The protective effect of a β_2 agonist against excessive airway narrowing in response to bronchoconstrictor stimuli in asthma and chronic obstructive lung disease

E H Bel, A H Zwinderman, M C Timmers, J H Dijkman, P J Sterk

Abstract

Beta₂ agonists reduce airway hyper-sensitivity to bronchoconstrictor stimuli acutely in patients with asthma and chronic obstructive lung disease. To determine whether these drugs also protect against excessive airway narrowing, the effect of inhaled salbutamol on the position and shape of the dose-response curve for histamine or methacholine was investigated in 12 patients with asthma and 11 with chronic obstructive lung disease. After pretreatment with salbutamol (200 or 400 μ g) or placebo in a double blind manner dose-response curves for inhaled histamine and methacholine were obtained by a standard method on six days in random order. Airway sensitivity was defined as the concentration of histamine or methacholine causing a 20% fall in FEV₁ (PC₂₀). A maximal response plateau on the log dose-response curve was considered to be present if two or more data points for FEV₁ fell within a 5% response range. In the absence of a plateau, the test was continued until a predetermined level of severe bronchoconstriction was reached. Salbutamol caused an acute increase in FEV₁ (mean increase 11.5% predicted in asthma, 7.2% in chronic obstructive lung disease), an increase in PC₂₀ (mean 15 fold in asthma, fivefold in chronic obstructive lung disease), and an increase in the slope of the dose-response curves in both groups. In subjects in whom a plateau of FEV₁ response could be measured salbutamol did not change the level of the plateau. In subjects without a plateau salbutamol did not lead to the development of a plateau, despite achieving a median FEV₁ of 44% predicted in asthma and 39% in chronic obstructive lung disease. These results show that, although beta₂ agonists acutely reduce the airway response to a given strength of bronchoconstrictor stimulus, they do not protect against excessive air-flow obstruction if there is exposure to relatively strong stimuli. This, together with the steepening of the dose-response curve, could be a disadvantage of beta₂ agonists in the treatment of moderate and severe asthma or chronic obstructive lung disease.

Airway hyperresponsiveness is an almost universal feature of patients with symptomatic asthma^{1,2} and is common among patients with chronic obstructive lung disease.^{3,4} Hyperresponsiveness is usually defined as an increased sensitivity of the airways to inhaled non-sensitising bronchoconstrictor stimuli. Increased sensitivity is reflected by a leftward shift of the dose-response curve for inhaled histamine or methacholine, so that a smaller dose of agonist produces the same response of the airways.⁵ There is accumulating evidence that airway hyperresponsiveness is a more complex functional abnormality that comprises more than just hypersensitivity.⁶ Apart from a leftward shift, the shape of the dose-response curve for inhaled stimuli also differs between asthmatic and normal subjects.⁷ In normal subjects the dose-response curve achieves a plateau at mild degrees of airway narrowing,^{7,8} whereas in asthmatic subjects excessive degrees of airway narrowing can be obtained without the achievement of a plateau response.⁷

From a clinical point of view excessive airway narrowing may be a more troublesome and hazardous feature than increased airway sensitivity as such.⁹ We might therefore argue that treatment of patients with airway hyperresponsiveness should be directed not only towards shifting the dose-response curve to the right but also towards preventing excessive airway narrowing.

Bronchodilators such as beta₂ agonists are recommended as first choice drugs in the management of asthma and chronic obstructive lung disease,¹⁰ and are the most effective drugs available for acutely shifting the bronchoconstrictor dose-response curves to the right.¹¹ It is uncertain, however, whether bronchodilators also protect against excessive airway narrowing. We have therefore studied the effects of inhaled salbutamol on the position and the shape of the dose-response curves for histamine and methacholine in hyper-responsive patients with asthma or chronic obstructive lung disease.

