the highest common dose of histamine or dry air for each subject before and after treatment and the percentage fall in FEV_1 at this dose on both occasions (histamine % fall and EVH % fall). The difference between pre- and post-treatment values (Δ % fall in FEV_1) was then calculated for all three challenge tests.

Baseline values for PD_{20} histamine, PV_{20} EVH, and exercise % fall for the two groups and change in PD_{20} histamine, PV_{20} EVH, and exercise % fall were compared by the rank sum test, and conservative 95% confidence intervals (CI) calculated.[29] Pearson's correlation was used to relate the changes following budesonide in PD_{20} histamine and PV_{20} EVH and, after logarithmic transformation, in Δ exercise % fall, Δ histamine % fall, and Δ EVH % fall in FEV_1.

Results

Baseline values for FEV_1, PD_{20}, PV_{20} and exercise % fall for all 40 subjects and for the 34 subjects who completed the study were similar for the two groups (table). The range of PD_{20} histamine pretreatment values for the 40 subjects (six doubling doses) was larger than the range for PV_{20} EVH values (2·5 doubling doses). PD_{20} histamine and PV_{20} EVH correlated with FEV_1 % predicted ($r = 0·4$, $p < 0·01$; $r = 0·4$, $p < 0·05$), whereas exercise % fall did not ($r = 0·1$, $p = 0·5$).

BUDESONIDE VERSUS PLACEBO

Median FEV_1 fell over the six weeks of placebo treatment from 3·44 to 3·22 l, and this change differed significantly from the increase in FEV_1 that followed budesonide treatment—from 3·53 to 3·81 l ($p < 0·001$). Median PD_{20} histamine increased with budesonide treatment from 0·48 to 2·81 μmol and with placebo from 0·27 to 0·40 μmol (fig 1). The increase in PD_{20} was significantly larger with budesonide than with placebo, by 2·4 doubling doses (95% CI 0·5 to 3·6, $p < 0·01$). Median PV_{20} EVH increased with budesonide treatment from 364 to 639 litres and with placebo from 347 to 419 litres. The difference between the increases for the two groups (0·6 doubling doses) was not significant (95% CI −0·05 to 1·05, $p = 0·07$). The median exercise % fall in FEV_1 decreased after budesonide treatment from 23·5% to 6·7% and after placebo from 25·6% to 22·1%, the difference of 13·3% (95% CI 3·5 to 27·5) being significant ($p < 0·01$).

COMPARISON OF CHANGE IN BRONCHIAL REACTIVITY TO HISTAMINE, EUCAPNIC HYPERVENTILATION, AND EXERCISE WITH BUDESONIDE TREATMENT

Changes in PD_{20} histamine and PV_{20} EVH correlated closely (fig 2: $r = 0·63$, $p < 0·01$). As the change in PV_{20} EVH would have been underestimated in seven of the 18 subjects because censored values were used, the relation between change in PV_{20} EVH and PD_{20} histamine was examined in the 11 subjects with no censored values for either challenge test. The correlation in these 11 subjects was slightly better (fig 2: $r = 0·76$, $p < 0·01$).

In the 18 subjects completing budesonide treatment the pretreatment median % fall in FEV_1 values for the eucapnic hyperventilation, exercise, and histamine challenge tests was 25·5%, 26·6%, and 24·5%. The median change in % fall in FEV_1 with budesonide treatment was similar for all three challenge tests (fig 3): Δ EVH % fall 18·9% (95% CI 16·7 to 24·2), Δ exercise % fall 17·5% (95% CI 11·2 to 28·1), Δ histamine % fall 16·6% (95% CI 13·7 to 18·7). These changes correlated significantly (Δ EVH % fall *v* Δ exercise % fall: $r = 0·63$, $p < 0·01$; Δ EVH % fall *v* Δ histamine % fall: $r = 0·46$, $p < 0·05$; Δ exercise % fall *v* Δ histamine % fall: $r = 0·48$, $p < 0·05$).

Discussion

In these subjects with mild to moderate asthma treatment with inhaled budesonide for six weeks reduced bronchial reactivity to eucapnic hyperventilation, exercise, and histamine. The

Median baseline values (95% confidence intervals) for the 40 subjects entering the study and for the 34 subjects completing the six week study

	Subjects starting study (n = 40)		*Subjects completing study (n = 34)*	
	Budesonide	*Placebo*	*Budesonide*	*Placebo*
FEV_1 (l)	3·53 (3·02–3·95)	3·44 (3·00–4·20)	3·63 (2·95–3·95)	3·57 (3·15–4·35)
FEV_1 (% pred)	95 (86–99)	95 (86–99)	96 (84–100)	98 (89–101)
PD_{20} (μmol)	0·48 (0·20–1·21)	0·27 (0·21–0·57)	0·53 (0·19–1·36)	0·45 (0·24–0·58)
PV_{20} (l)	365 (320–495)	349 (289–440)	366 (299–508)	409 (310–448)
Exercise (% fall)	23·5 (14·9–33·9)	25·6 (18·6–47·5)	26·6 (14·3–31·0)	22·5 (14·5–30·3)

EVH—eucapnic voluntary hyperventilation of dry air; PD_{20}—provocative dose of histamine causing a 20% fall in FEV_1; PV_{20}—provocative volume of dry air causing a 20% fall in FEV_1.

