

Airway response to salbutamol: effect of regular salbutamol inhalations in normal, atopic, and asthmatic subjects

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ABSTRACT This study was designed to determine whether resistance to the airway effects of the beta-agonist, salbutamol, would develop in three groups of subjects while taking large doses of inhaled salbutamol. Six normal non-atopic, six atopic non-asthmatic, and eight atopic asthmatic subjects were studied by an identical technique. The development of resistance was assessed from salbutamol dose-response studies in which the airway response was measured as specific airway conductance (sGaw). Further evidence was sought in the atopic and asthmatic subjects by measuring the airway response to a standard histamine inhalation challenge and the protective effect of 100 μg salbutamol on this challenge, and by six-hourly peak flow recordings. Subjects were assessed before and during four weeks in which they took inhaled salbutamol regularly in doses increasing to 500 μg qid in week 4. Normal subjects showed a progressive reduction in the bronchodilator (sGaw) response to salbutamol during the four weeks, indicating the progressive development of resistance. The atopic subjects, both asthmatic and non-asthmatic, showed no reduction in the response to salbutamol during the four weeks, nor any change in the response to histamine challenge or in regular peak flow readings. These results demonstrate that asthmatic patients do not develop bronchial beta-adrenoceptor resistance easily and suggests that they and atopic non-asthmatic subjects are less susceptible to its development than normal subjects.

When the sales of isoprenaline aerosols were found to have increased and decreased in parallel with the rise and fall in asthma deaths in the United Kingdom in the 1960s, a causative association was suggested¹ and though supported by circumstantial evidence was never proved.^{2,3} Explanations for a possible association included the provocation of cardiac arrhythmias by isoprenaline³ or alternatively the development of tolerance or resistance to this and other beta-agonists so that patients would then fail to respond to either endogenous or exogenous catecholamines during an acute episode of asthma.⁴ This possibility was supported by two retrospective studies of patients taking excessive amounts of inhaled isoprenaline who, when tested, showed little bronchodilator response to inhaled isoprenaline. Once isoprenaline inhaler usage was reduced or discontinued their bronchodilator response returned.^{5,6} The development of beta-adrenoceptor

resistance after beta-agonist treatment has been demonstrated for the tremor,⁷ metabolic,⁸⁻¹⁰ heart rate,^{4,11} and white cell cyclic-AMP^{12,14} responses to beta-agonists in both normal subjects and patients with asthma. The important question of whether patients with asthma develop bronchial beta-adrenoceptor resistance as a result of large or even moderate doses of beta-agonists has been investigated in more than 30 studies in the last decade. In the majority, drug-induced bronchial resistance could not be demonstrated,^{7,15-18} and when it did occur the changes were usually small.^{10,19-21} This is in contrast to the only study in normal subjects where bronchial beta-adrenoceptor resistance developed progressively over four weeks as subjects took increasing doses of inhaled salbutamol.²² This apparent difference between normal and asthmatic subjects may be a true difference, or may be the result of differences in technique and problems inherent in studying patients with asthma.

This study was therefore designed to compare three groups of subjects using an identical technique

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Table 1 Details of subjects in the three groups

		Normal	Atopy	Asthma
Sex	Male	5	4	7
	Female	1	2	1
Age (years)	Mean	31	32	29
	Range	18-57	23-50	22-53
% predicted	FEV ₁	117	113	91
	SEM	7.4	3.9	8.0

—normal subjects, atopic non-asthmatic subjects, and atopic asthmatic patients. Subjects were studied before and during four weeks in which they inhaled increasing doses of salbutamol.

Methods

SUBJECTS

All subjects agreed to participate after a full explanation of the protocol which was agreed by the Southampton Ethical Committee. Details of the six normal subjects, six atopic non-asthmatic subjects, and eight asthmatic subjects who participated are shown in table 1; all were non-smokers.

Normal subjects were healthy, had no respiratory symptoms, and no personal or family history of asthma or allergy. All had negative skin prick tests to five common allergens and less than 10% change in FEV₁ after 100 µg inhaled salbutamol.

The atopic non-asthmatic subjects gave a typical history of hay fever and had at least two positive skin prick tests to five common allergens, one being mixed grass pollen. They showed less than 10% change in PEF_R during recordings four times daily for four days and after 100 µg inhaled salbutamol, and less than 10% fall in FEV₁ after six minutes' exercise on a bicycle ergometer at 100 watts and after a two-minute inhalation of 0.5% histamine. They were studied in winter when asymptomatic.