Methods

SUBJECTS

Twelve patients with asthma and 11 with chronic obstructive lung disease volunteered to participate in the study. The asthmatic subjects (10 men and two women, mean age 24.9 (range

Lung Function Laboratory,
Department of Pulmonology,
University Hospital,
NL-2333 AA Leiden
E H Bel
P J Sterk
M C Timmers
J H Dijkman
Department of Medical Statistics,
State University,
NL-2333 CA Leiden
A H Zwinderman
The Netherlands

Reprint requests to: Dr Bel.
Accepted 11 October 1990

22–32) years) had a history of episodic chest tightness or wheezing, were atopic (more than one weal response to skinprick tests with 13 common allergens), and were lifelong non-smokers. Their mean forced expiratory volume in one second (FEV₁) was 82.7% (range 67.2–107.3%) of the predicted value,¹² whereas the postbronchodilator (400 µg inhaled salbutamol) FEV₁ and ratio of FEV₁ to vital capacity (FEV₁/VC) were within the predicted range.¹² The geometric mean provocative concentration of inhaled methacholine that caused a 20% fall in FEV₁ (PC₂₀) was 0.94 (range 0.11–2.86) mg/ml.⁵

The subjects with chronic obstructive lung disease (10 men and one woman, mean age 54 (range 30–71) years) were non-atopic and heavy smokers or ex-smokers with a smoking history of at least 30 pack years. The mean FEV₁ was 61.6% (range 44.5–82.1%) of the predicted value,¹² and the postbronchodilator FEV₁ or FEV₁/VC was below the predicted range.¹² The geometric mean PC₂₀ methacholine was 3.12 (range 0.54–7.7) mg/ml.

Subjects were taking inhaled bronchodilators alone, and these were withheld for at least eight hours before each inhalation test. No subject had ever received systemic or inhaled corticosteroids.

The study was approved by the Leiden University Hospital ethics committee and informed consent was obtained from all subjects.

STUDY DESIGN

Subjects underwent an inhalation challenge test on seven days within three weeks at the same time of day. At the first (screening) visit a control methacholine challenge was performed to assess baseline bronchial hyperresponsiveness and to investigate whether a maximal response plateau could be documented. If a plateau could not be measured, the challenge test was continued until a predetermined level of severe bronchoconstriction occurred: a fall in FEV₁ to below 50% (for asthma) or 60% (for chronic obstructive lung disease) of the pre-study postbronchodilator FEV₁; this was called the challenge limit. By the use of this challenge limit FEV₁ did not fall below 750 ml in any patient. Similar dose-response curves for inhaled methacholine or histamine were obtained on each of the next six days after double blind pretreatment with either 200 or 400 µg inhaled salbutamol or placebo in random order.

RESPIRATORY MEASUREMENTS

On each test day baseline FEV₁ and VC were determined as the mean of three reproducible measurements (within 5%), a dry rolling seal spirometer (Morgan, Spiroflow) being used. The subject then inhaled placebo or 200 or 400 µg salbutamol via a Volumatic spacer. After 15 minutes measurements of FEV₁ were repeated, and a methacholine or histamine challenge was performed by a standardised tidal breathing method.¹³ Dose-response curves were obtained after inhalation of doubling concentrations of acetyl-β-methylcholine chloride (0.03–256 mg/ml) in normal saline or histamine acid phos-

phate (0.03–64 mg/ml) in normal phosphate buffered saline. The aerosols were generated by a DeVilbiss 646 nebuliser (output 0.13 ml/min) and inhaled by tidal breathing for two minutes. The response was measured as change in FEV₁. The test was discontinued if the highest concentration of histamine or methacholine was inhaled or if the challenge limit determined at the screening visit was reached.

ANALYSIS

FEV₁ was plotted against log concentration of nebulised histamine or methacholine. The position of the dose-response curve was expressed in the traditional way as the provocative concentration of methacholine or histamine causing a fall in FEV₁ of 20% from post-drug baseline values (PC₂₀), and was calculated by linear interpolation of the two adjacent data points.¹³ To allow comparison of dose-response curves with different baseline FEV₁ values the slope and maximal response were analysed by expressing the response as a percentage of the predicted value.¹⁴ The slope of the sigmoid shaped dose-response curve was calculated by linear regression analysis of the data points used to calculate the PC₂₀ plus all consecutive data points up to the last point in the absence of a plateau, or up to the first point on the plateau. A maximal response plateau was considered to be present if two or more of the highest doses fell within a 5% response range; the maximum response was then calculated by averaging the consecutive data points on the plateau.⁸ If a plateau could not be measured, the last of the data points of the dose-response curve (lowest FEV₁) was used in the analysis, provided that the challenge limit ($\pm 5\%$), as determined at the screening visit, was reached. Analysis of variance and Student's unpaired and paired *t* tests were used to analyse differences in the natural log PC₂₀ or log slope between and within groups. McNemar tests were used to analyse differences in the lowest FEV₁.¹⁵ Values of *p* below 0.05 were considered statistically significant.

