Effect of prior bronchoconstriction on the airway response to histamine in normal subjects

KF CHUNG, PD SNASHALL

From the Department of Medicine, Charing Cross Hospital Medical School, London

ABSTRACT We have examined the effect of prior bronchoconstriction on the bronchial responsiveness to inhaled histamine in nine normal subjects. The airway response to increasing concentrations of histamine aerosol was assessed by measurement of specific airways conductance (sGaw) in a body plethysmograph. The threshold provocative dose of histamine needed to cause a 35% fall in starting sGaw (PD_{35}) and the steepest slope of the response were measured from cumulative log dose response curves. Histamine challenges were performed in duplicate after premedication with 0.9% sodium chloride (control) or methacholine aerosol on separate days. The mean starting sGaw did not change significantly after inhalation of 0.9% sodium chloride but methacholine caused a mean reduction in sGaw of 42%. Mean control PD_{35} values did not differ significantly from mean PD_{35} values after methacholine. The mean steepest slope of the response after methacholine was 47% lower than the mean control value. There was a significant linear relationship between starting sGaw and the steepest slope for the control and for the methacholine premedicated challenges. The reduction in slope after methacholine was accounted for by the fall in starting sGaw. Because histamine PD_{35} was not altered by prior bronchoconstriction, it is concluded that the bronchial hyperresponsiveness of asthmatic subjects to non-specific bronchoconstrictor stimuli is unlikely to be a direct consequence of their low starting airway calibre.

It has been proposed that one factor causing increased responsiveness of the airways of asthmatic subjects to bronchoconstrictor stimuli is their low starting airway calibre.¹ In a previous study,² in which the airway response to histamine aerosol was characterised as the dose required to lower specific airway conductance (sGaw) by 35% (that is, PD_{35}), we found that asthmatic subjects had a lower PD_{35} than normal subjects. On average, the asthmatic patients had lower airway conductance before bronchial challenge than normal subjects. When they were given bronchodilator (atropine or salbutamol) to bring prechallenge sGaw to within the normal range, PD_{35} also increased to within the normal range.

We wondered whether this increase in PD_{35} could have been a direct effect of bronchodilatation in the manner previously proposed.¹ The aim of the pres-

ent study was to examine the effect of a prior reduction in airway calibre on the bronchial response to histamine. We have investigated whether pretreatment of normal subjects with a long acting bronchoconstrictor agent, methacholine, would result in bronchial hyperresponsiveness.

Methods

Nine normal, non-asthmatic subjects (table 1) were studied. They all gave informed consent to the study, which was approved by the Charing Cross Hospital ethical committee. None of the subjects suffered from hayfever or had a respiratory infection at the time of the study or during the preceding month. Subjects were asked not to smoke and not to drink caffeine containing beverages within two hours of the start of each study.

Airway calibre was assessed by determination of airways resistance in a constant volume body plethysmograph (Fenyves and Gut, Basel, Switzerland). For each measurement, the subject panted at a frequency of 1-2 cycles per second,³ and thoracic

Address for reprint requests: Dr PD Snashall, Department of Medicine, Charing Cross Hospital Medical School, London W6 8RF.

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Table 1 Characteristics and baseline values of FEV₁ (means with SD in parentheses) and specific airway conductance (sGaw) of nine normal subjects.

Sex (M:F)	Age (y)	Atopic*	Smoker	Height (m)	FEV ₁ (l BTPS)		sGaw (s ⁻¹ kPa ⁻¹)
					Observed	Predicted†	
5:4	25.0 (2.6)	2	1	1.73 (0.07)	3.81 (0.44)	3.83 (0.57)	1.94 (0.40)

*Positive skin prick test responses to more than four common allergens.

†From Cotes JE. *Lung function: assessment and application in medicine*. Oxford: Blackwell, 1975:390.

gas volume (TGV) was measured simultaneously. Specific airways conductance (sGaw = (Raw × TGV)⁻¹ in s⁻¹kPa⁻¹) was calculated.

Histamine acid phosphate (molecular weight = 308) dissolved in water was delivered intermittently as an aerosol from a Hudson nebuliser attached to a breath activated "dosimeter,"⁴ delivering 8 μl of aerosol per puff. The nebuliser was triggered by a fall of mouth pressure at the onset of inspiration for 0.6 s. The same nebuliser was used throughout the experiment.