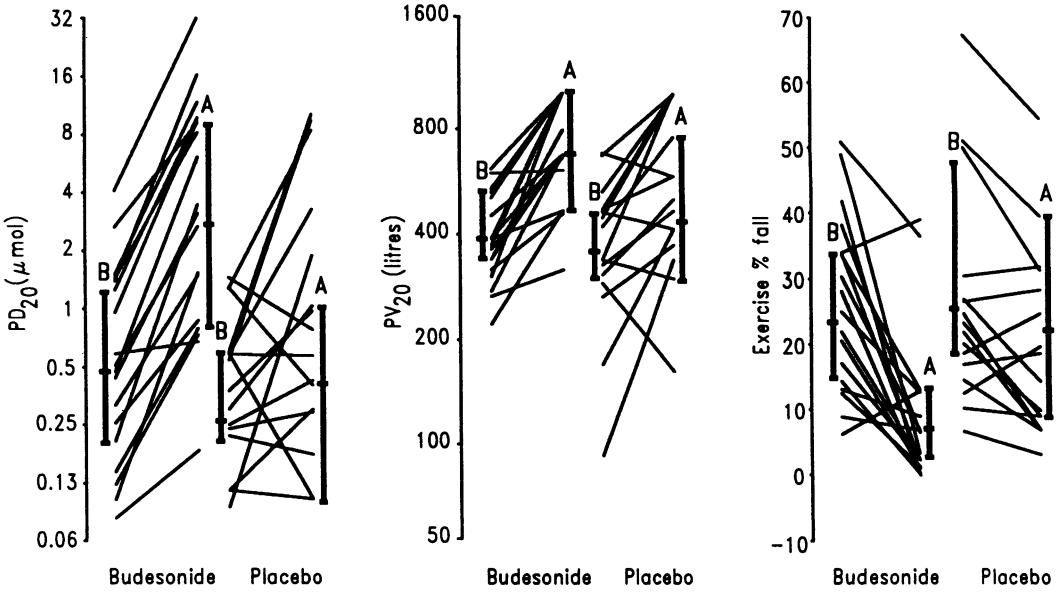

Figure 1 Individual values for the provocative dose of histamine and the provocative volume of dry air causing a 20% fall in FEV_1 (PD_{20} histamine, PV_{20} EVH) and the percentage fall caused by exercise before and after six weeks' treatment with budesonide and placebo: median values and 95% confidence intervals for the 20 subjects in each group before (B) and after (A) treatment.