Results

BRONCHODILATATION

The mean (SD) baseline FEV₁ was 83% (11%) predicted in the group with asthma and 62% (12%) predicted in the group with chronic obstructive lung disease. The mean (SD) increase in FEV₁ after 200 µg salbutamol was 11.5% (7.8%) predicted in the subjects with asthma and 6.9% (7.5%) predicted in those with chronic obstructive lung disease; after 400 µg salbutamol the mean increases were 11.0% (7.8%) and 7.2% (9.7%) predicted.

BRONCHOCONSTRICTOR DOSE-RESPONSE CURVES

Representative dose-response curves for histamine and methacholine after the different treatments in one subject with asthma and one subject with chronic obstructive lung disease are shown in figure 1.

AIRWAY SENSITIVITY

The geometric mean PC₂₀ (SD in doubling

Figure 1 Representative dose-response curves for histamine and methacholine in one subject with asthma (open symbols) and one subject with chronic obstructive lung disease (closed symbols) before (circles) and after salbutamol 200 μ g (squares) and 400 μ g (triangles).

concentrations) for histamine and methacholine after placebo and 200 and 400 μ g salbutamol are given in table 1. After placebo treatment the PC_{20} did not differ between histamine and methacholine for the group as a whole when expressed in mg/ml ($p > 0.1$). When expressed on a molar basis, however, the patients with chronic obstructive lung disease appeared to be more sensitive to histamine than methacholine ($p < 0.01$).

After 200 and 400 μ g salbutamol there was a significant increase in PC_{20} in both groups for both agonists ($p < 0.001$). The increase in PC_{20} was larger in the group with asthma (mean 15 fold, 3.9 doubling concentrations) than it was in those with chronic obstructive lung disease (fivefold, 2.3 doubling concentrations) for both histamine ($p < 0.02$) and methacholine ($p <$

0.02). There was a small salbutamol dose-response effect for the increase in PC_{20} , which achieved significance for histamine ($p < 0.05$) but not for methacholine ($p > 0.1$) in both groups. The increase in PC_{20} was not related to the increase in FEV_1 in either group for histamine or methacholine ($p > 0.05$), whereas it was inversely correlated with baseline PC_{20} ($p < 0.05$), except for methacholine in those with chronic obstructive lung disease.

EXCESSIVE AIRWAY NARROWING

The lowest FEV_1 values seen after inhaled histamine or methacholine in each subject (expressed as plateau value (p) or as the last of the data points on the dose-response curve) after placebo and 200 and 400 μ g salbutamol are shown in table 2.

After placebo it was possible to obtain a response plateau for histamine in only three patients with chronic obstructive lung disease, and in none of those with asthma. With methacholine a plateau was reached in four subjects with chronic obstructive lung disease and in three with asthma (fig 2). The plateau levels in these subjects did not change after 200 or 400 μ g salbutamol (except in subject 20) when assessed against the confidence intervals of the difference between repeated single plateau measurements ($\pm 7.8\%$) obtained in previous studies.⁸ The number of subjects who reached the predetermined challenge limit without a plateau response to histamine or methacholine did not differ between placebo and 200 or 400 μ g salbutamol ($p > 0.3$) (fig 1).

Table 1 Geometric mean PC_{20} (mg/ml)* (SD in doubling concentrations) histamine and methacholine

		<i>Salbutamol</i>		
		<i>Placebo</i>	<i>200 µg</i>	<i>400 µg</i>
Asthma				
PC ₂₀ histamine	1.27 (1.5)	15.51 (1.2)	20.99 (1.3)	
PC ₂₀ methacholine	0.94 (1.3)	13.90 (1.5)	14.77 (1.0)	
Chronic obstructive lung disease				
PC ₂₀ histamine	1.89 (1.4)	8.13 (1.0)	13.11 (0.9)	
PC ₂₀ methacholine	3.13 (1.2)	12.08 (1.4)	15.59 (1.5)	

*Two minutes' nebulisation of 1 mg/ml histamine corresponds with 0.85 μ mol histamine delivered; two minutes' nebulisation of 1 mg/ml methacholine corresponds with 1.33 μ mol methacholine delivered.