The asthmatic subjects gave a history of intermittent wheezing attacks for at least five years, had

positive skin prick tests to at least two of five common allergens, and a minimum 15% fluctuation in FEV₁ or PEF_R spontaneously or after 100 µg inhaled salbutamol. Their asthma had never necessitated hospital admission nor steroid therapy. All had recently been in good health requiring only an occasional inhalation of salbutamol (less than five in the preceding month). No other treatment was taken before or during the study by any subject.

INHALED SALBUTAMOL DOSE-RESPONSE STUDIES

Airway resistance was measured in a constant volume body plethysmograph and expressed as specific airway conductance (sGaw), the reciprocal of airway resistance divided by lung volume. For each measurement a set of 12 tracings was recorded on light sensitive paper. Each set was then coded and read blind by an independent observer to obtain a mean value for sGaw.²³ Measurements were made 10 minutes after the subject inhaled increasing doses of salbutamol from specially prepared metered aerosols providing cumulative doses of salbutamol from 10 to 600 µg.

HISTAMINE CHALLENGE STUDIES

Pilot studies on each asthmatic and each atopic subject determined the concentration of histamine required to cause a fall in FEV₁ of at least 20% and this concentration was used throughout the study. Histamine acid phosphate diluted in normal saline was inhaled from a Wright's nebuliser during tidal breathing, using compressed air at six litres/min. Measurements of sGaw were made before and immediately after a two-minute inhalation, and again after 30 minutes recovery. The subject then inhaled salbutamol (100 µg), sGaw was measured 10 minutes later and finally repeated after a second histamine challenge.

Table 2 Mean baseline values for sGaw and FEV₁ ± SEM

Salbutamol dose-response studies		Normal		Atopy		Asthma	
		Mean	SEM	Mean	SEM	Mean	SEM
sGaw	Control	1.69	0.11	1.90	0.10	1.35△	0.13
	Week 4	1.90	0.11	1.84	0.08	1.30	0.14
FEV ₁	Control	4.62	0.30	4.45	0.32	3.68△	0.36
	Week 4	4.62	0.28	4.21*	0.30	3.33*	0.37
Histamine challenge studies							
sGaw	Control			1.81	0.11	1.46△	0.14
	Week 4			1.76	0.12	1.48	0.23
FEV ₁	Control			4.38	0.36	3.69△	0.37
	Week 4			4.29	0.36	3.60	0.35

△ p < 0.05 asthma versus normal and atopic subjects. * p < 0.05 week 4 versus control. sGaw is in s⁻¹ kPa⁻¹ and FEV₁ in litres.

Table 3 *Salbutamol dose-response studies—mean change in sGaw ($s^{-1}kPa^{-1}$) and FEV₁ before and after 600 μ g salbutamol*

Control sGaw	Normal			Atopy			Asthma		
	Mean	SEM	% change	Mean	SEM	% change	Mean	SEM	% change
Control baseline	1.69	0.11		1.90	0.10		1.35	0.13	
600 μ g	2.71	0.09	60	2.47	0.13	30	2.23	0.21	65
Week 1 baseline	1.86	0.14		1.81	0.08		1.32	0.17	
600 μ g	2.72	0.07	46	2.52	0.16	39	2.37	0.31	80
Week 2 baseline	1.81	0.12		1.64	0.11		1.51	0.25	
600 μ g	2.56	0.13	41	2.37	0.24	45	2.56	0.28	70
Week 3 baseline	1.82	0.14		—	—		1.45	0.15	
600 μ g	2.48	0.12	36	—	—	—	2.26	0.20	56
Week 4 baseline	1.90	0.11		1.84	0.08		1.30	0.14	
600 μ g	2.40	0.10	26	2.59	0.32	41	2.51	0.25	93
<i>FEV₁</i>									
Control baseline	4.62	0.30		4.45	0.32		3.68	0.36	
600 μ g	4.88	0.29	5	4.57	0.33	3	4.02	0.39	9
Week 4 baseline	4.62	0.28		4.21	0.30		3.33	0.37	
600 μ g	4.90	0.28	6	4.47	0.34	6	3.86	0.32	16

PROTOCOL

Three control inhaled salbutamol dose-response studies were carried out on each subject on separate days. The asthmatic and atopic, non-asthmatic subjects also carried out a separate control histamine challenge study, and made regular six-hourly PEFr recordings at home for four days. When control studies were complete, subjects were asked to inhale salbutamol for four weeks, 100 μ g four times a day for the first week, and increasing to 300, 400, and 500 μ g four times a day during weeks 2, 3, and 4 respectively. An inhaled salbutamol dose-response study was carried out at the end of each week, 12 hours after the last salbutamol inhalation. During

week 4 the asthmatic and atopic subjects carried out a histamine challenge study and repeated the six-hourly PEFr recordings for four days.

