Subsensitivity of bronchodilator and systemic β_2 adrenoceptor responses after regular twice daily treatment with eformoterol dry powder in asthmatic patients

Donald M Newnham, Alison Grove, Denis G McDevitt, Brian J Lipworth

Abstract

Background **– There is controversy as to the role of long acting β_2 agonists such as eformoterol and, in particular, whether bronchodilator tolerance occurs during continuous therapy. The purpose of this study was to extend previous observations of bronchodilator subsensitivity with metered dose eformoterol aerosol in order to assess whether tolerance also occurs with a dry powder formulation of the same drug.**

Methods **– Sixteen asthmatic patients of mean age 33 (range 18–53) years and FEV_1 (% predicted) of 64 (3)%, of whom 13 were receiving inhaled corticosteroids, received regular treatment with eformoterol 24 μg twice daily or placebo twice daily (without β_2 agonists) given concurrently for four weeks in a randomised double blind cross-over design. An initial two week run-in was used when β_2 agonists were withdrawn and substituted with ipratropium bromide. Dose-response curves to eformoterol (cumulative dose 6–102 μg) for airways and systemic β_2 responses were constructed at the end of each treatment period.**

Results **– Baseline values for airways and systemic responses were similar. The peak delta FEV_1 response from the dose-response curve (as change from baseline) and the delta response for FEV_1 and FEF_{25-75} at six hours after the last dose were attenuated after eformoterol compared with placebo: peak delta FEV_1 response 1·00 l with placebo *v* 0·84 l with eformoterol (95% CI 0·04 to 0·28); at six hours 0·93 l with placebo *v* 0·58 l with eformoterol (95% CI 0·20 to 0·50); and for delta FEF_{25-75} at six hours 1·29 l/s with placebo *v* 0·87 l/s with eformoterol (95% CI 0·15 to 0·69). Morning peak expiratory flow rate was significantly improved during treatment with eformoterol (451 l/min) compared with placebo (399 l/min) (95% CI 21 to 82). Systemic β_2 responses were blunted after eformoterol, together with a reduction in lymphocyte β_2 receptor binding density.**

Conclusions **– Regular twice daily eformoterol dry powder may produce bronchodilator subsensitivity in terms of both peak and duration of response to cumulative repeated doses of eformoterol. Systemic β_2-mediated adverse effects also showed tolerance, which was mirrored by downregulation of lymphocyte β_2 adrenoceptors.**

(*Thorax* 1995;50:497–504)

Keywords: eformoterol, bronchodilator subsensitivity, asthma, β_2 adrenoceptor.

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

Reprints will not be available.

Received 29 September 1994
Returned to authors 24 November 1994
Revised version received 9 December 1994
Accepted for publication 12 January 1995

The ongoing debate regarding the use of β_2 agonists in asthma, together with the recent availability of long acting drugs such as eformoterol and salmeterol, has resulted in a critical reappraisal of their role as first line bronchodilator therapy.[1] In particular, it has been postulated that prolonged receptor occupancy with long acting β_2 agonists might conceivably result in the development of tolerance. In this respect it has recently been shown with salmeterol that tolerance occurs to its protective effects against methacholine and exercise induced bronchoconstriction.[2,3] We have also previously demonstrated that significant bronchodilator subsensitivity develops after four weeks of continuous therapy with inhaled eformoterol (Ciba-Geigy, Basle, Switzerland) in a dose of 24 μg twice daily given as a metered dose aerosol to patients with asthma.[4] However, it was felt that this preliminary study[4] might be open to criticism because of small patient numbers and a non-significant trend towards a higher baseline for forced expiratory volume in one second (FEV_1) 12 hours after treatment with eformoterol compared with placebo. It was therefore possible that this carryover effect on baseline FEV_1 with eformoterol could have a confounding effect on the subsequent bronchodilator dose-response curve. A dry powder delivery system for eformoterol has since become available and it was felt necessary to assess whether tolerance occurs with this new formulation.

The aim of the present study was to compare airways and systemic β_2 adrenoceptor responses after continuous therapy with inhaled eformoterol given as a dry powder or placebo to patients with stable, reversible, mild to moderate asthma. As in the previous study using the metered dose aerosol, an identical dose of eformoterol (24 μg twice daily) with the same duration of treatment (four weeks) was used. However, in order to obviate possible confounding effects of baseline on the dose-response curve, the latter was performed 24 hours after the previous dose rather than 12 hours as in the previous study.

Methods

PATIENTS

Eighteen patients were initially recruited. Of these, one dropped out during the run-in period and another was withdrawn during the second treatment period because of acute appendicitis. Sixteen non-smoking subjects (10 men) with a mean age of 33 (range 18–53) years and a mean duration of asthma of 15·6 years were recruited to completion and included in the analysis. All gave written informed consent before being randomised into the double blind, placebo controlled, crossover study, which was approved by Tayside medical ethics committee. A full physical examination, 12 lead electrocardiogram, biochemical and haematological parameters were normal prior to inclusion. At the first screening visit patients were required to have an FEV_1 of 40–80% of predicted normal with at least 15% reversibility of FEV_1 with inhaled salbutamol 200 μg. At the first screening visit patients had a mean (SE) FEV_1 as % predicted (and in litres) of 64 (3)%, range 45–83% (2·45 (0·20) l, range 1·17–3·80 l). Of the 16 patients recruited 13 were inhaling corticosteroids (either beclomethasone dipropionate or budesonide) in doses of 200 μg (one patient), 400 μg (four patients), 600 μg (one patient), 800 μg (one patient), 1000 μg (one patient), 1600 μg (two patients), and 2000 μg (three patients). All had been prescribed short acting β_2 agonists previously and were inhaling β_2 agonists as required prior to recruitment, at a dose of less than 600 μg salbutamol or 1000 μg terbutaline per day. In addition, one patient was inhaling salmeterol 100 μg twice daily which was discontinued at the first screening visit. Three patients were taking oral theophylline (175–625 mg daily), none had received oral prednisolone for at least three months, and none had had a recent exacerbation of asthma in the past six months. Before entry into the study the patients, who were all non-smokers, were given tuition in the use of the inhaler device which was required to deliver the dry powder formulation of eformoterol.

