Br. J. clin. Pharmac. (1983), **15**, 677–682

DOSE-RESPONSE CURVES TO INHALED β-ADRENOCEPTOR AGONISTS IN NORMAL AND ASTHMATIC SUBJECTS

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1 We have compared bronchodilator dose-response curves to inhaled salbutamol in seven normal and eight asthmatic subjects.

2 In all normal subjects maximal bronchodilatation measured by partial flow volume curves was achieved at a cumulative dose of 110 μg. The dose necessary to produce half maximal response (ED_{50}) was 23 ± 2 μg (mean ± s.e. mean) with a range of 18–28 μg.

3 In asthmatic subjects maximal bronchodilatation measured by FEV_1 and by maximal flow volume curves was achieved at significantly higher ($P < 0.01$) doses of salbutamol with a mean ED_{50} of 83 ± 28 μg and range of 25–251 μg. There was a significant ($P < 0.05$) correlation between ED_{50} and % predicted baseline FEV_1. This is more likely to reflect impaired access of drug for airway β-adrenoceptors than impaired β-adrenoceptor function in asthma.

4 In five asthmatic subjects dose-response curves to salbutamol and isoprenaline were compared and found to be similar, thus providing no evidence that salbutamol is a partial agonist *in vivo*, as it appears to be *in vitro*.

Introduction

β-Adrenoceptor agonists are the most widely used drugs in the treatment of asthma and a defect in airway β-adrenoceptor function has been suggested to explain the bronchial hyperreactivity of asthma (Szentivanyi, 1968). There have been many comparisons in cardiovascular and metabolic responses to β-adrenoceptor agonists between asthmatic and normal subjects, but changes in non-pulmonary β-adrenoceptors may not reflect changes in airway β-adrenoceptors. Yet there have been few studies in which airway β-adrenoceptor function in asthmatics has been compared to normal, partly because of the difficulty in measuring bronchodilator responses in normal subjects. We have investigated airway β-adrenoceptor function in asthma by comparing dose-response curves to inhaled salbutamol between asthmatics and normal subjects, in whom bronchodilator responses were measured by partial expiratory flow volume (PEFV) curves. As salbutamol and other β_2-selective adrenoceptor agonists appear to be partial agonists (Conolly & Greenacre, 1977; Minneman *et al.*, 1979; Davis *et al.*, 1980) *in vitro* we have also measured dose-response curves to inhaled isoprenaline, a full agonist, in the asthmatic subjects.

Methods

Subjects

Seven normal subjects (five male) aged 29–33 years were studied. None had a history of asthma but two were atopic. All had normal lung function. One normal subject smoked cigarettes occasionally: none were taking medication.

Eight asthmatic subjects aged 25–48 years (Table 1) were also studied. All had a previously documented increase in forced expiratory volume in 1 s (FEV_1) of greater than 20% to inhaled salbutamol. All asthmatic subjects were taking regular salbutamol therapy (200 μg inhaled four times daily) and three were taking inhaled beclomethasone (100 μg four times daily), but this was stopped 12 h before the study. Research Ethics Committee approval for the study was obtained and all subjects gave informed consent.

Protocol

Airway response in normal subjects was measured by PEFV curves as previously described (Barnes *et al.*, 1981). Studies were performed in an air-conditioned variable volume, pressure compensated body

0306–5251/83/0600–6770 $02.00

Table 1 Baseline lung function in asthmatic subjects prior to salbutamol dose-response study

Subject	*Sex*	*Age (years)*	*Height (m)*	*Skin test*	*FEV_1 (l)*	*FEV_1 (% predicted)*	*VC (l)*	*VC (% predicted)*
1	M	48	1.69	+	1.2	40	3.4	85
2	M	32	1.83	+	0.8	19	2.8	52
3	M	30	1.65	+	1.7	55	3.7	82
4	M	25	1.75	−	1.5	36	3.5	59
5	F	30	1.57	+	1.8	68	2.9	88
6	M	27	1.55	+	1.2	35	2.6	69
7	F	43	1.65	−	2.0	85	3.2	94
8	F	37	1.53	−	1.7	70	2.9	100
Mean		34	1.65		1.49	51.0	3.13	78.6
s.e. mean		2.8	0.04		0.14	7.8	0.14	6.0