STATISTICAL ANALYSIS

Salbutamol dose-response curves were constructed by plotting sGaw against the cumulative dose of inhaled salbutamol. The mean of three separate dose-response studies was used to obtain the control dose-response curve. Values of sGaw during the four weeks of treatment were compared for each dose of salbutamol by the Mann-Whitney U test.

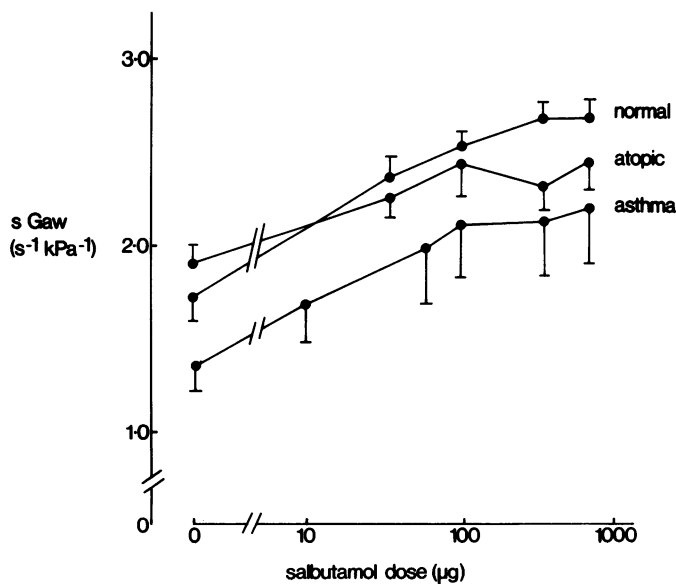


Fig 1 Mean sGaw values + SEM for three control dose-response studies for six normal, six atopic, non-asthmatic, and eight asthmatic subjects.

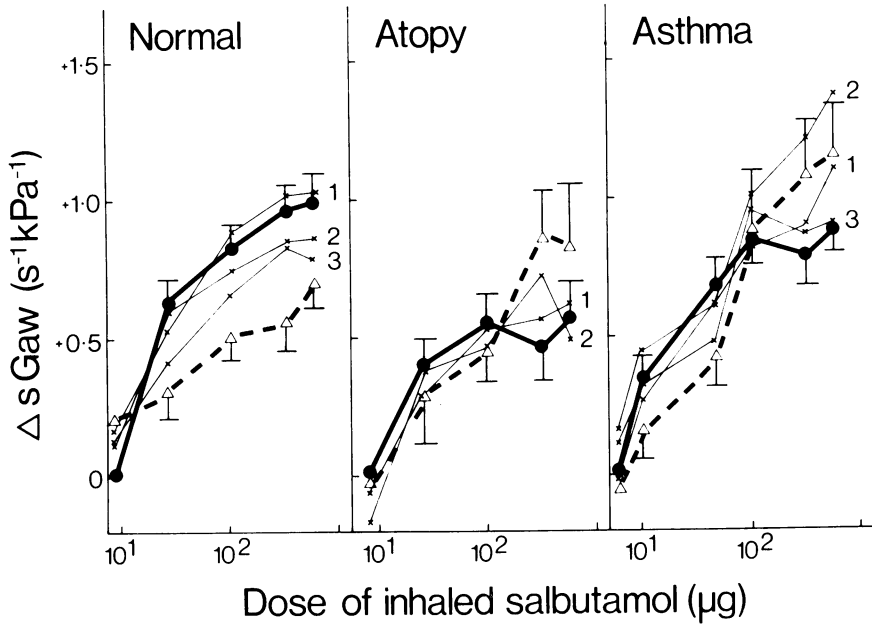
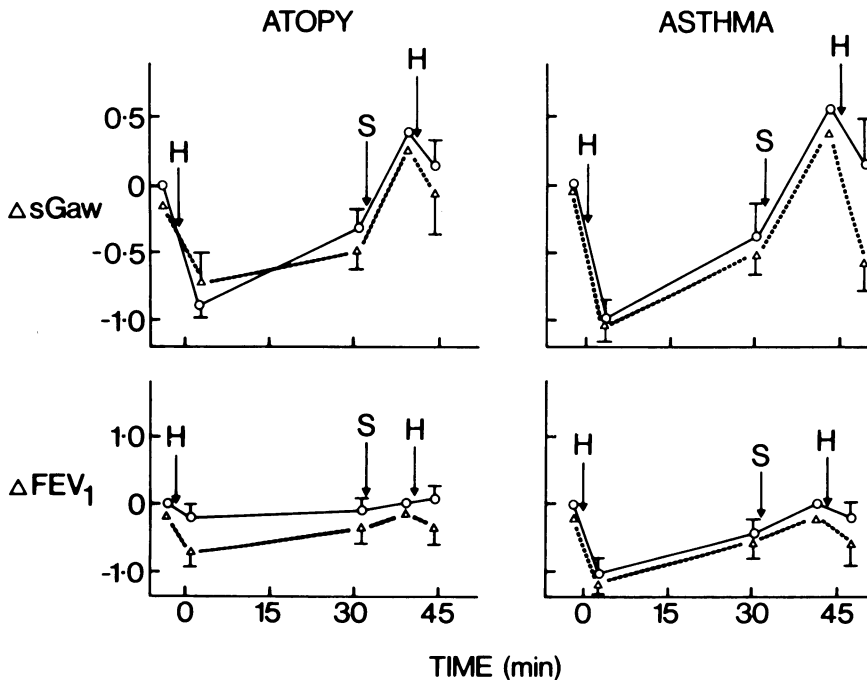


