Effects of prior treatment with salmeterol and formoterol on airway and systemic β_2 responses to fenoterol

Alison Grove, Brian J Lipworth

Abstract

Background – Previous studies have shown that both salmeterol and formoterol act as partial β_2 receptor agonists in terms of antagonising the extrapulmonary responses to fenoterol in normal subjects. The aim of the present study was to extend previous observations in evaluating the effect of prior treatment with salmeterol and formoterol on bronchodilator responses to fenoterol, a full β_2 receptor agonist, in patients with asthma.

Methods – Ten stable asthmatic patients of mean (SE) age 37 (3·7) years and forced expiratory volume in one second (FEV$_1$) 59·5 (4·1)% of predicted completed the study. One hour after inhaling single doses of placebo, salmeterol 25 µg, or formoterol 12 µg, dose-response curves to repeated doses of inhaled fenoterol were constructed (cumulative doses of 100–3200 µg). Measurements of airway and systemic β_2 receptor mediated responses were made at baseline, after inhalation of placebo, salmeterol, or formoterol, and after each dose of fenoterol.

Results – Salmeterol and formoterol produced significant bronchodilation compared with placebo (mean difference and 95% CI compared with placebo): FEV$_1$, salmeterol 0·41 (95% CI 0·13 to 0·69) l, formoterol 0·47 (95% CI 0·19 to 0·75) l. Salmeterol and formoterol had no significant effect on systemic responses compared with placebo. There were no significant differences in peak airway responses to fenoterol after treatment with salmeterol or formoterol compared with placebo (mean (pooled SE)): FEV$_1$, placebo 2·84 (0·03) l, salmeterol 2·87 (0·03) l, and formoterol 2·88 (0·03) l. There were no significant differences in the area under the dose-response curve for any of the parameters during the dose-response curve following treatment with salmeterol or formoterol compared with placebo. There was no difference in the slope of the dose-response curves to fenoterol for FEV$_1$ or forced expiratory flow (FEF$_{25-75}$) after treatment with salmeterol or formoterol compared with placebo, although there was a significant (p<0·05) attenuation of the slope in the dose-response curve for the peak expiratory flow rate (PEFR).

Conclusions – Prior treatment with low doses of salmeterol or formoterol does not significantly alter bronchodilator dose-response curves to repeated doses of fenoterol in stable asthmatic patients.

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Salmeterol and formoterol are both long acting β_2 receptor agonists used in the treatment of asthma. It has been shown in vitro that salmeterol is a partial β_2 receptor agonist[1][2] and, even at high concentrations, it is unable to produce the maximal bronchorelaxant response elicited by a full agonist such as isoprenaline. This difference can be expressed in terms of the intrinsic efficacy of the agonist, with a full agonist such as isoprenaline nominally being given an intrinsic activity of 1. Other agonists can then be compared with this standard. In vitro studies have shown that the intrinsic activity of salmeterol is of the order of 0·71 compared with isoprenaline.[3] The intrinsic activity of formoterol is also lower than that of isoprenaline with a value of 0·96,[3] thus formoterol is also a partial agonist – albeit a strong one.

From first principles it can be predicted that the presence of a partial agonist may inhibit the effects of an agonist with greater intrinsic activity.[4] In this respect we have previously shown that oral salbutamol inhibits the effects of endogenous adrenaline at extrapulmonary β_2 receptors in normal subjects.[5] In addition we have demonstrated that inhaled salmeterol and formoterol antagonise the extrapulmonary β_2 receptor responses to endogenous adrenaline and to exogenous inhaled fenoterol, also in normal subjects.[6]

The aim of the present study was to extend the findings of these previous studies in order to assess whether prior treatment with salmeterol or formoterol affects the airway and systemic β_2 responses to repeated doses of inhaled fenoterol in asthmatic subjects. Fenoterol was chosen to construct the dose-response curves as it is known to be a full agonist with greater intrinsic activity than either salmeterol or formoterol. Low doses of salmeterol and formoterol were used so as not to produce maximal bronchodilator activity prior to administering fenoterol.