After five measurements of resting sGaw over a period of 30 seconds, the subject took five slow, deep breaths of histamine aerosol, beginning from functional residual capacity. Inhalation of histamine aerosol was repeated at three minute intervals, the concentration of histamine being doubled with each repetition. The concentrations ranged from 1.63 to 208 mmol l⁻¹ (0.5–64 mg/ml) and the number of doses inhaled ranged from 6 to 9. Five sGaw measurements were made every two minutes after the inhalation of each concentration of histamine. Each challenge was terminated when sGaw had fallen by 50–70%, at which point the subject was aware of moderate chest tightness and wheeziness. This was promptly relieved by the inhalation of 200 μg salbutamol from an inhaler. Subjects avoided coughing and taking deep breaths, particularly during the phase of bronchoconstriction. The histamine challenge study took 20–25 minutes.

Each subject performed four histamine challenges on separate days. The first challenge was premedicated with 40 μl of 0.9% sodium chloride solution (control); the remaining three challenges were in random order. Two of these challenges were performed after premedication with inhaled methacholine hydrochloride 128 mmol l⁻¹ (25 mg/ml) to reduce starting sGaw by about 50%. Seven subjects received 40 μl (5.1 μmol) of methacholine and two subjects 80 μl (10.2 μmol). A further control challenge with saline as premedication was performed to assess reproducibility. In each case the premedicating aerosol was inhaled five minutes before the first dose of histamine. Measurements of sGaw were made two and four minutes before the

start of histamine challenge. The subjects were unaware of the nature of the premedicating solutions. At least 48 hours elapsed between successive challenge studies.

The arithmetic mean of each set of five measurements was plotted against the logarithm to base 10 of the cumulative dose of histamine delivered to the subject. Each dose response curve had an initial horizontal portion followed by a fall. As previously described,² we determined from each curve (a) starting sGaw, measured after inhalation of premedicating drug; (b) the cumulative dose of histamine that produced a 35% fall in sGaw (PD₃₅); and (c) the steepest slope of the response.

It was important to determine that methacholine was producing bronchoconstriction which was sustained for the 20–25 minutes required to define the dose response relationship to histamine. We therefore studied the duration of effect of methacholine on seven normal subjects, three of whom were subjects for the main experiment. Four of these subjects inhaled 40 μl and the remaining three subjects 80 μl of 2.5% methacholine hydrochloride solution to obtain a 40–50% fall in resting sGaw. Five measurements of sGaw were made at specific times after inhalation of methacholine—namely, at 2, 4, 6, and 10 minutes and subsequently at five minute intervals until the 40th minute.

The responses of the two control histamine challenges were compared with a paired *t* test and those of all four challenges were compared with a two factor analysis of variance.⁵ We accepted a *p* value of < 0.05 as indicating a significant difference. All results are quoted as means with standard deviations in parentheses.

Results

METHACHOLINE INDUCED BRONCHOCONSTRICTION (fig 1)

Methacholine reduced mean sGaw from 1.75 (0.54) s⁻¹kPa⁻¹ to 0.90 (0.29) s⁻¹kPa⁻¹ by 2 minutes after inhalation (fig 1). Mean sGaws at 4, 6, 10, 15, 20, and 25 minutes did not differ significantly from mean sGaw at 4 minutes (paired *t* test), indicating

Fig 1 Time course of the effect of methacholine on mean specific airway conductance in seven normal subjects (bars indicate SEM). Maximum bronchoconstriction was achieved within two minutes and was sustained for 25 minutes.

that bronchoconstriction was sustained for 25 minutes. By 30, 35, and 40 minutes mean sGaw had increased significantly ($p < 0.05$).

CONTROL AND METHACHOLINE PREMEDITATED HISTAMINE CHALLENGES (figs 2 and 3)

The mean initial sGaw for the first set of control challenges was $1.85 (0.28) \text{ s}^{-1} \text{ kPa}^{-1}$ and was not significantly changed after inhalation of 0.9%

Fig 2 Individual PD_{35} values for histamine, with means and SEM, after premedication with 0.9% sodium chloride (○) and methacholine (Δ), which caused a mean fall of 40% from the starting specific conductance.

Fig 3 Individual values of slopes of histamine dose response curves, with means and SEM, after premedication with 0.9% sodium chloride (○) and methacholine (Δ). After bronchoconstriction with methacholine there was a mean fall in slope of about 45% in both sets of challenges.

sodium chloride solution at $1.98 (0.28) \text{ s}^{-1} \text{ kPa}^{-1}$. The geometric mean PD_{35} was $7.19 \mu\text{mol histamine}$ and the mean steepest slope was $1.80 (0.33) \text{ s}^{-1} \text{ kPa}^{-1} \log^{-1} \mu\text{mol histamine}$ (table 2). The mean starting sGaw, mean PD_{35} , and mean steepest slope for the second set of control challenges were not significantly different from those of the first challenge ($p > 0.25$). The within subject coefficients of variation for PD_{35} and steepest slope were 27% and 24% respectively.