PROTOCOL

Following the initial screening visit subjects were washed out from β_2 agonist therapy with two puffs of ipratropium bromide, 40 μg per actuation (Atrovent Forte, Boehringer Ingelheim, Bracknell, UK) as a substitute for rescue requirements, during a run-in period of at least two weeks. At this second randomisation visit FEV_1 was measured (2·42 (0·20) l) and subjects were then randomised to receive concurrent treatment with inhaled eformoterol dry powder capsules 24 μg (as two 12 μg capsules) twice daily or matching inhaled placebo capsules twice daily for four weeks, whilst maintaining their inhaled steroid and other anti-asthma therapy at a constant dose. An ipratropium bromide inhaler was also available for rescue purposes during the two treatment phases in order to ensure that β_2 agonists were not used during the placebo period. The reason for not including a washout between the two treatments is that those receiving eformoterol second would have had four weeks of preceding placebo without β_2 agonists. Those receiving eformoterol first would have had a preceding two week run-in without β_2 agonists, and a four week placebo washout as their second treatment limb. The study treatment was taken twice daily between 07.00 and 09.00 hours and between 19.00 and 21.00 hours. The dose of eformoterol used in the present study is within the recommended range of daily dosage (24–48 μg daily). Morning and evening peak expiratory flow readings were recorded on a diary card using a Wright peak flow meter (Airmed, London, UK) as a measure of diurnal variability, with values recorded during the final week of each treatment period being used for the purposes of analysis. The number of recorded puffs of ipratropium rescue medication inhaled per day in the final week of treatment were also analysed. Patients were also required to mark on their diary card each time they used their study medication, and a count of unused capsules was made at the end of each treatment period.

Subjects attended the laboratory at the third and fourth visits at the same time between 08.00 and 09.00 hours, after eformoterol and placebo, having withheld their study medication for 24 hours, their rescue medication for at least 12 hours, and oral theophylline for 48 hours. An intravenous cannula was inserted and kept patent with bolus injections of heparinised saline. Cannula dead space of 2 ml was withdrawn before blood samples were collected. After a 30 minute period of supine rest, 30 ml of blood was collected for the determination of the parameters of lymphocyte β_2 adrenoceptor function. It was required that baseline FEV_1 (prior to the dose-response curve) must not differ from the value at the randomisation visit by more than 15%; this did not occur in any of the 16 completed patients. The dose-response curve was then constructed with inhaled eformoterol using doses of 6 μg, 24 μg, 24 μg and 48 μg – that is, a cumulative dose of 102 μg after the last dose – via the inhaler device, with the doses being separated by 50 minutes. Measurements of FEV_1, FEF_{25-75}, serum potassium (K), heart rate (HR), systolic and diastolic blood pressure (SBP, DBP), postural finger tremor (Tr) and ECG parameters (T-wave, Q–Tc) were undertaken over a 20 minute period at baseline (after the rest period), 30 minutes after each dose, and repeated at 1, 2, 4, and 6 hours after the final dose. In addition, at one hour after the last dose of the dose-response curve the patient's subjective grading of tremor and heart beat were measured using an analogue chromatic continuous scale from 0 (no sensation) to 100 mm (unbearable).[5] All subjects received 36 mmol effervescent potassium (Sando K, Sandoz Pharmaceuticals, Camberley, UK) at the end of each study day.

MEASUREMENTS

Airway responses

Measurements of FEV_1 and FEF_{25-75} were performed according to the American Thoracic Society criteria[6] using a Vitalograph compact

spirometer (Vitalograph Ltd, Buckingham, UK) with a pneumotachograph head and pressure transducer, and online computer assisted determination of FEV_1 and FEF_{25-75}. Forced expiratory manoeuvres were performed from total lung capacity to residual volume. The best FEV_1 value was taken from three consistent measurements and the FEF_{25-75} was taken from the best test of three consistent forced expiratory curves.[6] Calibration of the spirometer was performed with a 5 litre syringe on each study day. A coefficient of variation of less than 3% for three reproducible measurements of FEV_1 and 5% for FEF_{25-75} was considered as being acceptable.

Extrapulmonary responses

Serum potassium was 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 0·42% and 0·47%, respectively. The normal reference range for our laboratory is 3·5–5·5 mmol/l.

The electrocardiogram was recorded on a standard lead II using a Hewlett-Packard (Palo Alto, California, USA) monitor and printer with paper speed set at 50 mm/s and 0·5 mV/cm again. The following parameters were measured from the mean of five consecutive complexes: R–R interval (s), Q–T interval (ms) and T wave (mV). The Q–T interval was measured using the method described by Shamroth[7] to account for the presence of U waves. The formula of Bazett[8] was used to correct the Q–T interval for heart rate (Q–Tc). The heart rate was calculated from the R–R interval. Systolic and diastolic blood pressures were recorded by a semi-automatic sphygmomanometer (Dinamap Vital Signs Monitor, Critikon, Tampa, USA). All measurements were taken from the right arm at one minute intervals until recordings were constant. The mean of three consistent readings was used for the purpose of analysis.