plethysmograph (Mead, 1960). Expiratory flow was measured by pneumotachograph (Fleisch No. 4) with differential pressure transducer (Hewlett Packard 270). Volume was measured with a Krogh wet spirometer and was linear over the range used. Flow volume curves were displayed on a large screen XV storage oscilloscope (Tektronix 613) and a permanent record made with a direct writing hard-copy unit (Tektronix 4610). PEFV curves were initiated from the end of a normal tidal inspiration. After 90 to 120 s of tidal breathing subjects performed a forced expiratory manoeuvre from end inspiratory tidal volume down to residual volume (RV). A slow inspiration to total lung capacity (TLC) was then made so that vital capacity (VC) could be measured. Two further PEFV curves were performed with at least 30 s between separate manoeuvres. Flow was then measured at a volume which avoided any sudden change in contour, yet gave a sufficiently large signal and the same volume was used to measure flow in all subsequent PEFV curves in the same subject. The aim was to make measurements at 25% VC, but in some subjects 30% or 20% VC was chosen to avoid sudden changes in contour. The mean of three successive curves was calculated.

Pressurised canisters delivering metered doses of salbutamol of 10, 25 and 100 μg were used. Subjects held the canister 2 cm from the mouth and activated the canister at the start of a full inspiration from RV, followed by 10 s breath-hold at TLC. The same technique was used for each inhalation of bronchodilator aerosol. Subjects initially inhaled a placebo inhaler containing propellant only and three PEFV curves were then recorded 15 min later. Subjects then inhaled 10 μg salbutamol and the response again measured 15 min later. An inhalation of 25 μg was then taken giving a cumulative dose of 35 μg. Further records were then made at cumulative doses of 60, 110 and 410 μg. Flow at the chosen lung volume was then plotted against the cumulative dose of inhaled salbutamol on a logarithmic scale.

In asthmatic subjects FEV_1, VC and maximum expiratory flow at 70% of TLC ($Vmax_{70}$) were measured with a Krogh wet spirometer linked to a digital computer (Digico μ 16) for online data analysis. The highest values, corrected to BTPS, of three consecutive blows were taken. TLC was measured by body plethysmography before and after the dose response curves. Predicted normal values were taken from Cotes (1975). After inhalation of placebo aerosol airway responses were measured 15 min later, and subjects then inhaled 10 μg salbutamol using the same technique as described for normal subjects. The dose of salbutamol was then increased until a plateau of response (no increase after three successive doses) had been achieved, using cumulative doses of 10, 35, 60, 110, 210, 410, 810, 1610 and 3210 μg. A canister which delivered 400 μg was used for the highest cumulative doses. In five asthmatic subjects response to isoprenaline was also measured using the same cumulative doses on a separate day. The specially designed canisters of salbutamol were kindly supplied by Allen and Hanbury Ltd and of isoprenaline by Riker Ltd.

Dose-response lines between the lowest dose of β-adrenoceptor agonist and the dose giving the maximum response were determined by linear regression analysis and the cumulative dose giving 50% of maximum response (ED_{50}) was calculated.

Results

Normal subjects (Figure 1)

A maximal response in flow on the PEFV curve was attained at a cumulative dose of 110 μg and the ED_{50} was 22.8 ± 1.5 μg (mean ± s.e. mean, n = 7) with a range from 17.8 to 28.1 μg. There was no significant increase in VC after bronchodilator in normal subjects.

Asthmatic subjects (Figure 2)

The cumulative dose at which a maximal response in FEV_1 was achieved varied from 110 to 810 μg in seven

