Salmeterol tachyphylaxis in steroid treated asthmatic subjects

H Booth, R Bish, J Walters, F Whitehead, E H Walters

Abstract

Background – Tachyphylaxis to the protection afforded by salmeterol to bronchoconstrictor stimuli after regular use has been described in patients with mild asthma not receiving inhaled corticosteroids. The present study was performed to investigate whether airway tachyphylaxis occurs in symptomatic asthmatic subjects receiving inhaled corticosteroids, the group for whom salmeterol is recommended in clinical practice.

Methods – Thirty one adult patients with symptomatic chronic asthma who were receiving inhaled corticosteroids were randomised in a double blind manner and on a 2:1 basis to receive salmeterol 50 µg (n = 22) or placebo (n = 9) twice daily. Baseline forced expiratory volume in one second (FEV₁) was measured during the run-in period, on day 0, and after four and eight weeks of regular treatment (following a 36 hour test drug washout period). Airway responsiveness to methacholine was measured one hour after administration of the test drug on these occasions. Diary cards were kept throughout the study and for a two week follow up period.

Results – Baseline FEV₁ was not significantly different between the treatment groups or between visits. There was significant bronchodilatation one hour after salmeterol administration at 0, four, and eight weeks. No significant tachyphylaxis of the bronchodilator action of salmeterol was seen. Protection against methacholine induced bronchoconstriction reduced from 3.3 doubling dilutions after the first dose of salmeterol to two doubling dilutions after four and eight weeks of regular treatment. Symptom scores and "rescue" salbutamol use were significantly reduced during salmeterol treatment and daytime improvements were maintained into the follow up period.

Conclusions – Inhaled corticosteroids did not prevent tachyphylaxis to the protection afforded by salmeterol to methacholine induced bronchoconstriction. The clinical significance, if any, of these findings remains to be defined.

(Thorax 1996;51:1100-1104)

Keywords: tachyphylaxis, salmeterol, asthma.

bronchodilatation and protection against induced bronchoconstrictor stimuli – has been less consistently demonstrable. It has been described with short acting β_2 agonists but only in normal patients or in mild asthmatic subjects who require only short acting β_2 agonists for relief of symptoms.²⁻⁴ Airway tachyphylaxis may be expected to be more likely to develop with the newer longer acting β_2 agonists because of their higher potency and longer duration of action.⁵ Indeed, tolerance to the bronchodilator action of eformoterol⁶ and reduced protection to a variety of stimuli with regular salmeterol have been reported,^{7,8} but again these studies have been restricted to mild asthmatic subjects not receiving any regular inhaled corticosteroids. The interaction between corticosteroids and β_2 agonists is complex⁹ but reversal of tolerance to β_2 agonists by treatment with corticosteroids has been described.¹⁰

In a previous study we failed to find any statistically significant reduction in the protection afforded by salmeterol to methacholine induced bronchoconstriction after eight weeks of regular use in a group of moderate asthmatic subjects, most of whom were receiving inhaled corticosteroids.¹¹ In that study, however, we only performed bronchoconstrictor challenge at 12 hours after the last dose of regular salmeterol when protection is relatively weak, and there was no washout period as used in the study by Cheung *et al.*⁸

Current asthma guidelines recommend that the introduction of long acting β_2 agonists be considered when a patient continues to be symptomatic despite moderate to high doses of regular inhaled corticosteroid.¹² However, recent studies suggest they may be beneficially introduced earlier in the stepwise increase in asthma treatment.^{13,14} These studies highlight the rationale for performing the present study: firstly, that long acting β_2 agonists are likely to be used in conjunction with inhaled corticosteroids in the treatment of clinical asthma and studies of tachyphylaxis in mild patients using short acting β_2 agonists only when required are less clinically relevant; and secondly, the increasing importance of examining the safety of such drugs as their clinical indications for use are likely to increase.

The present study was undertaken to investigate whether tachyphylaxis of the airway effects of salmeterol occurs with regular treatment in a well defined group of asthmatic subjects all currently symptomatic despite maintenance inhaled corticosteroids, using a similar study design to that used by Cheung *et al.*⁸

Department of
Respiratory Medicine,
Alfred Hospital and
Monash Medical
School,
Victoria 3181,
Australia
R Bish
J Walters
F Whitehead
E H Walters

Department of
Thoracic Medicine,
The Middlesex
Hospital,
London W1N 8AA,
UK
H Booth

Correspondence to:
Professor EH Walters.