Finger tremor was recorded by a previously validated method[9] using an accelerometer transducer (Entran Ltd, Ealing, UK). Five recordings were measured and results stored on computer disc for subsequent spectral analysis of total tremor power >2 Hz (units of mg^2/s) using computer-assisted autocovariance. The mean of three consistent readings was subsequently analysed.

Lymphocyte β_2 adrenoceptors

Parameters of lymphocyte β_2 adrenoceptor function were measured as previously described.[10] Briefly, 30 ml of whole blood was collected into tubes containing ethylenediamine tetra-acetic acid diluted to 50 ml with phosphate buffered saline (PBS). Two equal aliquots were then centrifuged with 15 ml Lymphoprep (Nycomed Pharma AS, Oslo, Norway) and the lymphocyte layer subsequently removed. Following two further washes with PBS and centrifugation, the lymphocyte pellet was resuspended in 5 ml PBS prior to lymphocyte counting, with 5×10^6 cells being required for cyclic AMP stimulation by isoprenaline. Lymphocyte β_2 adrenoceptor binding density (Bmax) and dissociation constant for binding affinity (Kd) were determined using $(-)^{125}$I-iodocyanopindolol (ICYP, NEN-du Pont (UK) Ltd, Stevenage, UK) at eight concentrations of 5–160 pmol, with CGP 12177 (Ciba-Geigy, Basle, Switzerland) being added to half the tubes to prevent ICYP binding to receptor sites and to allow non-specific (non-receptor) binding to be evaluated. Resultant counts were determined by a gamma counter (LKB Wallac, Wallac OY Pharmacia, Turku, Finland) and specific (receptor) binding was calculated from total binding minus non-specific binding. The lymphocyte preparation was then suspended in PBS containing theophylline (100 μg) and bovine serum albumin before being maximally stimulated by increasing molar concentrations of isoprenaline (range 10^{-9}–10^{-3} M). A radioimmunoassay technique was used to evaluate the maximal cyclic AMP response to isoprenaline (Emax). The intra-assay coefficient of variation for analytical imprecision was 10·3% for Bmax, 5·9% for Kd, and 2·0% for Emax.

DATA ANALYSIS

Power calculations were based on a sample size of 12 patients in order to detect a 0·31 difference in delta FEV_1 response – that is, change from baseline from dose-response curve – and a 0·3 mmol/l difference in delta potassium response with a β error of 0·2 (80% power) and α error set at 0·05 (two tailed). In order to increase the power of the study to 90% a total of 16 patients were recruited to completion.

Since power calculations were based on change from baseline, all variables were analysed as delta responses, with data for finger tremor and Bmax having been transformed using logarithm to base 10 as both variables were not normally distributed. Data were analysed using a Statgraphics statistical software package (STSC Software Publishing Group, Rockville, USA). For all parameters comparisons between treatments were made by multifactorial analysis of variance using subjects, treatments, and period effects as within factors for the analysis. A p value of <0·05 (two tailed) was considered as being of significance. Values are shown in the text as means for each treatment and 95% confidence intervals for the differences between treatments. Since the response-time profile only provides a group mean response at a given time point, it is conceivable that peak effects may have occurred at different time points for each subject. Thus, FEV_1 and FEF_{25-75} responses for each individual were also analysed to ascertain the true peak response rather than the apparent peak from the response-time profile. For the lymphocyte β_2 adrenoceptor binding parameters – that is, Bmax and Kd – a technical problem with the cell harvester resulted in evaluable data being

Table 1 Mean (and between-treatment 95% confidence interval) baseline values after pretreatment with placebo or eformoterol before measurement of the dose-response curve

	Eformoterol	*Placebo*	*95% CI*	*p value*
FEV_1 (l)	2·44	2·40	−0·03 to 0·13	0·21
FEF_{25-75} (l/s)	1·80	1·73	−0·11 to 0·25	0·43
HR (beats/min)	69	68	−3 to 5	0·77
K (mmol/l)	3·91	3·93	−0·22 to 0·18	0·87
Tr (log units)	2·00	1·99	−0·18 to 0·19	0·96
Q–Tc (ms)	377	375	−10 to 14	0·72
T wave (mV)	0·26	0·27	−0·05 to 0·03	0·63
SBP (mm Hg)	117	118	−4 to 3	0·94
DBP (mm Hg)	65	66	−4 to 3	0·74

HR = heart rate; K = potassium; Tr = log tremor; Q–Tc = corrected Q–T interval; SBP, DBP = systolic and diastolic blood pressure.
p values eformoterol *v* placebo by ANOVA.