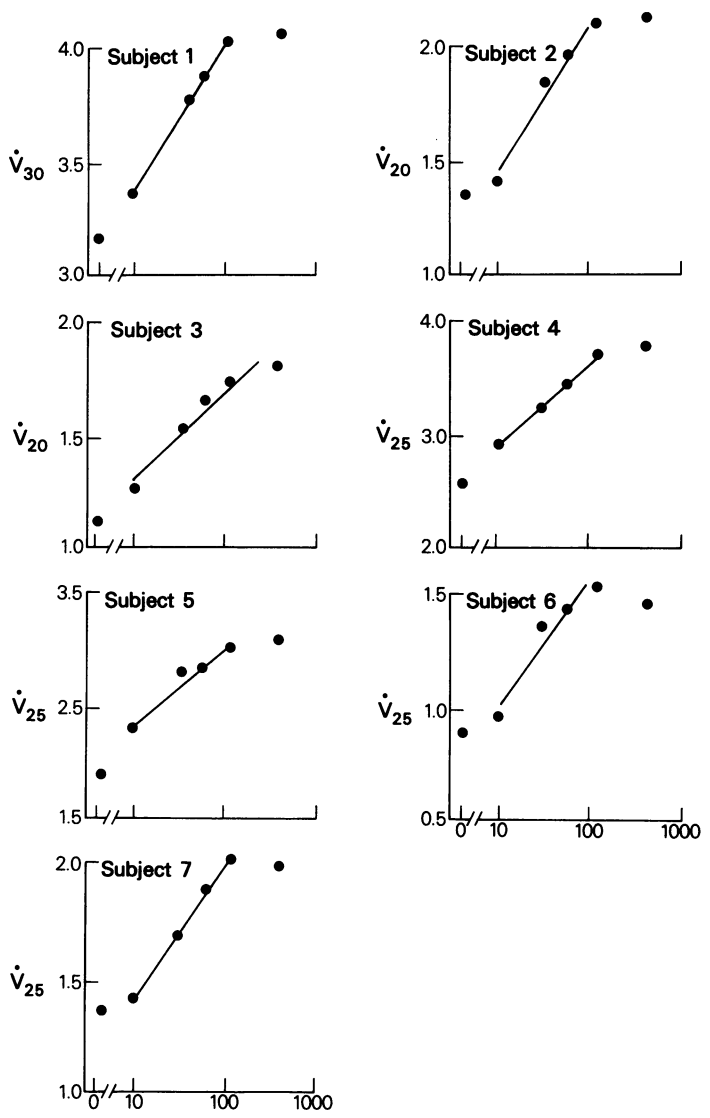

Figure 1 Dose-response curves to inhaled salbutamol in seven normal subjects. Maximum expiratory flow (l/s) from a partial expiratory flow volume curve at 20, 25 or 30% of vital capacity ($\dot{V}_{20}$, $\dot{V}_{25}$, $\dot{V}_{30}$) is plotted against cumulative dose of inhaled salbutamol on a logarithmic scale. Slopes were determined by linear regression.

patients; in the remaining patient a plateau was approached at a dose of 3210 μg salbutamol. The mean ED_{50} was 82.8 ± 28.2 μg, with a range from 25 to 251 μg, which was significantly higher ($P < 0.01$, Mann–Whitney U-test) than in normal subjects. There was a significant correlation between ED_{50} and the severity of airflow obstruction as measured by baseline % predicted normal FEV_1 ($r = 0.75$, $P < 0.05$). Analysis of $Vmax_{70}$ showed a similar large range of response (mean 114.6 ± 31.6, range 32–282 μg) in different subjects but the dose-response curves were less satisfactory because of the greater variability of response. There was no significant change in TLC after bronchodilator.

No significant change in either heart rate or blood pressure was recorded after salbutamol inhalation in either normal or asthmatic subjects.

