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In vitro responsiveness of human asthmatic bronchus to carbachol, histamine, β-adrenoceptor agonists and theophylline

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1 Responses of human bronchial strip preparations to contractile and relaxant agonists were measured in preparations from non-diseased and from asthmatic lung obtained 3–15 h post-mortem.

2 The potencies of carbachol and histamine were approximately two times less in asthmatic than in non-diseased bronchi. This was statistically significant for carbachol ($P < 0.05$), but not for histamine ($P > 0.05$). These results clearly indicate that the bronchial hyperreactivity to airway spasmogens observed in asthma is exclusively an *in vivo* phenomenon not involving increasing sensitivity of bronchial smooth muscle.

3 The potencies of the β-adrenoceptor agonists isoprenaline, fenoterol and terbutaline were significantly reduced by 4–5 fold in asthmatic bronchi compared with non-diseased airways. In contrast, theophylline was equipotent in the two populations of airway preparations. Thus, it appears that severe asthma is associated with decreased bronchial smooth muscle β_2-adrenoceptor function.

Keywords human bronchus asthma carbachol histamine theophylline β-adrenoceptor agonists

Introduction

Theories proposed to account for asthma and the accompanying non-specific bronchial hyperreactivity include enhanced responsiveness of bronchial smooth muscle to spasmogens and depressed activity of the β-adrenoceptors mediating bronchial relaxation (Szentivanyi, 1968; 1980; Boushey *et al.*, 1980; Cross, 1981).

In vivo metabolic and cardiovascular responsiveness to β-adrenoceptor agonists has been studied in both asthmatic and non-atopic subjects (Cookson & Reed, 1963; Parker, 1973), but inter-subject variability in nutritional and hormonal status, resting airway calibre and the influence of reflex mechanisms have made the interpretation of results difficult. Accordingly, *in vitro* studies using leukocytes from non-atopic and from asthmatic subjects have been used extensively to monitor β-adrenoceptor function and β-adrenoceptor density (Parker & Smith, 1973; Kariman & Lefkowitz, 1977; Galant *et al.*, 1978a,b; 1980; Brooks *et al.*, 1979; Morris, 1980). However, controversy remains as to whether the reduced β-adrenoceptor responsiveness and the reduced β-adrenoceptor density observed in lymphocytes harvested from asthma sufferers, were directly related to therapy with β-adrenoceptor agonists (Galant *et al.*, 1978a,b; 1980; Morris, 1980) and/or to the disease state (Parker & Smith, 1973; Brooks *et al.*, 1979). Furthermore, studies in lymphocytes may not always reflect the status of β-adrenoceptors in the lung (Harvey & Tattersfield, 1982; Tashkin *et al.*, 1982; Hasegawa & Townley, 1983). Clearly, an *in vitro* study of β-adrenoceptor function, as well as of responsiveness to airway spasmogens such as carbachol and histamine in asthmatic

airways is desirable. The present paper documents such a study, preliminary data having been previously reported (Paterson *et al.*, 1982).

Methods

Non-diseased bronchi were obtained at autopsy from the macroscopically normal lung of 32 subjects suffering sudden death from automobile accidents, heart disease, homicide or suicide. Bronchi were also obtained from six asthmatic subjects who died during attacks of bronchial asthma and from one other asthma sufferer who died as a result of a coronary occlusion. Airway preparations were dissected from the lungs 3–15 h post-mortem. Excised bronchi (2–3 mm i.d.) were dissected free of all parenchyma tissue and visible blood vessels and then cut into helical spiral strips. All preparations were suspended under 500 mg tension in Krebs-Henseleit solution, maintained at 37° C and aerated with 5% CO_2 in O_2. Changes in isometric tension were measured with a Grass-force-displacement transducer (FTO3C) coupled to a Rikadenki pen recorder (model 1328L). Preparations were allowed to equilibrate for 2 h with washing at 30 min intervals before any drug-induced effects were measured. Concentration-effect curves for both spasmogenic and relaxant agonists were constructed by cumulative drug administration and approximately 1 h was allowed to elapse between curves.

Almost all human bronchial preparations developed some tone spontaneously. However, unless otherwise stated, bronchial tone was also induced with a concentration of carbachol which caused 50% of the maximum response to this spasmogen (EC_{50}), before the effects of relaxant agonists were measured. This concentration was determined separately for each preparation from a cumulative concentration-effect curve. Cumulative concentration-effect curves to bronchial spasmogens were constructed in preparations with resting levels of tone.