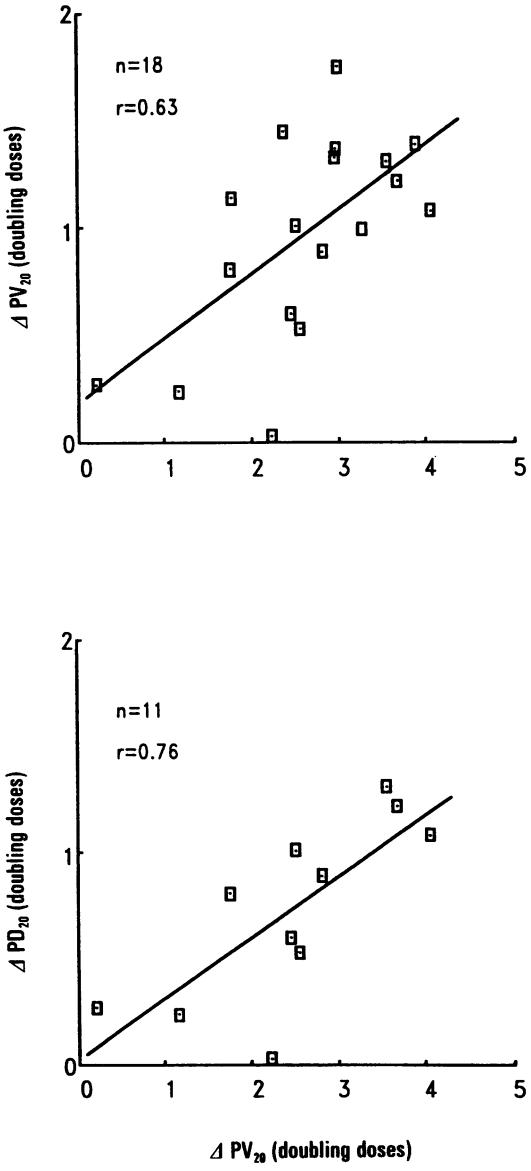

Figure 2 Scattergrams showing the correlation between change in PD_{20} histamine and change in PV_{20} EVH after treatment with budesonide in all 18 subjects completing the study (top) and in the 11 subjects for whom censored values were not required (bottom). For abbreviations see figure 1.

changes in bronchial reactivity to the three stimuli with budesonide treatment were closely correlated and were of a similar magnitude when assessed as the change in response to a fixed dose of stimulus.

In the analysis we had to deal with two problems that are common in longer term studies of bronchial reactivity in asthmatic patients. The first was that some subjects were unable to complete the study because of an exacerbation of asthma. Exclusion of data from these subjects from the analysis might have introduced bias as they were more likely to be in the placebo group and to have FEV_1 and PD_{20} values that were falling during the study; this would cause the mean values of the remaining subjects, in the placebo group in particular, to be increased. The use of medians and non-parametric ranking methods circumvents this problem.[20] The second problem was that after treatment some subjects did not show a 20% fall in FEV_1 with the highest dose of histamine or dry air given, so the highest dose given was assigned as the PD_{20} or PV_{20}. This would cause change with treatment to be underestimated if mean values and parametric methods had been used; by using medians and non-parametric methods these censored values were included without introducing bias.

In attempts to understand the mechanisms underlying bronchial reactivity to different stimuli it is important to be able to compare the effect of drugs such as corticosteroids on the response to different challenges. There is no agreement, however, on how this should be carried out and several approaches have been used. Comparing the change in response to a fixed dose of stimulus will give a valid measure only if the response before and after treatment is on the linear part of the dose-response curve. Measuring the shift in the dose-response curve circumvents this problem but is valid as a

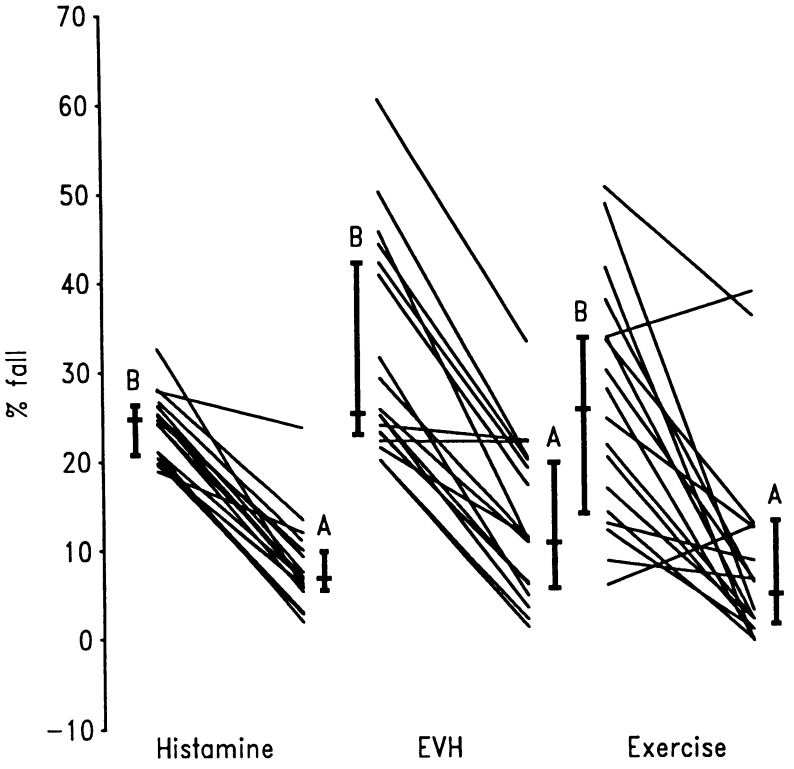

Figure 3 Individual values of % fall in FEV_1 after a fixed dose of histamine, eucapnic hyperventilation (EVH), and exercise before (B) and after (A) treatment with budesonide: median values and 95% confidence intervals for the 18 subjects completing budesonide treatment.

comparison between stimuli only if the dose-response relationships for the different stimuli are similar, so that a unit shift in the dose-response curve for one stimulus (one doubling dose, for example) is equivalent to the same unit shift for another stimulus. Another problem with this method is that with some stimuli it may be difficult to obtain a dose-response curve; this is the case with exercise, for example, because of the problem of refractoriness with repeated challenges.[23 24] Neither method is entirely satisfactory but using the same method to compare the responses to treatment is preferable to using different methods for different stimuli. We therefore compared the change in reactivity to histamine and eucapnic hyperventilation after inhalation of budesonide in terms of the shift in the dose-response curve, and we compared the change in reactivity to all three stimuli using the fixed dose-response method.