Methods

SUBJECTS

Ten stable asthmatic patients (five women) of mean (SE) age 37 (3·7) years and forced expiratory volume in one second (FEV$_1$) 2·04

Department of Clinical Pharmacology, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK
A Grove
B J Lipworth

Correspondence to:
Dr A Grove.

(0·23) l, 59·5 (4·1)% of predicted normal, completed the study. All patients were diagnosed as having asthma according to American Thoracic Society criteria.[8] At an initial screening visit patients were required to have an FEV_1 of less than 80% of predicted normal, and to demonstrate at least 15% reversibility to inhaled fenoterol 200 μg (Berotec 200 metered dose inhaler; Boehringer Ingelheim, Bracknell, Berkshire, UK). In addition, all subjects were required to have a normal physical examination, 12 lead ECG, haematology and biochemical screen. All subjects gave written informed consent to participate in the study which had been approved by the Tayside committee for medical ethics. At the time of the study all 10 patients were using inhaled corticosteroids in doses of 400–2400 μg daily, together with inhaled short acting bronchodilators on an as required basis. In addition, four patients were taking oral theophylline preparations.

PROTOCOL

Patients attended the laboratory between 08.00 and 09.00 hours on three occasions separated by at least one week. Before each visit bronchodilators were withheld for an appropriate period of time (that is, 48 hours for theophylline preparations and eight hours for short acting β_2 agonists). A cannula was inserted into a forearm vein to facilitate venous blood sampling. After 30 minutes supine rest, baseline measurements of heart rate, postural finger tremor, serum potassium levels, and spirometric parameters were performed. FEV_1 was required to be within 15% of that recorded at the initial screening visit. Subjects were then randomised to receive inhaled placebo, salmeterol 25 μg (Serevent metered dose inhaler, 25 μg per actuation; Allen and Hanburys, Uxbridge, Middlesex, UK), or formoterol 12 μg (Foradil metered dose inhaler, 12 μg per actuation, Ciba Geigy AG Basel, Switzerland) in a single blind crossover fashion. All drugs were administered via a large volume spacer device in an attempt to maximise lung delivery. After one hour further measurements of airway and systemic parameters were made. A dose-response curve to inhaled fenoterol (Berotec metered dose inhalers, 100 μg and 200 μg per actuation) was constructed using doses of 100 μg, 100 μg, 200 μg, 400 μg, 800 μg, and 1600 μg – that is, a total cumulative dose of 3200 μg. The fenoterol was also administered via the large volume spacer device. Dose increments were given at 20 minute intervals with measurements being made 15 minutes after each dose. On completion of the dose-response curve subjects received potassium supplements in the form of effervescent potassium 32 mmol (Sando-K, Sandoz Pharmaceuticals, Camberley, Surrey, UK).

MEASUREMENTS

Airway responses

Measurement of FEV_1, forced expiratory flow (FEF_{25-75}), and peak expiratory flow (PEF) were performed according to American Thor-acic Society criteria[9] using a Vitalograph compact spirometer with pneumotachograph head and pressure transducer, and on-line computer assisted determination of FEV_1, FEF_{25-75}, and PEFR. Forced expiratory manoeuvres were performed from total lung capacity to residual volume with measures being taken according to best test criteria.

Extrapulmonary responses

A standard lead II electrocardiogram was monitored and recorded with a Hewlett-Packard ECG monitor and printer (Palo Alto, California, USA) with paper speed set at 25 mm/s. Heart rate was calculated from the mean of five consecutive R–R intervals.

Finger tremor was recorded by a previously validated method[10] using an accelerometer transducer (Entran Ltd, Ealing, UK). Four recordings were made at each measurement and the results were stored on computer for subsequent analysis of total tremor power >2 Hz (mg^2/s) using computer assisted autocovariance. The mean of three consistent recordings was subsequently used in the analysis.