Table 2 Ultimate degree of airflow obstruction in response to methacholine and histamine expressed in lowest FEV₁ (% predicted)

Subject No	Pre-study FEV ₁ (post-bronchodilatation; % pred)	Challenge limit	PC ₂₀ histamine (mg/ml)	Histamine			Methacholine		
				Placebo	Salbutamol		Placebo	Salbutamol	
					200 µg	400 µg		200 µg	400 µg
Asthma									
1	120	60	4.24	60	59	57	59	*	59
2	100	50	1.82	46	*	*	51 (p)	51 (p)	*
3	86	43	4.98	35	39	*	49 (p)	*	46 (p)
4	99	50	4.31	42	*	*	47	*	*
5	97	49	0.17	43	51	37	43	36	41
6	87	44	1.31	38	40	36	42	43	39
7	91	46	0.47	38	42	37	35	42	42
8	97	49	0.91	42	45	48	48	46	42
9	100	50	2.88	44	50	*	38	44	48
10	79	40	1.12	38	*	*	37	41	36
11	100	50	0.52	40	48	44	44	35	48
12	100	50	0.70	28	*	*	52 (p)	43	51 (p)
Chronic obstructive lung disease									
13	83	50	5.01	46	50	*	50	48	50
14	52	31	6.18	36 (p)	34	32 (p)	38 (p)	33 (p)	36 (p)
15	66	40	2.56	33	32	30	32	38	37
16	82	49	0.32	35	30	39	44 (p)	40	31
17	69	41	0.54	37	38	36	25	39	25
18	77	46	2.87	39	47	40	46	45	49
19	78	47	2.45	41	41	50	42	45	45
20	70	42	2.24	42 (p)	42	51 (p)	52 (p)	44 (p)	42
21	52	31	0.99	33 (p)	34 (p)	30	28	30	30
22	54	32	0.85	28	33	28	29	29	35
23	83	50	6.00	44	45	40	43 (p)	40	39

*Challenge limit not obtained after highest dose of bronchoconstrictor.
(p) Plateau value.

SLOPE OF THE DOSE-RESPONSE CURVE

Values for the slopes were not normally distributed and were therefore log transformed before analysis (table 3). After placebo the slope of the histamine curve did not differ from the slope of the methacholine curve in either group ($p > 0.3$). The slopes of the histamine

and methacholine curves were, however, significantly steeper in patients with asthma than in those with chronic obstructive lung disease ($p < 0.05$; fig 1). After 200 µg and 400 µg salbutamol there was an increase in the slope of the curves for histamine and methacholine in each group ($p < 0.02$). There was a positive correlation between the increase in FEV₁ and the increase in slope of the methacholine and histamine curves in asthma ($p < 0.05$) and in chronic obstructive lung disease ($p < 0.01$).

Figure 2 Individual dose-response curves for inhaled methacholine (expressed in FEV₁, % predicted) in 12 asthmatic patients and 11 patients with chronic obstructive lung disease.

Discussion

This study shows that, although inhaled salbutamol increases baseline airway calibre acutely and decreases the sensitivity of the airways to bronchoconstrictor stimuli, it does not protect against excessive airway narrowing. On the contrary, the slope of the dose-response curves for histamine and methacholine were steeper after salbutamol, implying a more rapid

Table 3 Mean ln slopes* (SD) of histamine and methacholine dose-response curves

		<i>Salbutamol</i>	
	<i>Placebo</i>	<i>200 µg</i>	<i>400 µg</i>
Asthma			
Histamine	2.57 (0.40)	3.29 (0.29)	3.41 (0.33)
Methacholine	2.68 (0.36)	3.01 (0.38)	2.92 (0.30)
Chronic obstructive lung disease			
Histamine	2.25 (0.30)	2.74 (0.38)	2.80 (0.37)
Methacholine	2.18 (0.45)	2.42 (0.50)	2.54 (0.60)

*The natural log of slopes expressed as fall in FEV₁ (% predicted)/ln mg/ml is used.

increase in airflow obstruction should there be exposure to high doses of bronchoconstrictor stimuli. This raises questions about the value of β_2 agonists in the treatment of asthma and chronic obstructive lung disease.