Budesonide provided very effective protection against exercise induced asthma in our study, the percentage fall in FEV_1 after exercise being reduced from 23·5% to 6·7% following treatment. This degree of protection is similar to that seen in other recent studies.[13–15] We found a relatively large change in bronchial reactivity to histamine after treatment (2·4 doubling doses). This was greater than that seen in many early studies of histamine or methacholine reactivity after inhalation of corticosteroids[8–11] but is similar to the change in the study by Juniper *et al*[18] and in three recent uncontrolled studies.[16 17 19] When we analysed the change in histamine reactivity as the change in the percentage fall in FEV_1 to a fixed dose of stimulus, the magnitude of the reduction in the response to histamine (16·6%) was similar to that obtained with exercise (17·1%) in the same patients. Two studies[14 17] that have looked at the effect of budesonide on exercise induced bronchoconstriction and histamine reactivity in the same patients appear to show a larger effect on exercise (from 22% to 9% reduction in the fall in FEV_1) than on histamine (increase in PD_{20} less than one doubling dose). Neither study had a placebo control, however, and interpretation of results when different methods have been used to assess the response is difficult. Our results show a similar magnitude of effect on exercise and histamine induced bronchoconstriction when this is assessed by the same method.

There is no information on the effect of corticosteroids on bronchoconstriction induced by eucapnic hyperventilation. In our study budesonide caused a reduction in the percentage fall in FEV_1 with eucapnic hyperventilation (18·9%) similar to that seen with exercise (17·1%) and histamine (16·6%). In some patients, particularly after histamine inhalation and exercise, the response was small after budesonide, suggesting that it may not be on the linear part of the budesonide dose-response curve. This may have caused the percentage fall to be underestimated in some patients. Although the percentage fall in FEV_1 was similar for histamine and eucapnic hyperventilation the change in PD_{20} and PV_{20} after budesonide differed, being 2·4 doubling doses for histamine but only 0·6 doubling doses for PV_{20} EVH. This difference between eucapnic hyperventilation and histamine is similar to that seen with $beta_2$ agonists, where the shift in the dose-response curves for cold air is much less than that seen with histamine after the same dose of $beta_2$ agonist.[30 31] These data highlight the fact that the relation between change in percentage fall in FEV_1 and the shift of the dose-response curve (ΔPD_{20}, ΔPV_{20}) is different for histamine and eucapnic hyperventilation and show that a unit shift in the dose-response curve for one stimulus is not equivalent to a unit shift in the dose-response curve for another stimulus. This may be the reason why pretreatment PD_{20} histamine in the study group ranged over 6 doubling doses whereas the range of PV_{20} EVH in the same subjects was much smaller, only 2·5 doubling doses. Comparing the changes obtained with the three stimuli after budesonide treatment as change in the percentage fall in FEV_1 is, we believe, more valid in these circumstances than measuring the shift in the dose-response curve. When expressed in this way our results suggest that budesonide has a similar effect on bronchial reactivity to all three stimuli.

The mechanisms underlying the bronchoconstrictor response to eucapnic hyperventilation, exercise, and histamine appear to differ. Exercise and dry air hyperventilation are thought to act by cooling or drying (or both) of the airway, with consequent mediator release from inflammatory or epithelial cells.[32 33] Histamine, on the other hand, causes bronchoconstriction by a direct effect on airway smooth muscle via histamine H_1 receptors,[34 35] though other mechanisms, such as increased mucosal oedema or vagal activation,[36] may contribute. The correlation between the changes in bronchial reactivity to the three

stimuli after budesonide treatment and the similar magnitude of the changes obtained suggest that corticosteroids reduce bronchial reactivity by affecting mechanisms common to the three stimuli, rather than by acting on a more specific triggering mechanism. The increased bronchial reactivity seen in asthmatic patients is thought to be associated with the inflammatory changes—namely, epithelial shedding, inflammatory cell influx,[37] and the increase in airway wall thickness that is due to mucus, oedema, and collagen deposition below the basement membrane.[38 39] There is evidence that corticosteroids reduce the inflammatory response in the airways[40] and these changes may be responsible for the reduction in bronchial reactivity to all three stimuli.

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