Fig 2 Mean change in $sGaw$ from control baseline $sGaw$ for each salbutamol dose-response study for the normal, atopic, and asthmatic subjects. The bold lines are control values ●—● and week 4 △—△, with weeks 1, 2, and 3 in fainter lines X—X (week 3 was omitted in the atopic group).



H = histamine inhalation, S = 100 µg salbutamol inhalation

Fig 3 Histamine challenge studies. Mean change in $sGaw$ ($s^{-1}kPa^{-1}$) and FEV_1 (litres) + 1 SEM for six atopic and eight asthmatic subjects, before ○—○ and during week 4 △—△ of regular salbutamol inhalations.

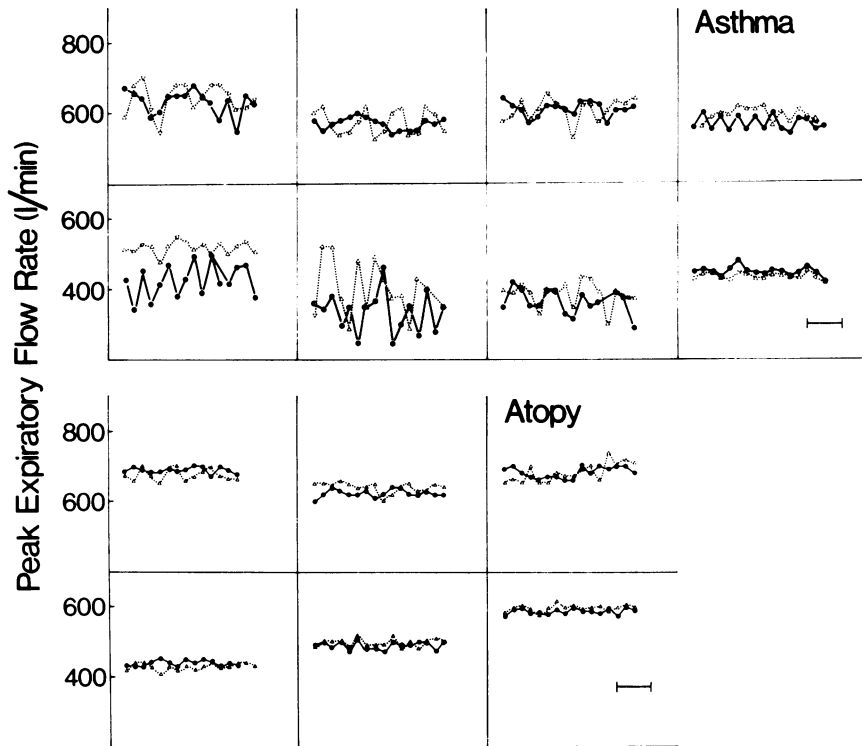


Fig 4 Individual peak flow values taken four times daily for four days for six atopic and eight asthmatic subjects before ●—● and during week 4 △—△ of regular salbutamol inhalations. — = 24 hours.