Serum potassium levels were measured by flame photometry (IL943 analyser, Instrumentation Laboratory Ltd, Warrington, UK) with analysis being performed in batches at the end of the study, and samples being assayed in duplicate. The coefficients of variation for analytical imprecision within and between assays were 0·41% and 1·04%, respectively. The normal reference range for serum potassium levels in our laboratory is 3·5–5·5 mmol/l.

STATISTICAL ANALYSIS

Data for finger tremor were transformed using logarithm to base 10 to achieve conformation with a normal distribution. Data were then analysed using a Statgraphics software package (STSC Software Publishing Group, Rockville, USA). Baseline values, and values following inhalation of placebo, salmeterol, and formoterol, were compared by multifactorial analysis of variance (MANOVA). Where the overall MANOVA was significant, Duncan's multiple range testing was used to establish where differences were significant. Peak values achieved during the dose-response curve by each individual were obtained, regardless of the doses at which they occurred, and were compared by MANOVA and Duncan's multiple range testing. Analysis of the responses at individual doses from the dose-response curve was not performed in order not to confound the error. The area under the curve for each parameter was also obtained using the trapezoidal rule and compared by MANOVA. In order to compare the overall dose-response curves least squares regression analysis was performed on the linear part of the curve for each individual and the resulting gradients were compared by analysis of variance.

A probability of $p < 0·05$ (two tailed) was taken to be of significance for all tests. Values are given in the text as means (pooled SE). Differences from placebo, where significant, were calculated as means and 95% confidence intervals.

Table 1 Mean (SE) baseline values before treatment with inhaled placebo, salmeterol 25 µg, or formoterol 12 µg

	Placebo	Salmeterol	Formoterol
FEV_1 (l)	1·93(0·09)	2·14(0·09)	2·10(0·09)
FEF_{25-75} (l/s)	1·26(0·14)	1·37(0·14)	1·40(0·14)
PEFR (l/min)	333(11)	358(11)	338(11)
HR (beats/min)	73(2)	71(2)	76(2)
Tremor (log units)	2·30(0·12)	2·31(0·15)	2·25(0·16)
Potassium (mmol/l)	3·88(0·06)	3·86(0·05)	4·02(0·06)

FEV_1 = forced expiratory volume in one second; FEF_{25-75} = forced expiratory flow; PEFR = peak expiratory flow rate; HR = heart rate.

Table 2 Mean (SE) airway and systemic parameters one hour after receiving inhaled placebo, salmeterol 25 µg, or formoterol 12 µg

	Placebo	Salmeterol	Formoterol
FEV_1 (l)	2·03(0·07)	2·44(0·07)*	2·50(0·07)*
FEF_{25-75} (l/s)	1·38(0·13)	1·96(0·13)*	1·83(0·13)*
PEFR (l/min)	347(11)	408(11)*	415(11)*
HR (beats/min)	71(2)	70(2)	72(2)
Tremor (log units)	2·23(0·09)	2·09(0·09)	2·27(0·10)
Potassium (mmol/l)	4·01(0·06)	3·93(0·06)	3·97(0·06)

Abbreviations as in table 1.
* $p<0.05$ compared with placebo.

Results

BASELINE VALUES (table 1)
There were no significant differences between baseline values at each visit for any of the parameters measured.

RESPONSES TO PLACEBO, SALMETEROL OR FORMOTEROL (table 2)
Both salmeterol and formoterol produced a significant increase in FEV_1, FEF_{25-75}, and PEFR compared with placebo (mean difference and 95% CI): FEV_1, salmeterol 0·41 (95%CI 0·13 to 0·69) l, formoterol 0·47 (95% CI 0·19 to 0·75) l; FEF_{25-75}, salmeterol 0·58 (95% CI 0·07 to 1·09) l/s, formoterol 0·46 (95% CI 0·04 to 0·87) l/s; PEFR, salmeterol 61 (95% CI 20 to 102) l/min, formoterol: 68 (95% CI 27 to 109) l/min. There were no significant differences between the bronchodilator responses to salmeterol 25 µg and formoterol 12 µg.