Received 11 October 1995
Returned to authors
16 January 1996
Revised version received
3 April 1996
Accepted for publication
25 April 1996

Tachyphylaxis or tolerance to the systemic side effects of β_2 agonists occurs readily.¹ However, tolerance to their airway actions – namely,

Figure 1 Study design.

Methods

SUBJECTS

All subjects had suffered from asthma for at least one year, had a prebronchodilator forced expiratory volume in one second (FEV₁) of at least 1.5 l, had demonstrated 15% reversibility in peak expiratory flow rate or FEV₁ at the screening visit, were receiving regular inhaled corticosteroids up to a maximum of 2000 µg/day, and had required at least 200 µg inhaled salbutamol for symptom relief during four of the first seven days of the run-in period.

All patients gave written informed consent to participate in the study which was approved by the Alfred Campus Joint Ethics Committee.

MEASUREMENTS

Airway responsiveness was measured using a standard dosimeter technique. Subjects performed three sets of six FEV₁ manoeuvres and then inhaled five breaths per dose from sequential doubling concentrations of methacholine chloride solution at five minute intervals. Cumulative doses ranged from 3.125 µg to 6400 µg. Aerosol was generated by a compressed air driven turbo jet nebuliser (Medic Aid, Pagham, UK) controlled by a breath activated dosimeter. Nebuliser output was calibrated to deliver 10 µl ($\pm 10\%$) of aerosol per inhalation. FEV₁ measurements were made using a dry bellows spirometer (Vitalograph) taking the mean of the best three of six readings. The methacholine test was completed when a 20% decrement from initial FEV₁ was attained. FEV₁ was plotted against log cumulative dose to obtain the dose of methacholine causing a 20% fall in FEV₁ (PD₂₀) by linear interpolation.

Day and night time symptom scores graded from 0 to 4, and the number of rescue doses of salbutamol used were recorded daily by subjects during the study and for two weeks immediately afterwards.

STUDY DESIGN

The study was of a randomised, double blind, placebo controlled, parallel group design and comprised a two week run-in period, an eight week treatment period, and then two weeks

of follow up (fig 1). A control methacholine challenge test was performed after one week of the run-in period (day -7) in subjects who fulfilled the entry criteria for the study. At the end of the run-in period (day 0) subjects were randomised, in the ratio of 2:1, to receive either salmeterol 50 µg or a matching placebo, both administered via a metered dose inhaler and large volume spacer (Volumatic) twice daily. The power of the study was increased by having a large actively treated group. On day 0 baseline FEV₁ was measured and the first dose of the patient's own test medication was then given by the investigator. A methacholine challenge test was performed one hour later. This sequence was repeated on days 28 and 56 of the treatment period but after a 36 hour "washout" period of each subject's test medication. Salbutamol was used as rescue medication but none was taken for at least eight hours before a methacholine challenge test. All testing was performed at the same time of day. The dose of inhaled corticosteroid for each subject was kept constant throughout the study.

DATA ANALYSIS

All PD₂₀ measurements were log₁₀ transformed before analysis. Changes in PD₂₀ from the run-in period were expressed in terms of doubling doses of methacholine calculated as $\Delta \log PD_{20} / \log_2$. Symptom scores were averaged over each study period and the values for the first and second month compared with each other and the run-in period. Continuous variable data collected in the run-in period for each treatment group were compared with non-paired *t* tests. Results of baseline FEV₁, FEV₁ one hour after test medication, PD₂₀ measurements, and symptom scores were analysed by repeated measures analysis of variance (MANOVA) using Genstat statistical software which took into account individual variations with time and between treatments. *p* values of <0.05 were considered significant.

Results

RUN-IN PERIOD

Twenty three subjects were randomised to receive salmeterol and 11 placebo. Three subjects were withdrawn from the study on or before day 28 of the treatment period, one from the salmeterol group who had persistently abnormal liver function tests, one from the placebo group because of inability to reattend, and another from the placebo group whose FEV₁ before the methacholine challenge was less than 1.5 l. Results from these subjects were excluded from further analysis.