This is the first study in which the protective effect of a β_2 agonist against excessive airway narrowing in response to bronchoconstrictor stimuli has been investigated. Most previous reports have focused on the influence of bronchodilators on the sensitivity of the airways to inhaled bronchoconstrictors.^{4 11 16-25} In these studies β_2 agonists have been shown to shift the dose-response curve by about 2-4 doubling doses in asthma^{11 16-23} and by about 0.5-2.5 doubling doses in chronic obstructive lung disease.^{24 25} The results of the present study confirm these data, showing a greater rightward shift of the dose-response curve in asthma than in chronic obstructive lung disease. Our results show that the shift of the dose-response curve is unrelated to the degree of bronchodilatation induced by salbutamol. This was not found by others,²² perhaps owing to differences in salbutamol doses. We were, however, able to confirm that the shift of the curve is greatest in the most sensitive subjects.^{18 21} The results of the present study show only a small dose-effect relationship of salbutamol and sensitivity to histamine and methacholine. This might be due to the relatively small increment in the salbutamol dose or to domination of the spasmogenic activity of methacholine and histamine over the relaxing capacity of salbutamol.²⁶ This is in accordance with the results from studies on airway smooth muscle *in vitro*, in which relative resistance of submaximal contractions to the relaxant effect of β_2 agonists has been observed.^{26 27} Overall our observations show the varying potency of the β_2 adrenergic bronchodilator salbutamol in protecting against hypersensitivity to bronchoconstrictor stimuli.

The main finding of the present study is the lack of any effect of salbutamol on excessive degrees of airflow obstruction to inhaled histamine or methacholine in asthma and chronic obstructive lung disease. In the subjects in whom a maximal response plateau could be measured, the β_2 adrenergic agent appeared not to change the presence of the plateau or its level. And in those subjects in whom a plateau could not be measured salbutamol pretreatment did not lead to the development of a plateau, despite achieving a median fall in FEV₁ down to 44% (asthma) and 39% (chronic obstructive lung disease) predicted, compared with 43% and 36% respectively after placebo. Despite a return of PC₂₀ values into the normal range after salbutamol in some instances, patients still had excessive airway narrowing in response to higher doses of histamine or methacholine. This is in sharp contrast to normal subjects, who at similar degrees of airway sensitivity show a plateau response at mild degrees of airway narrowing when exposed to high doses of histamine or methacholine.²⁸ As appears from modelling studies²⁹ and experimental evidence,⁶ the maximal degree of airway

narrowing is determined not only by intrinsic contractility of airway smooth muscle and the mechanical load the muscle has to overcome during contraction but also by the thickness of the airway wall. Swelling of the airway wall secondary to inflammatory processes has been reported both in asthma³⁰ and chronic obstructive lung disease.³¹ Apparently salbutamol did not acutely affect this later mechanism. This is in accordance with its mode of action as it is a functional antagonist of histamine and methacholine on smooth muscle contraction.

Besides position and shape, the slope of the dose-response curve is another index of the *in vivo* response to bronchoconstrictor stimuli. In the present study there was an increase in slope of the histamine and methacholine dose-response curve after salbutamol, both in the patients with asthma and in those with chronic obstructive lung disease. In previous studies investigating the effects of β_2 agonists on the slope of the dose-response curves for histamine and methacholine in asthma the results have been controversial,¹⁸⁻²¹ perhaps owing to the method of expressing the response (% fall from baseline or predicted value),²¹ or to there being a smaller number of data points on the curves than in the present study. The reasons for the increase in slope after inhalation of salbutamol are not clear. They may be related to differences in drug penetration and deposition between dilated and constricted airways,³² or to an increase in mucosal thickness induced by the higher doses of histamine and methacholine or by salbutamol itself.³³

Apart from the effect of β_2 agonists, this study shows differences between asthma and chronic obstructive lung disease in the position and shape of the dose-response curves for histamine and methacholine. Firstly, asthmatic patients were equally sensitive to histamine and methacholine, whereas patients with chronic obstructive lung disease were more sensitive to histamine than to methacholine, as found previously.³⁴ Secondly, there was a steeper slope of the dose-response curves in asthma than in chronic obstructive lung disease, fitting in with the characteristic symptoms of asthma (wide variations in airflow over short periods of time). Thirdly, only a few subjects with chronic obstructive lung disease reached a maximal response plateau on the dose-response curve for methacholine. The lack of a plateau in most of our subjects could be explained by increased airway wall thickness in chronic obstructive lung disease.³¹ Our findings are not, however, in agreement with an earlier study of smokers with hyperresponsive airways, most of whom showed a plateau in their dose-response curve for methacholine.³⁴ We have no explanation for these discrepant results. They could be due to different selection criteria of the subjects, yielding more severe airflow obstruction in our study.