In five of the subjects a dose-response curve to

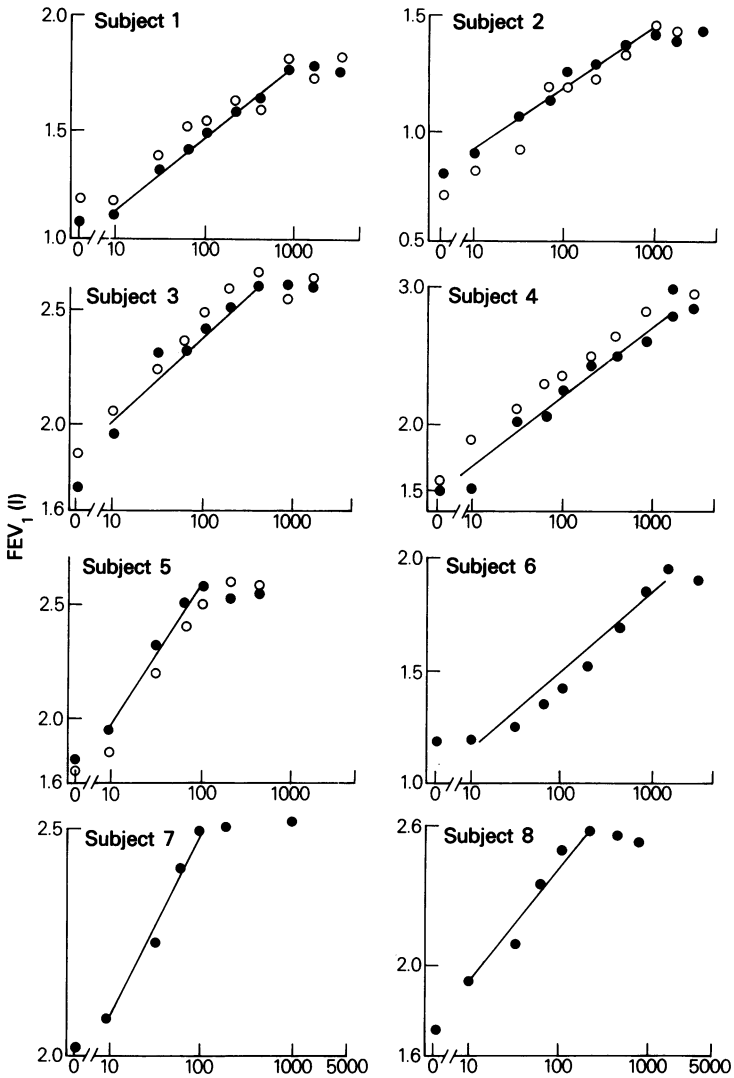

Figure 2 Dose-response curves to inhaled salbutamol (●) and isoprenaline (○) in eight asthmatic subjects. Forced expiratory volume (FEV_1) in litres is plotted against cumulative dose of inhaled β-adrenoceptor agonist on a logarithmic scale. Slopes were determined by linear regression.

inhaled isoprenaline was also obtained (Figure 2). There was no significant difference between mean maximal response and ED_{50} to isoprenaline compared with salbutamol.

Discussion

In normal subjects a maximum airway response to inhaled salbutamol was achieved with a cumulative dose of 110 μg. By contrast the dose required to produce a maximal bronchodilator response in asthmatic subjects was significantly higher and increased as the severity of bronchoconstriction increased.

In asthmatic subjects the bronchodilator response is relatively large and dose-response curves can be constructed using change in FEV_1. This is not possible with the small bronchodilator response found in normal subjects, so we have analysed dose-response

curves obtained measuring the airway response from changes in flow at small lung volume on PEFV curves, as recently described (Barnes *et al.*, 1981). In normal subjects VC does not increase with bronchodilation, therefore flows at a fixed volume can be simply compared by super-imposition of successive flow volume curves, without the need for a further measurement of absolute lung volume. This is not the case in subjects with asthma in whom VC increases with bronchodilation and total lung capacity may also alter. In subjects with asthma we found dose-response curves assessing airway response by FEV_1 were more consistent than curves using maximum expiratory flow at absolute lung volume. This is probably because the FEV_1 can be measured with greater reproducibility than a maximum flow rate and is not influenced by changes in VC. In contrast measurement of absolute lung volume, apart from the increase in equipment, adds its own error to the inherent poorer reproducibility of flow rate measurement. In practice, therefore, there are strong arguments for assessing airway responses using FEV_1 in subjects with asthma and PEFV curves in normal subjects. The mean ED_{50} value was higher when flow at low lung volume was used compared to FEV_1, making the discrepancy between normal and asthmatic subjects even greater. The only other method that has been used for measuring airway response and constructing dose-response curves in normal (Holgate *et al.*, 1977, 1980) and asthmatic subjects (Warrell *et al.*, 1970; Harvey *et al.*, 1981) has been the measurement of airway conductance with a body plethysmograph. This requires much more complex equipment and published studies have not consistently shown that a maximum airway response can be obtained.