The characteristics of the two treatment groups are shown in table 1. Subjects in the placebo group had a significantly longer history of asthma than those in the salmeterol group, possibly because of the slightly older age of the placebo group. There were no significant differences in lung function or airway responsiveness between the two treatment groups at randomisation. During the run-in period night time symptoms and salbutamol use were

Table 1 Mean (SE) characteristics of the treatment groups at randomisation

	Salmeterol (n = 22)	Placebo (n = 9)	p value
Age (years)	39.2 (3.2)	44.6 (3.1)	NS
Men (n)	11 (50%)	5 (56%)	
Atopic (n)	19 (86%)	9 (100%)	
Duration of asthma (years)*	21.5 (7-44)	36.3 (12-52)	0.02
Inhaled steroid dose (μ g)*	700 (200-2000)	800 (400-2000)	NS
FEV ₁ (l/min)	2.59 (0.13)	2.21 (0.14)	
% predicted FEV ₁	75.5 (2.7)	65.1 (5.2)	NS
PD ₂₀ (μ g)	12.5 (1.3)	9.0 (1.4)	NS
Night time			
Symptoms	0.38 (0.49)	0.57 (0.54)	0.02
Salbutamol use	0.75 (0.67)	1.52 (1.27)	0.02
Daytime			
Symptoms	1.08 (0.54)	1.09 (0.51)	NS
Salbutamol use	1.76 (0.79)	2.53 (1.42)	NS

* Data presented as median (ranges).

significantly higher in the placebo group, but there was no significant difference in daytime symptoms or salbutamol use between the two treatment groups.

TREATMENT PERIOD

Lung function

Baseline FEV₁ did not differ significantly between the two treatment groups during the study (fig 2). Significant bronchodilatation was seen one hour after administering salmeterol compared with placebo at each time point during the study (each $p < 0.001$). Tachyphylaxis of the bronchodilator action of salmeterol did not develop during the eight weeks of the study.

Airway responsiveness

Salmeterol significantly increased PD₂₀ measurements compared with placebo ($p = 0.01$, fig 3). The mean protection afforded against methacholine induced bronchoconstriction after a single dose of salmeterol was 3.3 doubling doses. After four and eight weeks of regular treatment there was still a significant increase in PD₂₀ measurements with salmeterol by a mean two doubling dilutions of methacholine (both $p < 0.001$), but this was significantly less than on day 0 ($p < 0.001$ at day 28, $p = 0.01$ at day 56). The FEV₁ measurement one hour after the trial medication had a significant effect

Figure 2 Mean (SE) forced expiratory volume in one second (FEV₁) at baseline and one hour after test medication following the first dose and after 28 and 56 days of regular treatment with salmeterol (solid bars) or placebo (open bars). * $p < 0.05$ before versus after salmeterol, and placebo versus salmeterol.

Figure 3 Mean (SE) change in PD₂₀ from the run-in period after the first dose and after 28 and 56 days of regular treatment with salmeterol (solid bars) or placebo (open bars). * $p < 0.05$ placebo versus salmeterol; ** $p < 0.05$ day 28/56 versus day 0.

on methacholine challenge results ($p < 0.01$). Significant tachyphylaxis was still found, however, even if the results were adjusted for this covariate.

PD₂₀ values for each individual are shown in table 2.

Symptom scores

In the salmeterol treated group night time and daytime symptoms ($p < 0.01$ and $p < 0.001$, respectively) and rescue salbutamol use (both $p < 0.001$) were significantly reduced, particularly in the second four weeks of treatment (table 3). No significant changes in any diary card parameter were seen in the placebo treated group during the study.

FOLLOW UP PERIOD

Night time symptom scores returned to pre-treatment levels but the reduction in night and daytime rescue medication and daytime symptoms in the salmeterol treated group was continued into the follow up period (table 3; all $p < 0.05$).

Discussion

We have shown, in a well defined group of symptomatic asthmatic subjects receiving inhaled corticosteroids, that a degree of tachyphylaxis to the protection afforded against methacholine induced bronchoconstriction occurs. The effect is variable and does not occur at all in some individuals. For the group as a whole, reasonably good protection is maintained. When it occurs, this fall off in protection is evident by the end of the first month with no further change over the second month of treatment. No evidence of tachyphylaxis to salmeterol induced bronchodilatation one hour after administration developed.