Up to 12 bronchial preparations were dissected from a given specimen of human lung. Concentration-effect curves for particular agonists were constructed in some or all of these preparations. Mean values for concentrations of agonists producing 10, 30, 50, 70 and 90% of the maximum response (E_{max}) were then obtained for bronchi from a particular lung sample. Final mean curves were constructed from values from separate lung samples. Agonist potencies were determined as mean pD_2 values where $pD_2 = -\log_{10}$ agonist concentration producing 50% E_{max}.

The probability of differences between mean pD_2 values was determined by Student's two-tailed, non-paired *t*-test and considered significant if $P < 0.05$.

Drugs

The drugs used were isoprenaline hydrochloride, theophylline, carbamylcholine chloride (Sigma); histamine maleate (SKF); terbutaline sulphate (Astra); salbutamol hydrochloride (ICI); rimiterol hydrochloride (Riker); fenoterol hydrobromide (Boehringer Ingelheim). All drug solutions were freshly prepared daily in 0.9% NaCl solution containing ascorbic acid 20 $\mu g\ ml^{-1}$.

Results

Of the seven asthmatic subjects from whom lung samples were obtained at post-mortem, six died during an attack of bronchial asthma and one from a coronary occlusion before medical aid arrived. Macroscopic examination revealed hyper-inflation of these lung samples as well as impacted mucus in peripheral bronchioles. These properties are characteristic of severe asthma. Light microscopic examination also revealed histological abnormalities including bronchiolar smooth muscle hypertrophy, exfoliated bronchial lumen cells and eosinophil infiltration of airways, that were compatible with severe asthma.

Table 1 summarizes the relevant characteristics of the deceased asthmatics including their recent drug therapy and the post-mortem age of the lung samples obtained from them. Subject 1 had voluntarily withdrawn from all medical treatment for asthma approximately 2 years before death. Subject 2 had asthma that was apparently well controlled and only used a salbutamol inhaler on rare occasions. Subject 3 suffered from poorly controlled severe asthma requiring regular drug therapy. Subjects 4 and 5 had labile asthma that was controlled by regular bronchodilator therapy. Subject 6 obtained salbutamol 'over the counter' as self-medication for asthma. This subject's general medical practitioner was unaware of her asthma and was treating her with propranolol for hypertension. Bronchial preparations from the lungs from this subject were not used in assessments of the relaxant potencies of β-adrenoceptor agonists or theophylline. Subject 7 suffered severe asthma and received regular bronchodilator therapy. In addition, he was being treated for epilepsy. There was no significant difference between non-diseased and asthmatic groups with respect to either the mean age of subjects or the post-mortem age of lung samples ($P > 0.05$; Table 2).

Table 1 Relevant characteristics of deceased donor asthmatic subjects

Subject	*Sex*	*Age (years)*	*Recent drug therapy*	*Cause of death*	*Post-mortem age of tissue (h)*
1	Female	18	Nil	Bronchial asthma	12
2	Female	52	salbutamol on rare occasions	Bronchial asthma	14
3	Female	78	salbutamol theophylline beclomethasone dipropionate digitalis	Bronchial asthma	13
4	Male	8	salbutamol theophylline	Bronchial asthma	9
5	Female	17	salbutamol theophylline beclomethasone dipropionate	Bronchial asthma	12
6	Female	40	salbutamol propranolol	Bronchial asthma	12
7	Male	60	salbutamol theophylline carbamazepine thioridazine	Coronary occlusion	7

Table 2 Comparison of subject age and post-mortem age of lung samples for non-diseased and asthmatic groups

	Subject age (years)	*Post-mortem age of lung sample (h)*
Non-diseased ($n = 32$)	39.8 ± 4.1	8.6 ± 0.6
Asthmatic ($n = 7$)	39.0 ± 9.8*	11.3 ± 0.9*

Results expressed as mean ± s.e. mean.
*Not significantly different cf value for non-diseased group, $P > 0.05$ (non-paired t-test).

Carbachol and histamine

Both carbachol and histamine caused concentration-dependent contraction of non-diseased and of asthmatic human bronchi (Figure 1). The ratio of the EC_{50} values for these spasmogens in the two populations of bronchi showed that carbachol was 2.6 times less potent in asthmatic than in non-diseased airways ($P < 0.05$, non-paired t-test), while the potency of histamine was similar in the two groups ($P > 0.4$). Furthermore, the maximum response (mg) to carbachol and to histamine was not significantly different in non-diseased and asthmatic bronchi ($P > 0.05$).