For the first methacholine premedicated challenge the mean initial sGaw fell from $1.96 (0.43)$ to $1.13 (0.30) \text{ s}^{-1} \text{ kPa}^{-1}$ after methacholine ($p < 0.005$). The geometric mean PD_{35} was $6.71 \mu\text{mol histamine}$ ($-3.5 + 7.4 \text{ SD}$) and the mean steepest slope was $0.96 (0.39) \text{ s}^{-1} \text{ kPa}^{-1} \log^{-1} \mu\text{mol histamine}$.

A similar degree of bronchoconstriction was obtained for the second methacholine premedicated challenge, with mean sGaw falling from $1.93 (0.40)$ to $1.13 (0.25) \text{ s}^{-1} \text{ kPa}^{-1}$ after methacholine. Geometric mean PD_{35} was $7.08 (-2.5 + 3.9 \text{ SD}) \mu\text{mol histamine}$ and mean steepest slope was $0.91 (0.39) \text{ s}^{-1} \text{ kPa}^{-1} \log^{-1} \mu\text{mol histamine}$. There was no significant difference between the PD_{35} values obtained for all four sets of challenges (F ratio = 0.15, $p > 0.25$) but there was a significant difference between the four sets of slopes (F ratio = 19.7, $p < 0.0001$). Thus prior bronchoconstriction with methacholine did not alter PD_{35} but resulted in a significant fall in slope.

The dose response curves obtained from four sub-

Table 2 Individual starting values of sGaw, PD₃₅, and steepest slopes for histamine challenges

Subject No	1st control			2nd control			1st postmethacholine*				2nd postmethacholine‡			
	Starting sGaw*	PD ₃₅	Steepest slope	Starting sGaw*	PD ₃₅	Steepest slope	Baseline sGaw†	Starting sGaw*	PD ₃₅	Steepest slope	Baseline sGaw†	Starting sGaw*	PD ₃₅	Steepest slope
1	1.60	10.4	1.30	1.64	7.8	1.43	1.58	1.15	7.0	1.18	1.37	0.95	9.9	0.55
2	1.98	10.1	2.06	2.13	11.4	1.92	2.88	1.64	10.9	1.75	2.63	1.38	4.9	0.99
3	1.37	6.4	1.40	1.56	3.9	1.03	1.38	0.62	4.2	0.46	1.37	0.78	5.6	0.54
4	2.10	9.4	1.79	1.96	6.8	2.13	2.07	1.20	20.8	0.96	2.12	1.39	8.1	0.87
5	2.10	5.5	1.95	2.46	5.2	2.44	1.74	1.06	3.1	1.20	1.83	1.14	5.7	1.24
6	2.10	3.1	2.18	2.10	4.9	1.87	2.13	1.02	2.6	0.56	2.07	0.77	5.2	0.42
7	1.96	10.4	2.18	1.80	13.5	1.33	1.98	0.95	9.1	0.72	2.09	1.42	8.6	1.59
8	1.62	3.3	1.51	2.05	2.9	1.99	1.74	0.99	3.9	1.00	1.80	1.20	4.6	1.22
9	1.83	14.0	1.81	2.13	11.4	1.48	2.12	1.50	14.6	0.84	2.09	1.11	17.6	0.72
Mean	1.85	7.19§	1.80	1.98	6.68§	1.74	1.96	1.13	6.71§	0.96	1.93	1.13	7.08§	0.91
SD	0.27	-2.9, +5.1	0.33	0.28	-2.8, +4.5	0.45	0.43	0.30	-3.5, +7.4	0.39	0.40	0.25	-2.5, +3.9	0.39

*Measured four minutes after inhalation of 0.9% sodium chloride (control) or methacholine.

†Measured before methacholine.

‡All subjects inhaled 40 µl of methacholine 2.5% except for subjects 7 and 9, who inhaled 80 µl.

§Geometric mean.

jects are shown in figure 4 and the values for starting sGaw, PD₃₅, and steepest slope for each subject are shown in table 2.

RELATIONSHIP BETWEEN STARTING sGaw AND SLOPE AND BETWEEN STARTING sGaw AND PD₃₅ (figs 5 and 6)

There was a significant linear relationship between starting sGaw and slope for the 18 control and for the 18 methacholine premedicated challenges (fig 5). There was no significant difference in the slopes of these two regression lines ($p > 0.1$), but their positions were significantly different ($p < 0.001$). There was no significant linear relationship between starting sGaw and PD₃₅ (fig 6).