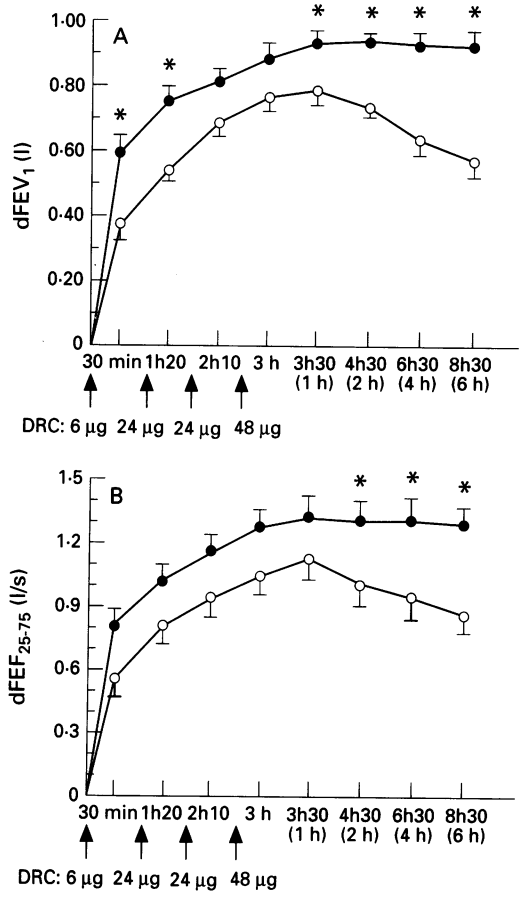

Figure 1 Response-time profiles for (A) FEV_1 and (B) FEF_{25-75} shown as delta (d) from baseline after treatment for four weeks with either placebo (●) or eformoterol 24 µg twice daily (○). Measurements were made over a 20 minute period beginning 30 minutes after each dose, with increments during the dose-response curve (DRC) made every 50 minutes. Times are given following inhalation of the first dose of eformoterol (6 µg) and in brackets for time after inhalation of the last dose (48 µg). Asterisks denote a significant difference (p<0·05) between the value after treatment with placebo compared with eformoterol. Values are shown as means and pooled SE.

available in only seven subjects. Data for lymphocyte β_2 adrenoceptor isoprenaline-induced maximal cyclic AMP response (Emax) were, however, obtained in all 16 subjects.

Results

Baseline values for FEV_1 and FEF_{25-75} before the dose-response curve did not differ significantly between the four week eformoterol treatment period and placebo (means and 95% CI for eformoterol *v* placebo): FEV_1 2·44 l *v* 2·40 l (−0·03 to 0·13), and FEF_{25-75} 1·80 l/s *v* 1·73 l/s (−0·11 to 0·25). There were no significant differences between baseline values for any of the extrapulmonary parameters measured (table 1).

BRONCHODILATOR RESPONSES

The dose-response curves (as change from baseline) after pretreatment with either placebo or eformoterol showed dose-dependent increases in delta FEV_1 and delta FEF_{25-75} (fig 1). A right shift of the dose-response curve for both delta FEV_1 and delta FEF_{25-75} occurred after treatment with eformoterol compared with placebo, and there was significant attenuation of the bronchodilator response which was greatest at six hours after the final dose (fig 1). Peak responses for delta FEV_1 were significantly (p = 0·01) blunted after eformoterol compared with placebo, with mean values being 0·84 l and 1·01 respectively (95% CI for difference 0·04 to 0·28). The peak responses for delta FEF_{25-75} were not significantly different, with mean values of 1·22 l/s after eformoterol and 1·45 l/s after placebo (95% CI −0·08 to 0·52). Responses for delta FEV_1 and delta FEF_{25-75} at six hours after the final dose were significantly attenuated after eformoterol compared with placebo. The values of delta FEV_1 and FEF_{25-75} at six hours for eformoterol *v* placebo were: 0·58 *v* 0·93 l (95% CI 0·20 to 0·50), p = 0·0002, and 0·87 *v* 1·29 l/s (95% CI 0·15 to 0·69), p = 0·006, respectively.

The peak absolute values for FEV_1 and FEF_{25-75} from the dose-response curve were not, however, significantly attenuated: FEV_1 3·29 *v* 3·40 l (95% CI −0·03 to 0·25), FEF_{25-75} 3·03 *v* 3·19 l/s (95% CI −0·14 to 0·46) eformoterol *v* placebo. The absolute values for FEV_1 and FEF_{25-75} at six hours were both significantly attenuated after eformoterol compared with placebo: FEV_1 3·02 *v* 3·32 l (95% CI 0·13 to 0·46), p = 0·001; FEF_{25-75} 2·68 *v* 3·02 l/s (95% CI 0·11 to 0·58), p = 0·007.

SYSTEMIC RESPONSES

Response-time profiles showed a right shift of the dose-response curve after pretreatment with eformoterol compared with placebo for hypokalaemic, chronotropic, tremor, Q–Tc and T wave responses (figs 2 and 3), with significant blunting of the individual peak responses (table 2). There was also significant (p<0·001) attenuation of the log Tr response six hours after the final dose. No significant differences were demonstrated between the two treatments for systolic and diastolic blood pressure.

The patient's subjective assessment of tremor showed significant blunting after eformoterol compared with placebo: 20·1 *v* 36·8 mm (95% CI 5·1 to 28·7), p = 0·01. Subjective assessment of heart beat was not, however, significantly blunted after eformoterol (6·0 mm) compared with placebo (10·1 mm).