Isoprenaline

Isoprenaline caused a concentration-dependent relaxation of carbachol-induced tone in 78 bronchial preparations from 22 non-diseased lungs and in 16 preparations from four separate samples of asthmatic lung (Figure 2a). Isoprenaline was on average approximately five times less potent in asthmatic than in non-diseased bronchi. In two preparations with spontaneous tone from asthmatic lung samples 1 and 2, isoprenaline was approximately 30 and 3 fold less potent respectively, than in non-diseased bronchi. Figure 2b shows isoprenaline concentration-effect curves in six non-diseased bronchial preparations and in a preparation from asthmatic lung sample 3, where responses were calculated as a % of the carbachol-induced tone. Note that carbachol was administered at a concentration causing only 30% of the maximal contraction, and that these preparations had gained some tone spontaneously. Clearly, the isoprenaline concentration-effect curve in the asthmatic bronchus is displaced to the right relative to the mean curve obtained in non-diseased bronchi. The maximal relaxation produced by isoprenaline was also greatly reduced. This was not due to an inability of the smooth muscle to relax, since theophylline (1×10^{-3} M) added cumulatively after the last isoprenaline dose, caused a further marked relaxation. Similarly, incomplete relaxation to isoprenaline was observed in bronchi from asthmatic lung samples 1 and 2.

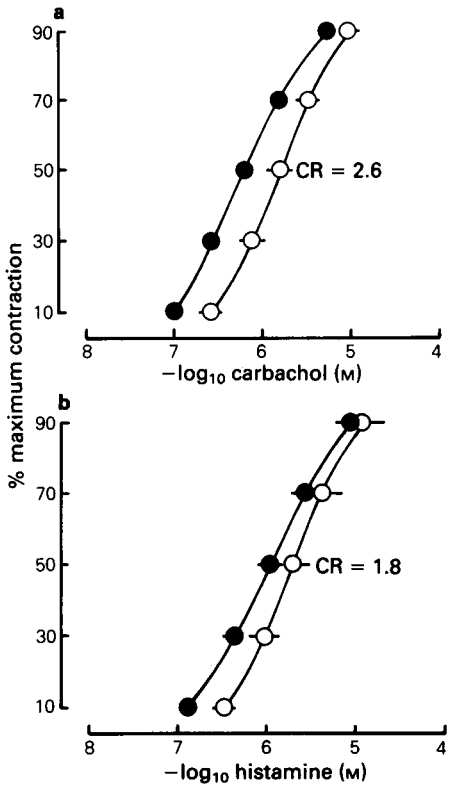

Figure 1 Mean cumulative $\log_{10}$ concentration-effect curves to (a) carbachol and (b) histamine in human non-diseased (•) and asthmatic (○) isolated bronchial preparations. Responses were calculated as a percentage of the maximal response to the spasmogen. Horizontal bars represent standard errors of mean concentrations of spasmogens producing 10, 30, 50, 70 and 90% of the response maximum from N samples of human lung, which provided *n* bronchial preparations. For carbachol in non-diseased lung, N = 26 and *n* = 130 and in asthmatic lung, N = 6 and *n* = 53. For histamine, N = 4 for both non-diseased and asthmatic lung and *n* = 19 and 7 respectively. CR = concentration ratio at the 50% response level.