Results

BASELINE VALUES FOR SGAW AND FEV₁

Mean baseline values for sGaw and FEV₁ for the three groups of subjects are shown in table 2. There was no significant difference in control values for sGaw and FEV₁ between the normal and atopic subjects, but both groups had significantly higher baseline values than the asthmatic subjects ($p < 0.05$). The small differences in mean baseline sGaw between control period and week 4 were not significant for any of the three groups. The small fall in baseline FEV₁ from control to week 4 was significant however for both the atopic (4.45 to 4.21 litres, $p < 0.05$) and asthmatic group (3.68 to 3.33 litres, $p < 0.05$), but no change occurred in the normal subjects.

CONTROL SALBUTAMOL DOSE-RESPONSE STUDIES (TABLE 3, FIG 1)

There was a progressive increase in sGaw with increasing doses of salbutamol in all three groups; 60% in normal subjects ($p < 0.001$), 30% in atopic

subjects ($p < 0.005$), and 65% in asthmatic subjects ($p < 0.003$). The corresponding increases in FEV₁ over baseline were 5% (normal), 3% (atopic), and 9% (asthmatic).

SALBUTAMOL DOSE-RESPONSE STUDIES DURING FOUR WEEKS' REGULAR SALBUTAMOL (TABLE 3, FIG 2)

After regular inhalation of salbutamol, normal subjects showed a progressive decrease in the airway response to salbutamol, so that by week 4, 600 μ g salbutamol produced only a 26% increase in sGaw compared with 60% in the control period ($p < 0.03$). This reduction was significant at all doses on the dose-response curve ($p < 0.03$). The FEV₁ response was 6% in week 4 compared with 5% in the control study.

In contrast, atopic subjects maintained their response to salbutamol; after four weeks' regular treatment an increase in sGaw of 41% after 600 μ g salbutamol was not significantly different from the 30% in the control study. There was a 6% increase in FEV₁ in week 4 compared with 3% in the control

study. Similarly, asthmatic subjects also maintained their response to salbutamol with a 93% increase in sGaw in week 4 compared with 65% in the control study after 600 µg salbutamol ($p > 0.4$). There was a 16% increase in FEV₁ in week 4 compared with 9% in the control study ($p = 0.08$).

HISTAMINE CHALLENGE STUDIES

Control baseline values of sGaw and FEV₁ were lower in the asthmatic than the atopic subjects. Neither group showed any significant change in baseline sGaw or FEV₁ between control period and week 4 (table 2), nor any difference in the sGaw or FEV₁ response to histamine or in the protection afforded by salbutamol against histamine challenge (fig 3).

PEAK EXPIRATORY FLOW RATES

Mean values for PEFR in the control studies were 585 ± 9.7 and 494 ± 9.8 l/min respectively for the atopic, non-asthmatic, and asthmatic groups (102 and 83% predicted). There was no significant change in the mean values during week 4 (593 ± 9.8 and 519 ± 9.2 l/min) nor any consistent change in individual peak expiratory flow patterns (fig 4).

Discussion

This is the first prospective study to compare airway responsiveness to a beta-agonist in normal and asthmatic subjects before and during treatment with large doses of inhaled salbutamol using the same technique. By choosing patients with mild and relatively stable asthma the problem of fluctuating airway calibre was reduced. The results suggest that normal subjects develop resistance after doses of inhaled salbutamol which do not cause resistance in asthmatic subjects. Baseline sGaw was lower in the asthmatic patients in the control study but the percentage increase in sGaw in response to salbutamol was similar in both groups (60 and 65%). The atopic subjects had a smaller response to salbutamol (30%), possibly because of their higher baseline sGaw, though this did not differ significantly from sGaw in the normal subjects.

After regular inhaled salbutamol the response of the normal and asthmatic subjects differed, with normal subjects developing a progressive fall in their airway response to salbutamol, while the asthmatic subjects clearly maintained their response. The extent and time-course of beta-adrenoceptor resistance in the normal subjects is very similar to that demonstrated in the only previous airway study of normal subjects.²² The atopic non-asthmatic subjects responded like the asthmatic patients, maintaining their airway responsiveness to salbutamol. The