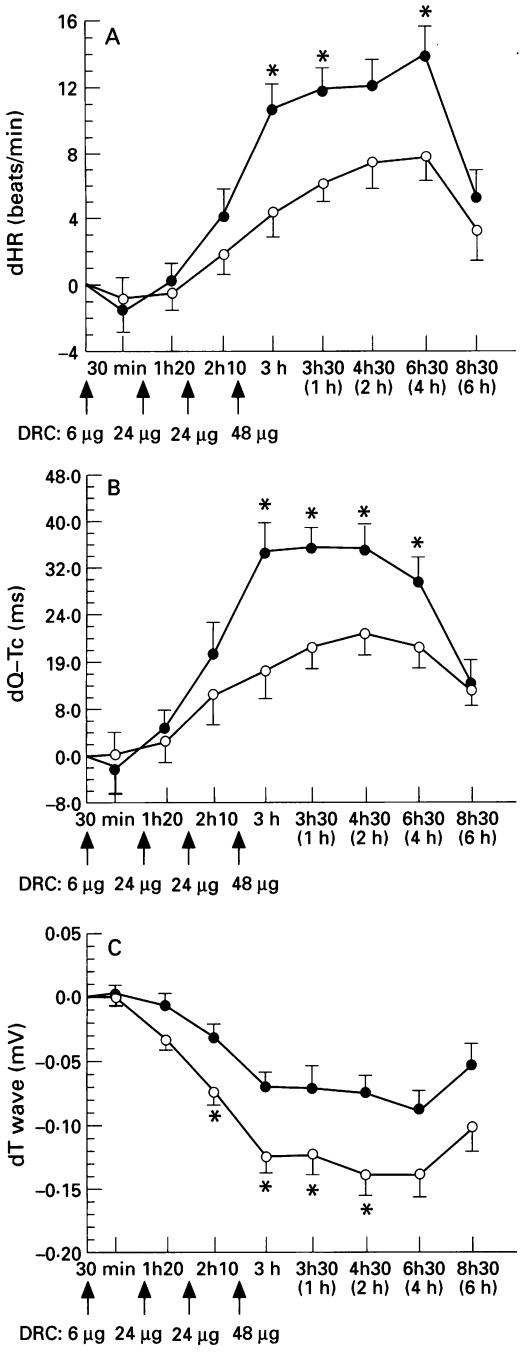

Figure 2 Response-time profiles for (A) heart rate (HR), (B) Q–Tc interval, and (C) T wave amplitude, shown as change from baseline as in fig 1.

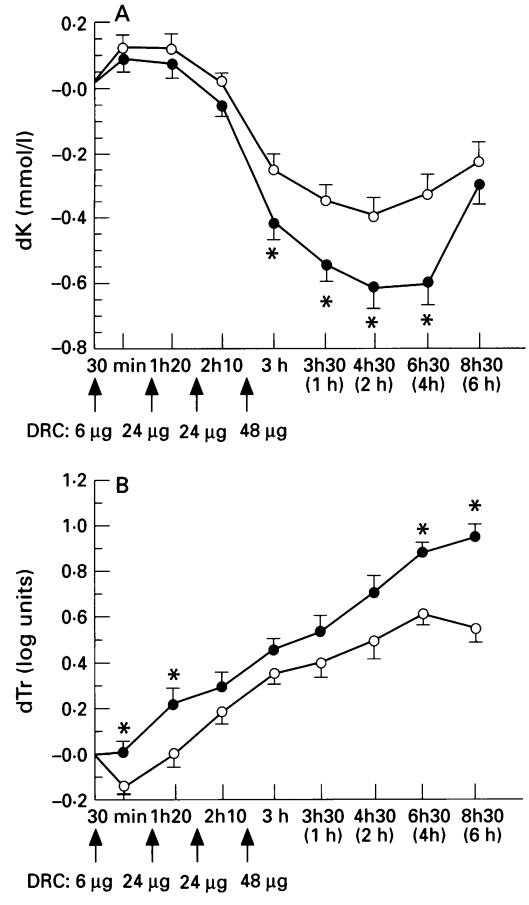

Figure 3 Response-time profiles for (A) potassium (K) and (B) finger tremor (Tr), shown as change from baseline as in fig 1.

LYMPHOCYTE β_2 ADRENOCEPTOR FUNCTION

Parameters of Bmax and Kd showed a significant reduction after treatment with eformoterol compared with placebo (fig 4), with the mean values for eformoterol *v* placebo being 0·035 *v* 0·214 fmol/10^6 cells (95% CI 0·17 to 0·33), p<0·01, for Bmax (n=7), and 17·29 *v* 25·96 pmol/l (95% CI 0·64 to 25·50), p<0·05, for Kd (n=7). There was a non-significant trend towards an attenuated Emax response between the two treatments (n=16) of 2·08 *v* 2·66 pmol/10^6 cells (95% CI −0·27 to 1·49).

Table 2 Mean change from baseline of peak systemic responses following treatment with placebo or eformoterol and 95% confidence intervals for the differences between the two treatments

	Placebo	*Eformoterol*	*95% CI*	*p value*
dHR (beats/min)	18	12	3 to 9	0·005
dK (mmol/l)	0·69	0·45	0·04 to 0·42	0·02
dTr (log units)	1·06	0·71	0·18 to 0·50	0·005
dQ–Tc (ms)	47	30	6 to 28	0·004
dT wave (mV)	0·16	0·11	0·02 to 0·08	0·02

HR=heart rate; K=serum potassium; Tr=tremor; Q–Tc=corrected Q–T interval; T wave: amplitude.
p values eformoterol *v* placebo by ANOVA.

PEAK EXPIRATORY FLOW RATES

Mean peak expiratory flow rates during the last week of treatment were significantly (p<0·01) higher with eformoterol than with placebo in the morning: (451 *v* 399 l/min (95% CI 21 to 82)) and in the evening (464 *v* 425 l/min (95% CI 15 to 64)). Rescue medication was required less frequently during treatment with eformoterol than with placebo: one puff/day *v* three puffs/day (95% CI 1 to 4), p<0·005.