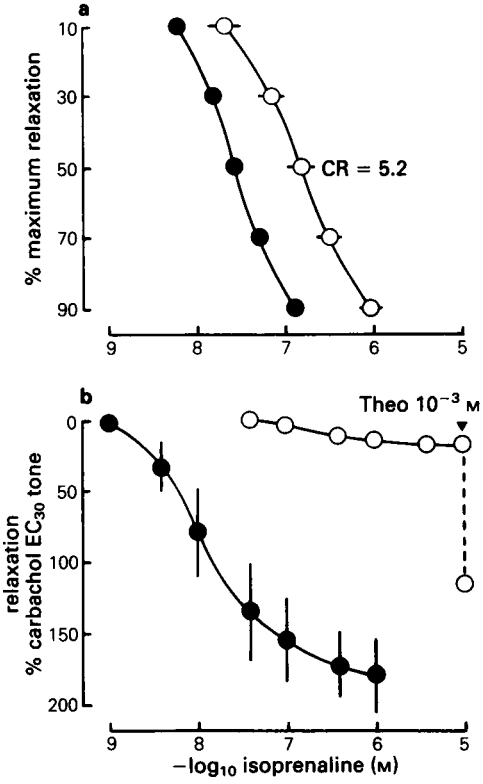

Figure 2 (a) Mean cumulative $\log_{10}$ concentration-effect curves to isoprenaline in human non-diseased (•) and asthmatic (○) isolated bronchial preparations. All preparations were pre-contracted with a concentration of carbachol producing 50% of the maximal response to this spasmogen. Responses were calculated as a percentage of the maximal relaxation to isoprenaline in each preparation. Horizontal bars represent standard errors of mean concentrations of isoprenaline producing 10, 30, 50, 70 and 90% of the maximal relaxation to isoprenaline from 22 non-diseased lung samples (78 preparations) and four asthmatic lung samples (16 preparations). CR = concentration ratio at the 50% response level. (b) Mean cumulative $\log_{10}$ concentration effect curves to isoprenaline in six bronchial preparations from three non-diseased lung samples (•) and a single preparation of asthmatic bronchus (○). All preparations developed tone spontaneously and were also precontracted with a concentration of carbachol causing a 30% maximal contraction. Relaxation responses were calculated as a percentage of the carbachol-induced tone. Theo = theophylline. Vertical bars represent standard errors of mean responses to isoprenaline.

Other β-adrenoceptor agonists and theophylline

Figure 3 shows cumulative concentration-effect curves to the β_2-selective adrenoceptor agonists fenoterol, terbutaline, rimiterol and salbutamol and to the non-β-adrenoceptor agonist bronchodilator theophylline, in non-diseased preparations and in bronchi from asthmatic subjects 4, 5 and 7. Fenoterol was four fold less potent in asthmatic than in non-diseased bronchi. However, the two mean concentration-effect curves produced for terbutaline showed that its potency was on average 3.5 fold less in asthmatic than in non-asthmatic airways preparations, while those of salbutamol and theophylline were not statistically different in the two types of airway