Neither salmeterol nor formoterol significantly increased heart rate or finger tremor, or lowered serum potassium levels compared with placebo.

DOSE-RESPONSE CURVES TO FENOTEROL
Because of the significant confounding effect of salmeterol and formoterol on baseline airway parameters compared with placebo, the dose-response curves are shown as absolute values, rather than as changes from baseline (figs 1 and 2). Dose-dependent increases in FEV_1, FEF_{25-75}, and PEFR were seen, together with dose-dependent increases in heart rate and finger tremor and a fall in serum potassium levels. Regression analysis revealed no significant differences in FEV_1 or FEF_{25-75} responses after treatment with salmeterol or formoterol compared with placebo, although there was a statistically significant ($p<0.05$) attenuation in the slope for PEFR responses after treatment with both salmeterol and formoterol compared with placebo. Regression analysis revealed no significant differences in systemic responses after treatment with salmeterol or formoterol com-
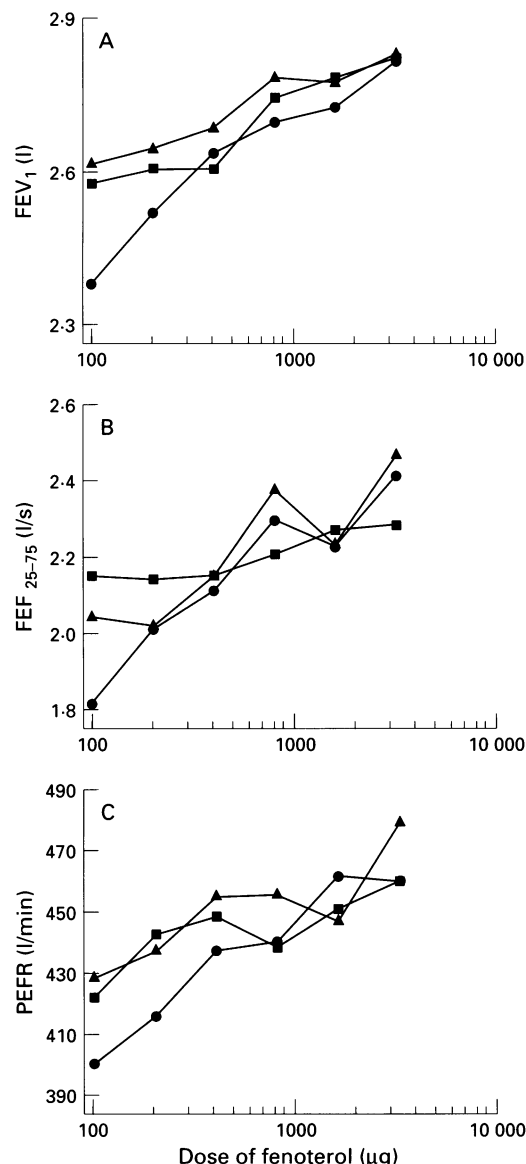

Figure 1 Dose-response curves to inhaled fenoterol (cumulative doses of 100–3200 µg) constructed one hour after inhaling placebo (●), salmeterol 25 µg (■), or formoterol 12 µg (▲) for (A) FEV_1, (B) FEF_{25-75}, and (C) PEF.

pared with placebo. There were no significant differences in the area under the dose-response curve for airway or systemic responses following treatment with salmeterol or formoterol compared with placebo.

Compared with placebo there were no significant differences in the peak values for FEV_1, FEF_{25-75}, or PEFR obtained during the dose-response curves following treatment with salmeterol or formoterol. Likewise, there were no significant differences in the peak systemic responses after treatment with salmeterol or formoterol compared with placebo (table 3).