Discussion

The results of the present study showed that, compared with placebo, there was significant blunting of the peak delta FEV_1 response to eformoterol from the dose-response curve after continuous treatment for four weeks with inhaled eformoterol given twice daily as a dry powder. Furthermore, there was evidence to suggest that subsensitivity also developed to the duration of the bronchodilator effect of eformoterol six hours after the last dose of the dose-response curve. Indeed, this was observed for both delta FEV_1 and delta FEF_{25-75}, sug-

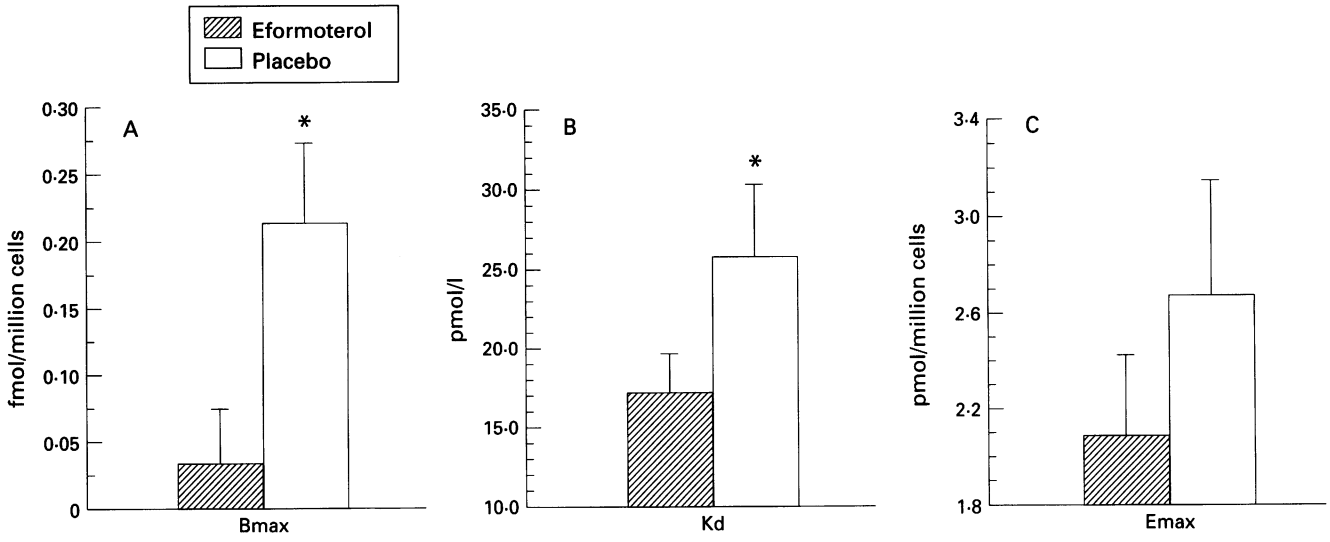

Figure 4 Parameters of lymphocyte β_2 adrenoceptor function after eformoterol v placebo for (A) log Bmax, (B) Kd, and (C) Emax. Asterisk denotes a significant difference between eformoterol and placebo.

gesting that subsensitivity may have developed in both large and small airways.

It is important to point out that the magnitude of difference in delta FEV_1 at six hours after the last dose (0·35 l) was twice that of peak delta FEV_1 (0·16 l), suggesting that tolerance to the duration of response may be the more relevant finding. These data are in agreement with results from our previous study with metered dose aerosol[4] which also showed that the magnitude of difference in the bronchodilator response between eformoterol and placebo was largest after six hours. However, in the present study baseline values for FEV_1 and FEF_{25-75} were not significantly different, and hence this is unlikely to have been a confounding factor on the subsequent dose-response curve. It is therefore likely that the observed bronchodilator subsensitivity was a real effect.

The clinical relevance of these findings is difficult to assess. However, since eformoterol is fast acting it is conceivable that patients might use it repeatedly for rescue relief of bronchoconstriction, as might occur during an acute attack. It would therefore be interesting to know how continuous exposure to eformoterol affects the acute bronchodilator response to repeated puffs of inhaled salbutamol. Since salbutamol is a weaker β_2 agonist than eformoterol, it is probable that subsensitivity would be more likely to be uncovered under conditions of submaximal receptor stimulation as might occur if salbutamol was used to construct the dose-response curve. Since peak flow data were only available for the last week of each treatment, we cannot say whether the effects of eformoterol 24 μg twice daily were maintained or attenuated during the four week treatment period. However, it was evident that morning and evening peak flows were much higher during eformoterol than with placebo at the end of the four week treatment period, suggesting that its effects were probably maintained.

We have therefore now described the development of subsensitivity to the bronchodilator effects with both aerosol and dry powder formulations of inhaled eformoterol in asthmatic patients. There is evidence to suggest that tolerance does develop to the protective action of inhaled long acting β_2 agonists against bronchoconstrictor stimuli such as methacholine and exercise.[2,3] However, previous studies which have attempted to evaluate subsensitivity to the bronchodilator effects of both eformoterol and salmeterol have been difficult to interpret as a consequence of inadequate run-in and washout periods without β_2 agonists[11-13] or the absence of dose-response curves.[11,14] It should be emphasised that in vitro data using precontracted guinea pig trachea or human bronchus have demonstrated eformoterol to be a full agonist, with greater intrinsic activity than salmeterol which acts as a partial agonist at the β_2 adrenoceptor.[15-18] However, it remains unclear whether such differences in intrinsic activity will be relevant in terms of a greater propensity for inducing β_2 adrenoceptor downregulation with eformoterol compared with salmeterol.