The results of the present study have clinical implications. Firstly, β_2 agonists only partially protect against airway narrowing in patients with asthma or chronic obstructive lung disease exposed to strong stimuli. Even when airway sensitivity has returned to normal

after inhalation of salbutamol, potent bronchoconstrictor stimuli will result in excessive airflow obstruction with a steeper dose-response slope. Secondly, the degree of protection by salbutamol, as shown by the rightward shift of the dose-response curve, is not increased by simply increasing the dose of drug from 200 to 400 μg . We believe that, taken together, these results may be important in view of increased deaths from asthma³⁵ and the recently reported association with another beta₂ agonist, fenoterol.³⁶ Beta₂ agonists provide immediate relief of symptoms, and are therefore popular. They do not, however, prevent excessive degrees of airway narrowing and are therefore not efficacious as the sole drug for treating moderate to severe asthma or chronic obstructive lung disease. In this respect drugs that have been shown to decrease the maximal degree of airway narrowing, such as inhaled corticosteroids,³⁷ are more promising.

We are grateful to Professor F E Hargreave for his valuable comments on the study protocol. We also thank Mrs A Middelham for preparation of the manuscript. The study was supported by grant 84.36 of the Netherlands Asthma Foundation and a grant from Glaxo BV, The Netherlands.

- 1 Hargreave FE, Dolovich J, O'Byrne PM, Ramsdale EH, Daniel EE. The origin of airway hyperresponsiveness. *J Allergy Clin Immunol* 1986;78:825-32.
- 2 Holgate ST, Beasley R, Twentyman OP. The pathogenesis and significance of bronchial hyperresponsiveness in airways disease. *Clin Sci* 1987;73:561-72.
- 3 Pride NB, Taylor RG, Lim TK, Joyce H, Watson A. Bronchial hyperresponsiveness as a risk factor for progressive airflow obstruction in smokers. *Bull Eur Physiopathol Respir* 1987;23:369-75.
- 4 Kamsdell JW, Nachtwey FJ, Moser KM. Bronchial hyper-reactivity in chronic obstructive bronchitis. *Am Rev Respir Dis* 1982;126:829-32.
- 5 Cockcroft DW. Nonallergic airway responsiveness. *J Allergy Clin Immunol* 1988;81:111-8.
- 6 Sterk PJ, Bel EH. Bronchial hyperresponsiveness: the need for a distinction between hypersensitivity and excessive airway narrowing. *Eur Respir J* 1989;2:267-74.
- 7 Woolcock AJ, Salome CM, Yan K. The shape of the dose-response curve to histamine in asthmatic and normal subjects. *Am Rev Respir Dis* 1984;130:71-5.
- 8 Sterk PJ, Daniel EE, Zamel N, Hargreave FE. Limited bronchoconstriction to methacholine using partial flow-volume curves in nonasthmatic subjects. *Am Rev Respir Dis* 1985;132:272-7.
- 9 Macklem PT. The clinical relevance of respiratory muscle research. *Am Rev Respir Dis* 1986;134:812-5.
- 10 Cockcroft DW. Airway hyperresponsiveness: therapeutic implications. *Ann Allergy* 1987;59:405-14.
- 11 Tattersfield AE. Effect of beta-agonists and anticholinergic drugs on bronchial reactivity. *Am Rev Respir Dis* 1987;136:S64-8.
- 12 Quanjer PhH, ed. Standardized lung function testing. *Bull Eur Physiopathol Respir* 1983;19(suppl 5):1-95.
- 13 Hargreave FE, Sterk PJ, Ramsdale EH, Dolovich J, Zamel N. Inhalation challenge tests and airway responsiveness in man. *Chest* 1985;87(suppl):202-6.
- 14 Eiser NM. Bronchial provocation tests. In: Nadel JA, Pauwels R, Snashall PD, eds. *Bronchial hyperresponsiveness: normal and abnormal control, assessment and therapy*. Oxford: Blackwell, 1987:173-254.
- 15 Armitage P. *Statistical methods in medical research*. 6th ed. Oxford: Blackwell, 1983:116-31, 189-97.