Discussion

In these studies we have reduced airway conductance before histamine challenge into the range previously observed in asthmatic subjects.² This manoeuvre did not increase bronchial responsiveness to histamine; PD₃₅ for normal subjects remained five times higher than that of asthmatics. These findings do not support the hypothesis that the hyperreactivity of the asthmatic is a consequence of his reduced starting airway calibre.

The effect of bronchoconstriction on the slope of the response was predictable from our previous findings.^{2,6} We have found with both histamine and methacholine challenges a positive linear correlation between starting airway conductance and slope for

Fig 4 Histamine dose response curves from four subjects. Each performed four challenge tests—two after inhalation of 0.9% sodium chloride (○) and two after methacholine (Δ). Histamine PD₃₅ was not altered by prior bronchoconstriction but the slope of the response was reduced in proportion to the decrease in specific conductance.

Fig 5 Relationship between starting specific conductance and steepest slope for challenges after 0.9% sodium chloride ($\circ$, $n = 18$) and methacholine (Δ , $n = 18$). The linear regression lines for placebo challenges ($y = 1.14x - 0.40$, $r = 0.78$, $p < 0.001$) and for methacholine premedicated challenges ($y = 1.04x - 0.24$, $r = 0.74$, $p < 0.001$) are shown.

both spontaneous changes and drug induced increases in airway calibre. Thus bronchodilatation results in an increase in the steepest slope, and we have now shown a reduction in slope after bronchoconstriction. The relationship between starting conductance and slope was quantitatively similar to

Fig 6 Relationship between starting specific conductance and PD_{35} for challenges after 0.9% sodium chloride ($\circ$, $r = 0.02$, $p > 0.25$) and after methacholine (Δ , $r = 0.37$, $p > 0.05$).

our previous findings, a halving the prechallenge airway conductance resulting in an approximate halving of the slope. This is an important relationship because it implies that when dose response curves from control challenges and after bronchoconstriction are adjusted by expressing airway conductance as percentage changes of the starting value they will be roughly parallel or superimposed (fig 7), and if this is so then a single value for the intercept PD_{35} describes their position. Since the regression lines shown in figure 5 do not pass through the origin, "normalisation" will result in a small error.

Our observation that prior bronchoconstriction did not alter bronchial responsiveness to histamine is interesting in the light of factors which have been suggested to increase the normalised slope and decrease the intercept of the dose response curve. The hyperbolic relationship between airways resistance and calibre (Poiseuille's law), the presence of a thicker bronchial wall,⁷ and the increase in activity of airway irritant receptors with bronchoconstriction⁸ may result in an amplification of the bronchoconstrictor response and therefore in a steeper slope. In the bronchoconstricted state airway smooth muscle may be operating on a steeper part of its length tension curve, such that less histamine may be needed to induce a response and a greater degree of airway narrowing would result.¹ Differences in aerosol deposition may also be expected to alter the PD_{35} . A more central deposi-

Fig 7 Histamine dose response curves for one subject with specific conductance expressed as percentages of starting $sGaw$. Challenges were obtained after premedication with 0.9% sodium chloride ($\circ$ — $\circ$) and after methacholine (Δ — Δ). These curves (obtained from subject 9—see fig 4) have been superimposed after "normalisation" for the starting value of $sGaw$.

tion of inhaled aerosol is seen in the bronchoconstricted state⁹ and Ruffin and colleagues¹⁰ have shown that asthmatic subjects needed much less histamine to cause a response when aerosol was deposited in the central airways than when the histamine is deposited in central and peripheral airways.

The relationship between airway calibre and responsiveness is more complex in the asthmatics and it may be difficult to dissociate changes in airway calibre from accompanying changes in airway responsiveness if both events result from similar factors. Thus during an attack of clinical asthma both the airway narrowing and airway hyperresponsiveness may be caused by bronchial inflammation and oedema. In our previous studies of asthmatic patients, in a stable clinical condition,^{2,6} we found no relationship between prechallenge airway conductance and the PD₃₅ for histamine or methacholine, but in a group of patients with more severe asthma we may expect to find that the most severely affected have the lowest airway calibre and the highest responsiveness.¹¹⁻¹³ The present study on normal subjects, however, suggests that these two factors can be separated, as does the demonstration of an increased responsiveness in normal subjects after upper respiratory tract viral infections¹⁴ or brief exposure to pollutants^{15,16} without any accompanying changes in airway calibre. Our results suggest that the airway hyperresponsiveness of the asthmatic is not the consequence of a reduction in starting airway calibre per se.

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