It is interesting to note that two previous placebo controlled chronic dosing studies using short acting β_2 agonists[19,20] have demonstrated a maintained peak bronchodilator effect, but in the non-placebo controlled study by Repsher *et al*[21] subsensitivity developed to the duration of bronchodilator response with salbutamol, a finding observed in the present study with eformoterol. What is the possible mechanism for bronchodilator subsensitivity with eformoterol? It is possible with twice daily dosing that 24 hour β_2 adrenoceptor occupancy results in downregulation of airway β_2 receptors. It may also be relevant that the dose of eformoterol (48 μg daily) was at the top of the recommended dose range (24–48 μg daily) and it is therefore possible that there may be a reduced propensity for inducing β_2 adrenoceptor subsensitivity at lower doses.

It should be mentioned that, although bronchodilator subsensitivity was demonstrated to inhaled eformoterol, a clinically significant improvement in delta FEV_1 both at peak (0·84 l) and at six hours after the last dose (0·58 l) was observed after regular therapy with eformoterol when compared with the initial baseline value. Furthermore, as in our previous study,[4] morning and evening peak flow values were significantly higher and rescue requirements significantly lower after treatment with eformoterol compared with placebo. The improved peak flow values might conceivably lead to a delay in patients seeking medical attention during an acute exacerbation, and possibly result in a perceived false sense of security. However, it should be emphasised that it may not be possible to extrapolate these findings to patients with severe asthma in terms of producing a reduced response to nebulised salbutamol during an acute attack. It is also worth noting that most of our patients were receiving inhaled corticosteroid which did not prevent the development of bronchodilator subsensitivity.

The subsensitivity of the bronchodilator response was mirrored by blunting of lymphocyte β_2 receptor binding density. It has been shown that lymphocyte β_2 adrenoceptor function in moderately severe asthmatic subjects with naive β_2 receptors (after washout with ipratropium bromide) is not significantly different from that in normal individuals.[10] Hence, it can be concluded that the β_2 adrenoceptor downregulation in the present study must have occurred as a consequence of exogenous β_2 agonist exposure. The downregulation and change in affinity of lymphocyte β_2 adrenoceptors is in keeping with the findings of our previous study,[4] but is in contrast to the study of Van Schayck *et al*[22] with regular inhaled salbutamol where downregulation did not occur. There is uncertainty as to whether changes in β_2 adrenoceptor density on peripheral mononuclear leucocytes may reflect what happens in lung β_2 adrenoceptors.[23] However, recent data have suggested that downregulation of mononuclear leucocyte β_2 adrenoceptors closely mirrors effects on lung β_2 receptors as assessed by positron emission tomography,[24] although the latter may not truly reflect the previously shown finding that the long acting β_2 agonist salmeterol increases the β_2 adrenoceptor association constant,[25] in keeping with the attenuated Kd in our two studies with eformoterol. Although systemic corticosteroids are known to upregulate lymphocyte β_2 adrenoceptors,[26 27] it would appear that, in the present study, inhaled steroids did not prevent downregulation from occurring.

In vitro downregulation of β_2 adrenoceptors was also reflected in attenuation of peak extrapulmonary responses, and this was similar to effects observed previously with the metered dose formulation of eformoterol.[4] However, it is likely that extrapulmonary β_2 adrenoceptor tachyphylaxis would be reversed by administration of systemic corticosteroids[26 27] as, for example, during an acute asthma attack. The magnitude of systemic β_2 effects might also be blunted in this setting as a consequence of attenuated lung deposition and hence reduced absorption across the lung vascular bed.[28–31] In terms of duration of systemic β_2 responses our results showed that effects were beginning to wear off by six hours after the last dose of the dose-response curve. This suggests that there may be differences in duration of action between airway and systemic β_2 adrenoceptors, which may be a reflection of the respective differences in local drug concentration.

In summary, we have shown significant bronchodilator subsensitivity after continuous therapy with eformoterol given twice daily as a dry powder formulation, which is in keeping with our previous study using an aerosol formulation. Similar changes were also observed in terms of lymphocyte β_2 adrenoceptor downregulation and tolerance of systemic β_2 responses. Placebo controlled studies are now required to further evaluate bronchodilator responsiveness with other long acting β_2 agonists such as salmeterol, after regular treatment.

The authors wish to thank Mrs J Thomson for carefully typing this manuscript, and Ciba-Geigy AG for their support of the study.

These data have previously been presented in part in abstract form to the British Thoracic Society, Manchester, June 1994.