- 16 Casterline CL, Evans R, Ward GW. The effect of atropine and albuterol aerosols on the human bronchial response to histamine. *J Allergy Clin Immunol* 1976;58:607-13.
- 17 Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Protective effect of drugs on histamine-induced asthma. *Thorax* 1977;32:429-37.
- 18 Bandouvakis J, Cartier A, Roberts R, Ryan G, Hargreave FE. The effect of ipratropium and fenoterol on methacholine- and histamine-induced bronchoconstriction. *Br J Dis Chest* 1981;75:295-305.
- 19 Salome CM, Schoeffel RE, Woolcock AJ. Effect of aerosol and oral fenoterol on histamine and methacholine challenge in asthmatic subjects. *Thorax* 1981;36:580-4.
- 20 Salome CM, Schoeffel RE, Yan K, Woolcock AJ. Effect of aerosol fenoterol on the severity of bronchial hyperreactivity in patients with asthma. *Thorax* 1983;38:854-8.
- 21 Chung KF, Morgan B, Keyes SJ, Snashall PD. Histamine dose-response relationships in normal and asthmatic subjects. The importance of starting airway caliber. *Am Rev Respir Dis* 1982;126:849-54.
- 22 Chung KF, Snashall PD. Methacholine dose-response curves in normal and asthmatic man: effect of starting conductance and pharmacological antagonism. *Clin Sci* 1984;66:665-73.
- 23 Britton J, Hanley SP, Garrett HV, Hadfield JW, Tattersfield AE. Dose related effects of salbutamol and ipratropium bromide on airway calibre and reactivity in subjects with asthma. *Thorax* 1988;43:300-5.
- 24 Yan K, Salome CM, Woolcock AJ. Prevalence and nature of bronchial hyperresponsiveness in subjects with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1985;132:25-9.
- 25 Greenspon LW, Parrish B. Inhibition of methacholine-induced bronchoconstriction in patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1988;137:281-5.
- 26 Ariens EJ. Pharmacology of airway smooth muscle. In: Nadel JA, Pauwels R, Snashall PD, eds. *Bronchial hyperresponsiveness: normal and abnormal control, assessment and therapy*. Oxford: Blackwell, 1987:7-22.
- 27 Gunst SJ, Stropp JQ, Flavahan NA. Muscarinic receptor reserve and beta-adrenergic sensitivity in tracheal smooth muscle. *J Appl Physiol* 1989;67:1294-8.
- 28 Sterk PJ, Timmers MC, Dijkman JH. Maximal airway narrowing in humans in vivo: histamine compared with methacholine. *Am Rev Respir Dis* 1986;134:714-8.
- 29 Moreno RH, Hogg JC, Paré PD. Mechanics of airway narrowing. *Am Rev Respir Dis* 1986;133:1171-80.
- 30 James AL, Paré PD, Hogg JC. The mechanics of airway narrowing in asthma. *Am Rev Respir Dis* 1989;139:242-6.
- 31 Bosken C, Wiggs B, Paré PD, Hogg JC. Small airway dimensions in smokers with obstruction to airflow. *Am Rev Respir Dis* 1990;142:563-70.
- 32 Dolovich MB, Sanchis J, Rossman C, Newhouse MT. Aerosol penetrance: a sensitive index of peripheral airways obstruction. *J Appl Physiol* 1976;40:468-71.
- 33 Laitinen LA, Laitinen A, Widdicombe J. Effects of inflammatory and other mediators on airway vascular beds. *Am Rev Respir Dis* 1987;135:S67-70.
- 34 Du Toit JJ, Woolcock AJ, Salome CM, Sundrum R, Black JL. Characteristics of bronchial hyperresponsiveness in smokers with chronic airflow limitation. *Am Rev Respir Dis* 1986;134:498-501.
- 35 Sears MR. Increasing asthma mortality—fact or artifact? *J Allergy Clin Immunol* 1988;82:957-60.
- 36 Crane J, Flatt A, Jackson R, et al. Prescribed fenoterol and death from asthma in New Zealand, 1981-83: case-control study. *Lancet* 1989;i:917-22.
- 37 Bel EH, Timmers MC, Dijkman JH, Sterk PJ. The effect of long-term treatment with inhaled corticosteroids on the maximal degree of airway narrowing to methacholine in asthmatic subjects. *Am Rev Respir Dis* (in press).