Human airway smooth muscle β_2 receptors have two actions – bronchodilatation and protection against induced bronchoconstriction – and appear to be relatively resistant to receptor

Table 2 Individual PD₂₀ results (µg methacholine)

	Run-in period	Day 0	Day 28	Day 56
Salmeterol group				
1	32.5	1725.0	126.3	347.5
2	32.5	100.0	100.0	62.8
3	10.6	47.0	103.8	61.3
4	4.4	68.5	18.1	13.3
5	10.5	125.0	26.9	38.0
6	41.8	181.0	34.0	28.4
7	3.9	19.3	1.7	3.7
8	14.4	80.5	44.0	29.1
9	57.5	325.0	750.0	550.0
10	29.9	237.5	810.0	637.5
11	22.0	200.0	170.0	88.8
12	2.1	48.5	10.4	19.8
13	13.5	331.3	76.0	111.3
14	5.8	237.5	55.3	47.8
15	4.1	34.8	35.5	23.5
16	1.6	19.4	12.5	17.4
17	3.0	28.4	29.5	21.8
18	2.1	13.3	3.0	2.1
19	58.3	650.0	96.3	126.3
20	12.5	31.4	6.6	28.8
21	15.9	362.5	76.3	52.3
22	400.0	3825.0	887.5	812.5
Geometric mean (SE)	12.5 (1.3)	122.2 (1.4)	48.5 (1.4)	49.5 (1.4)
Placebo group				
1	2.6	7.6	4.1	1.8
2	9.0	6.0	6.0	17.8
3	25.8	15.8	173.8	160.0
4	23.5	14.8	17.8	31.8
5	17.8	12.5	5.2	27.6
6	2.1	10.6	6.0	13.8
7	5.0	11.8	4.5	1.7
8	12.1	8.8	27.2	41.0
9	12.5	12.5	14.0	20.5
Geometric mean (SE)	9.0 (1.4)	10.7 (1.1)	11.7 (1.5)	16.4 (1.6)

Table 3 Mean (SD) results of diary card data

	Salmeterol	Placebo
Night time symptoms:		
Run-in	0.38 (0.49)	0.57 (0.54)
0-28 days treatment period	0.19 (0.35)*	0.54 (0.50)
29-56 days treatment period	0.16 (0.31)*	0.67 (0.64)
Follow up period	0.41 (0.67)	0.70 (0.63)
Night time salbutamol use:		
Run-in	0.75 (0.67)	1.52 (1.27)
0-28 days treatment period	0.38 (0.59)*	1.25 (1.27)
29-56 days treatment period	0.23 (0.45)*	1.27 (1.26)
Follow up period	0.66 (0.73)*	1.25 (1.10)
Daytime symptoms:		
Run-in	1.08 (0.54)	1.09 (0.51)
0-28 days treatment period	0.50 (0.54)*	1.07 (0.53)
29-56 days treatment period	0.43 (0.52)*	1.17 (0.76)
Follow up period	0.67 (0.66)*	1.12 (0.41)
Daytime salbutamol use:		
Run-in	1.76 (0.79)	2.53 (1.42)
0-28 days treatment period	0.81 (1.01)*	2.33 (1.42)
29-56 days treatment period	0.59 (0.86)*	2.36 (1.55)
Follow up period	1.25 (1.47)*	2.52 (1.22)

* p<0.05 compared with run-in period.

desensitisation compared with β_2 receptors on non-airway tissues.¹ However, we have shown that a degree of tachyphylaxis to methacholine induced bronchoconstriction only, and not bronchodilatation, occurs within four weeks of starting regular salmeterol treatment – a finding which agrees with other workers.⁸ Although Grove and Lipworth¹⁵ recently reported such an effect on salbutamol induced bronchodilatation after regular salmeterol, their data would suggest a major confounding effect of a sustained elevation of baseline FEV₁, thus allowing less room for improvement (data analysed as change in FEV₁ or peak expiratory flow rate with maximal values achieved being unchanged from baseline).

The mechanism of protection against induced bronchoconstriction by β_2 agonists is poorly understood, as is the reason that tachyphylaxis appears to develop more readily to this action compared with bronchodilatation.

It may be that the loss of bronchodilatation is masked by the position of salmeterol on the maximum plateau of the dose response curve. Alternatively, it has been suggested that the extra protection afforded by short acting β_2 agonists against indirectly acting bronchoconstrictor stimuli (such as adenosine 5'-monophosphate (AMP)³ and allergen⁴) compared with methacholine is mediated by β_2 receptors on inflammatory cells, particularly mast cells. Downregulation of these β_2 receptors may explain the disparity between tachyphylaxis to bronchodilatation and protection. This hypothesis is supported by the report of a greater degree of tachyphylaxis developing to the protection afforded by terbutaline to AMP compared with methacholine induced bronchoconstriction with its regular use.³ However, Soler *et al*¹⁶ found no difference in the degree of protection afforded by salmeterol 14 hours after dosing to AMP and histamine induced bronchoconstriction, suggesting that at this time point its action is mediated solely by functional antagonism at the smooth muscle β_2 receptor. It will be important to investigate changes in airway responsiveness to indirectly acting bronchoconstrictor stimuli with the regular use of long acting β_2 agonists.