Discussion
The results of the present study show that prior treatment with low doses of salmeterol and formoterol have no significant effects on the bronchodilator or systemic β_2 receptor mediated responses to fenoterol either in terms of
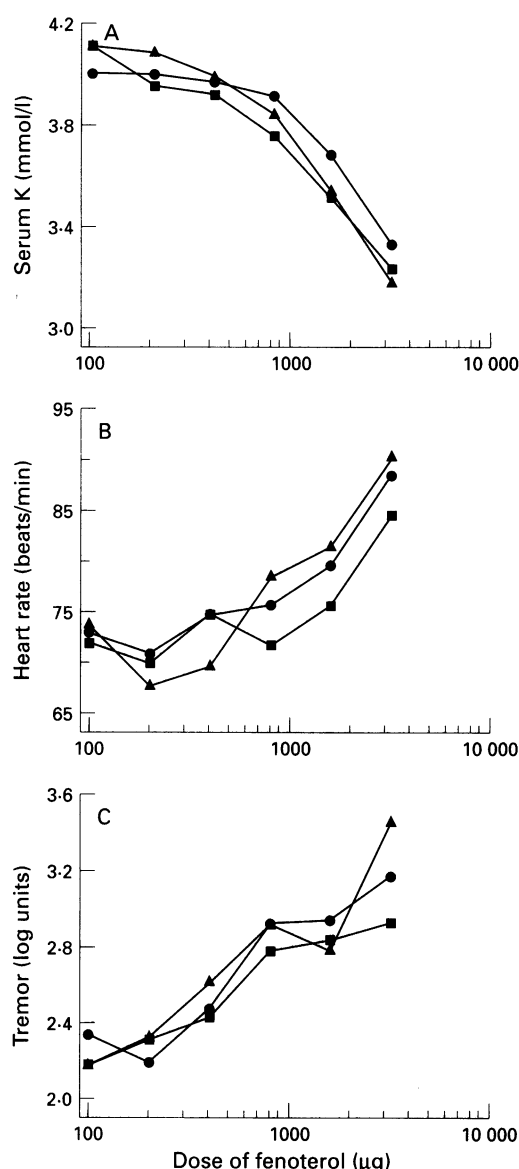
Figure 2 Dose-response curves to inhaled fenoterol (cumulative doses of 100–3200 µg) constructed one hour after inhaling placebo (●), salmeterol 25 µg (■) or formoterol 12 µg (▲) for (A) serum potassium, (B) heart rate, and (C) finger tremor.

the peak values achieved or in the overall dose-response curves.

In contrast to the present study, we have previously shown in normal subjects that inhaled salmeterol 300 µg and formoterol 72 µg antagonise the extrapulmonary β_2 receptor mediated responses to both endogenous adrenaline released during exercise and inhaled fenoterol.[6] This apparent discrepancy may be

explained by the difference in doses of long acting β_2 agonist used in the two studies. In the present study it is likely that a relatively low degree of receptor occupancy occurred, and this did not therefore antagonise the effects of fenoterol. The dose-response curves for systemic β_2 effects may, however, be suggestive of a non-significant trend towards a reduction in heart rate and finger tremor after treatment with salmeterol compared with placebo. Another factor worth considering is that receptor occupancy may be greater at steady state after chronic dosing as compared with single dosing. However, as β_2 receptor downregulation and associated subsensitivity to salbutamol occurs after chronic dosing with salmeterol,[11] it would not be possible to separate out this phenomenon from that of β_2 receptor antagonism.