1 Lipworth BJ. Risks versus benefits of inhaled β_2-agonists in the management of asthma. *Drug Safety* 1992;7:54–70.
2 Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dykman JH, Sterk PJ. Long term effects of long-acting β_2-adrenoceptor agonist, salmeterol, on airway hyper responsiveness in patients with mild asthma. *N Engl J Med* 1992;**327**:1198–203.
3 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.
4 Newnham DM, McDevitt DG, Lipworth BJ. Bronchodilator subsensitivity after chronic dosing with eformoterol in patients with asthma. *Am J Med* 1994;**97**: 29–37.
5 Grossi E, Borghi C, Cerchiari EL, Della-Puppa T, Francucci B. Analogue continuous chromatic scale (ACCS): a new method for pain assessment. *Clin Exp Rheum* 1983;**1**: 337–40.
6 American Thoracic Society. Standardization of spirometry – update. *Am Rev Respir Dis* 1987;**136**:1285–98.
7 Shamroth L. The Q–T interval. In: *An introduction to electrocardiography.* Oxford: Blackwell Scientific Publications, 1982:141–4.
8 Bazett HC. Analysis of the time relations of electrocardiograms. *Heart* 1920;7:353–70.
9 Lipworth BJ, McDevitt DG. Beta-adrenoceptor responses to inhaled salbutamol in normal subjects. *Eur J Clin Pharmacol* 1989;**36**:239–45.
10 Newnham DM, Coutie WJR, McFarlane LC, Lipworth BJ. Comparison of parameters of in vitro lymphocyte β_2-adrenoceptor function in normal and asthmatic subjects. *Eur J Clin Pharmacol* 1994;**45**:535–8.
11 Wallin A, Melander B, Rosenhal L, Sandstrom T, Wahlander L. Formoterol, a new long-acting β_2-agonist for inhalation twice daily, compared with salbutamol in the treatment of asthma. *Thorax* 1990;**45**:259–61.
12 Ullman A, Hedner J, Svedmyr N. Inhaled salmeterol and salbutamol in asthmatic patients: an elevation of asthma symptoms and the possible development of tachyphylaxis. *Am Rev Respir Dis* 1990;**142**:571–5.
13 Arvidsson P, Larsson S, Lofdahl C-G, Mellander B, Wahlander L, Svedmyr N. Formoterol, a new long-acting bronchodilator for inhalation. *Eur Respir J* 1989;**2**:325–30.
14 Pearlman DS, Chervinsky P, La Force 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.
15 Ball DI, Brittain RT, Coeleman RA, Denyer LOH, Jack D, Johnson M. Salmeterol, a novel, long-acting β_2-adrenoreceptor agonist: characterization of pharmacological activity in vitro and in vivo. *Br J Pharmacol* 1991;**104**: 665–71.
16 Advenier C, Zhang Y, Naline E, Grandordy BM. Effects of formoterol in isolated human bronchus. *Am Rev Respir Dis* 1991;**143**:A651.
17 Linden A, Bergendal A, Ullman A, Skoogh B-E, Hofdahl C-G. Salmeterol, formoterol and salbutamol in the isolated guinea-pig trachea: differences in maximum relaxant effect and potency but not in functional antagonism. *Thorax* 1993;**48**:547–53.

18 Kallstrom BL, Sjoberg J, Waldeck B. The interaction between salmeterol and β_2-adrenoceptor agonists with higher efficacy on guinea-pig trachea and human bronchus in vitro. *Br J Pharmacol* 1994;**113**:681–92.
19 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.
20 Lipworth BJ, Clark RA, Dhillon DP, McDevitt DG. Comparison of the effects of prolonged treatment with low and high doses of inhaled terbutaline on β_2-adrenoceptor responsiveness in patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1990;**142**:338–42.
21 Repsher LH, Anderson JA, Bush RK, Fallier C, Kass I, Kemp JP, *et al.* Assessment of tachyphylaxis following prolonged therapy with inhaled albuterol aerosol. *Chest* 1984;**85**:34–8.
22 Van Schayck CP, Visch MB, van Herwaarden CLA, Dorpeling H, van Wed C. Increased bronchial hyperresponsiveness after inhaling salbutamol during one year is not caused by desensitisation to salbutamol. *J Allergy Clin Immunol* 1990;**86**:793–800.
23 Hauck RW, Bohm M, Gengeback S, Sunder-Plassman L, Fruhmann G, Erdmann E. β_2-adrenoceptors in human lung and peripheral mononuclear leukocytes of untreated and terbutaline-treated patients. *Chest* 1990;**98**:376–81.
24 Quing F, Hayes MM, Rhodes CG, Ind PW, Jones T, Hughes JMB. The effects of chronic salbutamol therapy on human β-adrenergic receptors: peripheral mononuclear leucocytes compared to lung tissue. *Thorax* 1994;**49**:1046–7P.
25 Nishikawa M, Mak JCW, Barnes PJ. Selective $\beta_2$agonists, salbutamol, salmeterol, and formoterol down regulate β_2-adrenoceptors. *Am J Respir Crit Care Med* 1994;**149**:A483.
26 Brodde OE, Brinkmann M, Schermuth R, O'Hara N, Daul A. Terbutaline induced desensitization of human lymphocyte beta-2 adrenoceptors. Accelerated restoration of beta-adrenoceptor responses by prednisolone and ketotifen. *J Clin Invest* 1985;**76**:1096–101.
27 Brodde OE, Howe U, Egerszegi S, Konietzko N, Michel MC. Effect of prednisolone and ketotifen on β_2-adrenoceptors in asthmatic patients receiving β_2-bronchodilators. *Eur J Clin Pharmacol* 1988;**34**:145–50.
28 Collier JG, Dobbs RJ, Williams I. Salbutamol aerosol causes a tachycardia due to the inhaled rather than the swallowed fraction. *Br J Clin Pharmacol* 1980;**9**:273–4.
29 Kung M, Croley SW, Phillips BA. Systemic cardiovascular and metabolic effects associated with the inhalation of an increased dose of albuterol; influence of mouth rinsing and gargling. *Chest* 1987;**91**:382–7.
30 Lipworth BJ, McDevitt DG, Struthers AD. Systemic beta-adrenoceptor responses to salbutamol given by metered-dose inhaler alone and with pear-shaped spacer attachment: comparison of electrocardiographic, hypokalaemic and haemodynamic effects. *Br J Clin Pharmacol* 1989;**27**:837–42.
31 Newnham DM, McDevitt DG, Lipworth BJ. Comparison of the extrapulmonary β_2-adrenoceptor responses and pharmacokinetics of salbutamol given by standard metered-dose inhaler and modified actuator device. *Br J Clin Pharmacol* 1993;**36**:445–50.