The results of this study contrast with those in a group of mild to moderate asthmatic subjects, most of whom were taking inhaled corticosteroids, in whom methacholine challenge tests were performed 12 hours after the last dose of salmeterol.¹¹ Although the power of this earlier study was only 60%, the failure to demonstrate any airway tachyphylaxis at 12 hours compared with one hour may reflect changes in the pharmacodynamics of salmeterol with chronic dosing. There may be a difference between the intense early protection afforded by salmeterol and the lesser but prolonged and better sustained protection over 12 hours, the latter being more resistant to tachyphylaxis. However, Ramage *et al*⁷ reported tachyphylaxis of the protection afforded by salmeterol to exercise induced bronchoconstriction six and 12 hours after dosing with regular use.

The design and results of our study are similar to those of Cheung *et al*,⁸ although in a group of asthmatic subjects who were all symptomatic and receiving maintenance inhaled corticosteroids for whom salmeterol may be clinically indicated. It has been thought that one beneficial action of corticosteroids is to reverse or prevent tolerance developing to β_2 agonists. Indeed, in vitro, corticosteroids increase the levels of β_2 receptors on the cell surface and they have been shown to reverse the airway tachyphylaxis induced in normal volunteers to high dose inhaled salbutamol.¹⁷ The present study has failed to show that inhaled corticosteroids, even at high dose in some individuals, prevent the development of tachyphylaxis to methacholine induced bronchoconstriction to salmeterol in a group of symptomatic asthmatic subjects. However, Cheung *et al*⁸ reported the degree of protection to methacholine induced bronchoconstriction to drop from 3.3 doubling doses to one doub-

ling dose after four weeks of treatment with salmeterol. This is more pronounced than the results of the present study and suggests that inhaled corticosteroids may prevent the development of some airway tachyphylaxis. Whether corticosteroids given systemically can completely prevent tolerance developing, as has been reported in the animal model,¹⁸ needs further study.

It is apparent from our data that it is only a subgroup of asthmatic subjects, albeit probably a majority, who readily develop tachyphylaxis to β_2 agonists. The reason for this is unclear. However, recent evidence suggests that genetic variations of the β_2 adrenergic receptor confer different receptor expression and function on airway smooth muscle cells,¹⁹ and particular polymorphisms may be associated with more or less susceptibility to downregulation.²⁰ Future studies should concentrate on those individuals who develop the most profound degree of tachyphylaxis to ensure that any clinical deterioration in this small number of people is not missed by lack of change in group mean data.

It should be emphasised that, after eight weeks of regular treatment, salmeterol still affords large and significant protection against methacholine induced bronchoconstriction (approximately two doubling doses). The clinical significance of the small degree of tachyphylaxis remains uncertain. There was no tachyphylaxis of the beneficial effects of salmeterol on symptom scores as evidenced by our diary card data, and symptom scores continued to improve in the second four weeks of the treatment period. Similarly, there was no deterioration in baseline lung function, which tended to improve over the eight weeks of the study. That there is no diminution in clinical efficacy or lung function with regular salmeterol therapy is supported by larger clinical studies.^{21,22} Indeed, the addition of salmeterol to moderate doses of inhaled corticosteroids has been reported to give better asthma control than doubling the corticosteroid dose.^{13,14} However, longer term studies are needed to ensure that progressive tachyphylaxis does not occur and that this does not get translated into clinical vulnerability to unpredictable attacks.

The authors wish to thank Mrs Alison Avery, Department of Medical Statistics, Newcastle University, Newcastle upon Tyne, UK for her statistical help in the preparation of this manuscript. This study was supported by a grant-in-aid from Glaxo Group Research and Development.