The primary aim of the present study was to evaluate the effect of these long acting β_2 agonists on bronchodilator responses to fenoterol. If higher doses of salmeterol or formoterol had been administered it is likely that they themselves would have produced the maximum bronchodilation in an individual, making it impossible to assess interactions with fenoterol because of confounding effects on airway geometry. The effect on airway geometry is clearly evident from the shape of the dose-response curves, in that a plateau response for FEV_1 and PEFR was achieved at a lower dose of fenoterol following active treatment. It should be pointed out that, irrespective of prior treatment, the final airway response achieved was comparable. This is not, however, an explanation for failure to demonstrate antagonism of systemic effects where a ceiling in response did not occur.

In vitro studies are not subject to such constraints. In a study using precontracted guinea pig trachea and human bronchus, high concentrations of salmeterol ($0.1–1.0$ µmol/l) inhibited relaxant responses to a variety of other β_2 agonists with higher intrinsic efficacy.[12]

Interestingly, it was noted that the degree of inhibition produced appeared to vary depending on the agonist used – for example, responses to fenoterol were inhibited less than responses to salbutamol. These findings represent a deviation from conventional theories governing agonist/antagonist interactions which predict that an antagonist should produce a parallel shift to the right of the dose-response curve for a given agonist. In other words, the magnitude of the shift should be determined by properties of the antagonist rather than the agonist. The inference is that antagonism may have been observed had salbutamol rather than fenoterol been used to construct the dose-response curve.

In this respect, Smyth and co-workers examined the interaction between salmeterol and salbutamol in vivo.[13] They found that salmeterol in doses of 50–200 µg had no significant effect on bronchodilator or systemic responses to salbutamol in a group of mild asthmatics. Even after receiving the lowest dose of salmeterol, it was evident that salbutamol in a cumulative dose of 3600 µg produced very little additional increase in FEV_1. Both the present study and that of Smyth *et al* assessed

Table 3 Mean (SE) peak values for airway and systemic parameters obtained during a dose-response curve to inhaled fenoterol (total dose 3200 µg) constructed one hour after receiving inhaled placebo, salmeterol 25 µg, or formoterol 12 µg

	Placebo	Salmeterol	Formoterol
FEV_1(l)	2·84(0·03)	2·87(0·03)	2·88(0·03)
FEV_1 (%)	85·4	86·3	86·4
FEF_{25-75} (l/s)	2·54(0·09)	2·45(0·09)	2·64(0·09)
FEF_{25-75} (%)	61·9	59·8	64·5
PEFR (l/min)	473(6)	474(6)	491(6)
PEFR (%)	101·7	102·0	104·2
HR (beats/min)	90(3)	87(3)	92(3)
Tremor (log units)	3·42(0·17)	3·16(0·13)	3·42(0·17)
Potassium (mmol/l)	3·34(0·06)	3·24(0·06)	3·18(0·06)

Abbreviations as in table 1.

interactions between long and short acting agonists at the peak effect of the long acting drug.[14][15] It may also be relevant to assess such interactions towards the end of a normal dose interval – that is, 12 hours after administering the drug. After 12 hours it would be expected that the long acting drug would be producing significant but not maximal bronchodilation.

The results of the present study, together with the work of Smyth *et al*, are reassuring in that they suggest that the presence of a long acting β_2 agonist does not attenuate the airway response to shorter acting agonists with higher intrinsic efficacies, at least in stable asthmatic subjects under conditions of basal broncho-motor tone. It is worth noting, however, that in vitro studies have suggested that differences in intrinsic activities between agonists become more pronounced in the presence of increased bronchial tone.[16] In human bronchus pre-contracted with acetylcholine, for example, salmeterol behaves as an even weaker agonist with an intrinsic activity of 0·62 compared with 0·71 at basal tone.[16] A similar pattern was also demonstrated with formoterol. Thus it is conceivable that in patients with acute severe asthma, with a higher degree of bronchial tone, relatively weak partial agonists such as salmeterol may antagonise the bronchodilator effects of salbutamol or fenoterol. This issue may merit further investigation, particularly in view of concerns raised over a possible increase in asthma deaths in patients prescribed salmeterol.[17]

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