There are several possible explanations for the finding that larger doses of β-adrenoceptor agonists are required to produce maximal bronchodilatation in asthmatic compared to normal subjects. Previous β-adrenoceptor therapy may have caused tachyphylaxis of airway β-adrenoceptors as has been demonstrated with metabolic responses (Jenne *et al.*, 1977; Morris *et al.*, 1977; Nelson *et al.*, 1977) and a reduction in polymorph β-adrenoceptor density (Galant *et al.*, 1980). Although such tachyphylaxis has been demonstrated in airway β-adrenoceptor responses in normal subjects (Holgate *et al.*, 1977), no such desensitization has been convincingly shown in asthmatics (Svedmyr *et al.*, 1976; Harvey *et al.*, 1981), but the variability of airway responses in asthma may have obscured such a defect. It is possible that the difference between asthmatics and normal subjects could be due to the disease process itself. Although it is now accepted that at least some of the diminution β-adrenoceptor function in asthma can be attributed to tachyphylaxis as a result of previous adrenergic therapy (Conolly & Greenacre, 1976), there is some evidence for reduced β-adrenoceptor responsiveness or reduction in lymphocyte β-adrenoceptor density even in untreated asthmatics, the magnitude of which is related to the severity of bronchoconstriction (Brooks *et al.*, 1979; Kariman, 1980). The correlation between initial bronchoconstriction and dose of inhaled salbutamol required to produce 50% maximal bronchodilatation may similarly reflect decreasing airway β-adrenoceptor function as disease severity increases. Another explanation may be that access of inhaled salbutamol to airway β-adrenoceptors is impaired. The proportion of an inhaled aerosol which reaches the airway in normal subjects is probably less than 10% of the inhaled dose (Davies, 1975) but in the presence of airways obstruction aerosol deposition is more central and even less of the inhaled drug reaches peripheral airways (Dolovich *et al.*, 1976). In severe airway obstruction less salbutamol would therefore reach peripheral airway β-adrenoceptors but as large airways dilate, progressively more drug reaches smaller airways. This could be investigated further by studying dose-response curves to infused salbutamol when access to airway β-adrenoceptors may be less influenced by airway narrowing, although the disturbance of pulmonary perfusion in asthma may still make interpretation difficult (Taplin & Chupra, 1978).

In vitro salbutamol is a partial agonist producing only 55% activation of pulmonary adenylate cyclase compared with isoprenaline (Minneman *et al.*, 1979), and similar findings have been reported in human lymphocytes (Conolly & Greenacre, 1976). In human isolated airways also salbutamol behaves as a partial agonist in reversing histamine-induced bronchoconstriction (Davis *et al.*, 1980). In an earlier study in which salbutamol and isoprenaline were compared in asthmatic patients no difference in bronchodilator response was found, although higher doses were not studied and maximal responses were not attained (Warrell *et al.*, 1970). In the present study the dose-response curves to inhaled isoprenaline and to inhaled salbutamol were similar providing no evidence that salbutamol functions as a partial agonist *in vivo*. The discrepancy between the *in vivo* and *in vitro* findings could be explained if the maximal bronchodilator response *in vivo* is restricted by the surrounding lung and occurs when only a small fraction of airway β-adrenoceptors is activated, so that no difference between a full and partial agonist would be seen at this low receptor occupancy (Furchgott, 1972). Studies with isolated airways *in vitro* and with pulmonary membranes using radioligand binding have demonstrated that pulmonary β-adrenoceptors are not homogeneous (Conolly & Greenacre, 1976; Rugg *et al.*, 1978); although most receptors are of the β_2-subtype there are a significant proportion of β_1-adrenoceptors, which are bronchodilator, particularly in the larger airways (Furchgott *et al.*, 1975). Theo-

retically, therefore, salbutamol, which is β_2-selective, may be a less effective bronchodilator than isoprenaline which would activate both β_1- and β_2-adrenoceptors, but in practice we have found no significant difference between these drugs.

The clinical implication of these studies is that with more severe asthma larger doses of inhaled β-adrenoceptor agonist are necessary to produce a maximal airway response, so using a standard inhaled dose for all patients may be inappropriate.

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(Received December 23, 1982, accepted March 14, 1983)