- 1 Lipworth BJ, Struthers AD, McDevitt DG. Tachyphylaxis to systemic but not to airway responses during prolonged therapy with high dose inhaled salbutamol in asthmatics. *Am Rev Respir Dis* 1989;140:586-92.
- 2 Harvey JE, Tattersfield AE. Airway response to salbutamol: effect of regular salbutamol inhalations in normal, atopic, and asthmatic subjects. *Thorax* 1982;37:280-7.
- 3 O'Connor BJ, Aikman SL, Barnes PJ. Tolerance to the nonbronchodilator effects of inhaled β_2 agonists in asthma. *N Engl J Med* 1992;327:1204-8.
- 4 Cockcroft DW, McParland CP, Britto SA, Swystun VA, Rutherford BC. Regular inhaled salbutamol and airway responsiveness to allergen. *Lancet* 1993;342:833-7.
- 5 Johnson M. The preclinical pharmacology of salmeterol: bronchodilator effects. *Eur Respir Rev* 1991;1:253-6.
- 6 Newnham DM, McDevitt DG, Lipworth BJ. Bronchodilator subsensitivity after chronic dosing with eformoterol in patients with asthma. *Am J Med* 1994;97:29-37.
- 7 Ramage L, Lipworth BJ, Ingram CG, Cree IA, Dhillon DP. Reduced protection against exercise induced bronchoconstriction after chronic dosing with salmeterol. *Respir Med* 1994;88:363-8.
- 8 Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long-term effects of a long-acting β_2 -adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. *N Engl J Med* 1992;327:1198-203.
- 9 Mak JCW, Nishikawa M, Barnes PJ. Glucocorticosteroids increase β_2 adrenergic receptor transcription in human lung. *Am J Physiol* 1995;268:L41-6.
- 10 Tashkin DP, Connolly ME, Deutsch RI, Hui KK, Littner M, Scarpace P, et al. Subsensitization of β -adrenoceptors in airways and lymphocytes of healthy and asthmatic subjects. *Am Rev Respir Dis* 1982;125:185-93.
- 11 Booth H, Fishwick K, Harkawat R, Devereux G, Hendrick DJ, Walters EH. Changes in methacholine induced bronchoconstriction with the long acting β_2 agonist salmeterol in mild-to-moderate asthmatic patients. *Thorax* 1993;48:1121-4.
- 12 British Thoracic Society. Guidelines for management of asthma in adults: I - Chronic persistent asthma. *BMJ* 1990;301:651-4.
- 13 Greening AP, Ind PW, Northfield M, Shaw G. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. *Lancet* 1994;344:219-24.
- 14 Woolcock A, Lundback B, Ringdal OLN, et al. Comparison of the effect of addition of salmeterol with doubling the inhaled steroid dose in asthmatic patients. *Am J Respir Crit Care Med* 1994;149:A280.
- 15 Grove A, Lipworth BJ. Bronchodilator subsensitivity to salbutamol after twice daily salmeterol in asthmatic patients. *Lancet* 1995;346:210-6.
- 16 Soler M, Joos L, Bollinger CT, Elsasser S, Perruchard AP. Bronchoprotection by salmeterol: cell stabilization or functional antagonism? Comparative effects on histamine and AMP-induced bronchoconstriction. *Eur Respir J* 1994;7:1973-7.
- 17 Holgate ST, Baldwin CJ, Tattersfield AE. β -adrenergic agonist resistance in normal human airways. *Lancet* 1977;iii:375-7.
- 18 Roberts JM, Jacobs MM, Cheng JB, Barnes PJ, O'Brien AT, Ballard PJ. Fetal pulmonary beta-adrenergic receptor characterization in the human and in vitro modulation by glucocorticoids in the rabbit. *Paediatr Pulmonol* 1985;1: S69-76.
- 19 Green SA, Hall IP, Turki J, Liggett SB. Genetic basis of β_2 -adrenergic receptor function in airway myocytes. *Am J Respir Crit Care Med* 1995;151:A582.
- 20 Reihnsaus E, Innis M, MacIntyre N, Liggett SB. Mutations in the gene encoding for the beta 2-adrenergic receptor in normal and asthmatic subjects. *Am J Respir Cell Mol Biol* 1993;8:334-9.
- 21 Pearlman DS, Chervinsky P, LaForce C, Seltzer JM, Southern DL, Kemp JP, et al. A comparison of salmeterol with albuterol in the treatment of mild-to-moderate asthma. *N Engl J Med* 1992;327:1420-5.
- 22 Lundback B, Rawlinson DW, Palmer JBD. Twelve month comparison of salmeterol and salbutamol as dry powder formulations in asthmatic patients. *Thorax* 1993;48:148-53.