absence of any detectable resistance by week 4 in the salbutamol dose-response studies in these patients was further supported by the lack of any consistent change in the airway response to inhaled histamine or the protection afforded by salbutamol against a histamine challenge. Peak expiratory flow rates in both atopic and asthmatic subjects also showed no change in either mean values or diurnal pattern during the study. We have no way of ensuring that all subjects took regular salbutamol as prescribed, but all were considered reliable, all used a similar number of inhalers, and the findings were consistent within each group. The findings also fit with the clinical impression that patients with asthma rarely develop bronchial resistance to beta-agonists when inhaling amounts close to or above the recommended maximum dose. These results, therefore, suggest a true difference between normal subjects and asthmatic subjects, with atopic non-asthmatic subjects behaving like asthmatic subjects in this respect. The difference is probably a relative one since the studies of Van Metre⁵ and Reisman⁶ strongly suggest that bronchial beta-adrenoceptor resistance did develop in patients taking very large doses of beta-agonists—up to one inhaler a day.

The results in the asthmatic patients agree with most previous prospective studies in asthma in which comparable doses of beta-agonists have been used. These have usually been unable to demonstrate the development of bronchial beta-adrenoceptor resistance;^{7 15–18} in contrast to studies on non-bronchial beta-adrenoceptor responses (tremor, heart rate, lymphocyte and leucocyte cyclic-AMP, and intermediary metabolites) where resistance has frequently developed.^{7 10–14} Doses of beta-adrenoceptor agonists which result in impairment of tremor, lymphocyte cyclic-AMP, or metabolic responses in asthmatic patients may not impair airway responses,^{7 12 24} even when given by inhalation.²⁴ This suggests that tissues vary in the ease with which they develop resistance, with bronchial tissue being considerably less susceptible than other tissues in asthmatic patients.

The reduction in baseline FEV₁ after four weeks' salbutamol treatment in the atopic and asthmatic subjects was unexpected and not associated with a significant reduction in sGaw. The changes may be transient since they were not found before histamine challenge in week 4, nor was there any concurrent fall in PEFR or clinical deterioration. These results could be a chance finding, although a similar fall in PEFR and FEV₁ has been reported previously after regular adrenergic therapy.^{21 25} Since the changes affected FEV₁ rather than sGaw they may reflect narrowing of small airways. A possible explanation would be increased bronchial mucus production

since this has been demonstrated in animals and man after treatment with beta-adrenoceptor agonists.²⁶⁻²⁷ Alternatively, sudden withdrawal of large doses of beta-agonists may upset the autonomic control of mast cells,²⁸ allowing a transient increase in mediator release.

The reason why normal and asthmatic subjects differ in their response to large doses of beta-agonists is not clear. Although asthmatic patients in this study showed no change in airway responsiveness to salbutamol after four weeks' regular salbutamol, the plasma cyclic-AMP response to intravenous salbutamol in the same subjects was reduced.²⁴ If plasma cyclic-AMP levels reflect intracellular cyclic-AMP levels in bronchial smooth muscle, failure to develop bronchial beta-adrenoceptor resistance suggests that there may be a protective mechanism independent of the beta-adrenoceptor. For example, increased intracellular steroid levels might induce a cyclic-AMP dependent protein kinase to augment the effect of intracellular cyclic-AMP.²⁹ Alternatively, our findings may be the result of changes in the beta-adrenoceptor since plasma cyclic-AMP levels may not reflect concentrations of cyclic-AMP in bronchial smooth muscle, particularly in view of the differing susceptibilities of different tissues to develop resistance.

Recent beta-adrenoceptor radioligand binding studies have suggested that asthma may be associated with a reduced number of beta-adrenoceptors,³⁰ or a relative increase in the alpha to beta-adrenoceptor ratio.³¹⁻³² Most of these changes are likely to be caused by previous treatment with beta-agonists since patients with asthma on no treatment have a normal number of leucocyte beta-adrenoceptor binding sites,³³ and excess agonist, both in vitro³⁴ and in vivo³⁵ in normal subjects cause a reduction in leucocyte beta-adrenoceptor numbers. The number of beta-adrenoceptor radioligand binding sites does not necessarily reflect functional activity. An 85% reduction in leucocyte dihydroalprenolol binding sites was found in asthmatic subjects after moderate doses of oral terbutaline for only six days³⁵ in marked contrast to the complete lack of any functional impairment in airway beta-adrenoceptor responsiveness in both our study and in previous studies of oral terbutaline.⁷ Also, the increased alpha/beta adrenoceptor ratio in sensitised guinea-pig lung was not associated with any change in the adenylate cyclase response to isoprenaline.³² The findings of leucocyte radioligand binding studies cannot be extrapolated to beta-adrenoceptor function in the airways, and do not help to determine the cause of the changes we observed.

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