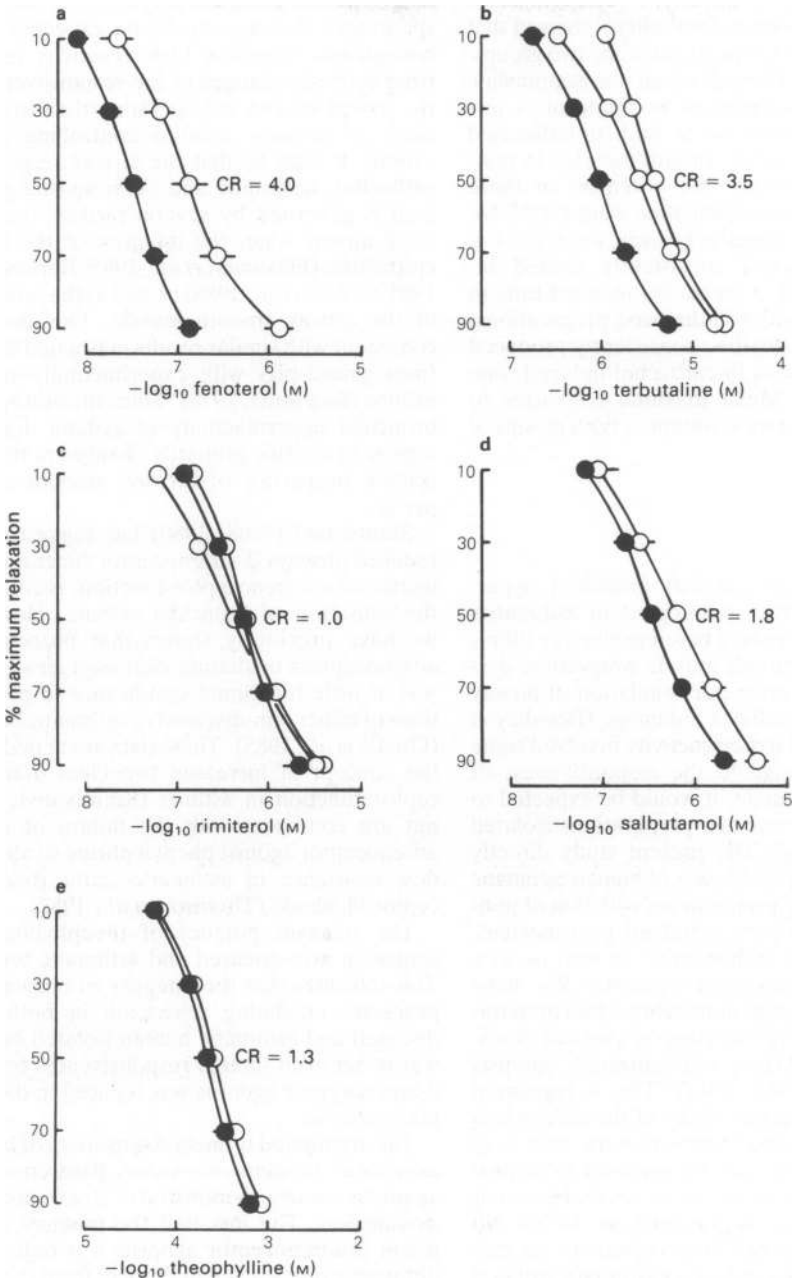

Figure 3 Mean cumulative $\log_{10}$ concentration-effect curves to bronchial relaxant agonists from N samples of human lung which provided *n* bronchial preparations from non-diseased (●) and asthmatic (○) lung. All preparations were precontracted with a concentration of carbachol producing 50% of the maximal response to this spasmogen. Responses were calculated as a percentage of the maximal relaxation produced by the agonist in each preparation. Horizontal bars represent standard errors of mean concentrations of relaxant agonists producing 10, 30, 50, 70 and 90% of the maximal response to: (a) fenoterol, non-diseased, N = 7, *n* = 20; asthmatic, N = 3, *n* = 11; (b) terbutaline, non-diseased N = 4, *n* = 19; asthmatic, N = 2, (individual mean curves shown) *n* = 9; (c) rimiterol, non-diseased, N = 4, *n* = 17; asthmatic, N = 2, (individual mean curves shown), *n* = 8; (d) salbutamol, non-diseased, N = 7, *n* = 23; asthmatic, N = 3, *n* = 11; (e) theophylline, non-diseased, N = 8, *n* = 21; asthmatic, N = 3, *n* = 11.

($P > 0.05$; non-paired *t*-test). Overlapping concentration-effect curves for rimiterol showed that this agonist was also equipotent in the two groups. The potency order for relaxation was isoprenaline = fenoterol > salbutamol = rimiterol > terbutaline > theophylline in both non-diseased and asthmatic airways smooth muscle. In non-diseased preparations, the potencies of these agonists relative to isoprenaline were 1:1:13:18:33:7621 and in asthmatic bronchi were 1:1:4:4:22:1850. Salbutamol consistently caused incomplete reversal of carbachol-induced tone in both asthmatic and non-diseased preparations. Conversely, theophylline consistently produced maximum decreases in carbachol-induced tone below baseline. Mean maximal responses to theophylline (mg) were similar in both groups of preparations.

Discussion

It has been suggested that bronchial hyperreactivity to airway spasmogens in asthmatics may be due to decreased baseline airway calibre, altered airway smooth muscle properties, dysfunction in the autonomic regulation of airway calibre or to epithelial cell damage (Boushey *et al.*, 1980). If such hyperreactivity involved some fundamental change to the responsiveness of airway smooth muscle, it would be expected to be observed in bronchial preparations isolated from human lung. The present study directly compared the responsiveness of human asthmatic isolated bronchial preparations with that of non-diseased preparations, obtained post-mortem, to carbachol and to histamine as well as to a range of β-adrenoceptor agonists. We have previously shown that human bronchial preparations obtained post-mortem responded sensitively to both relaxant and contractile agonists (Goldie *et al.*, 1982, 1984). This is consistent with data showing that many of the deleterious changes to bronchial ultrastructure that may occur post-mortem, can be reversed by appropriate incubation of tissue in Krebs-Henseleit solution (Ferguson & Richardson, 1978). No evidence of bronchial hyperreactivity to carbachol or histamine in human asthmatic isolated bronchi was found. Indeed, there was a small but significant rightward shift in the concentration-effect curve to carbachol in asthmatic bronchi while the potency of histamine was not significantly different in the two groups. The most significant element in these findings is not the fact that spasmogen potency was slightly reduced or unaltered in asthmatic airways, but that no evidence at all was found of enhanced spasmogen potency in these preparations. Increased spasmogen potency would be expected if the non-specific bronchial hyperreactivity resulted from intrinsic changes in the responsiveness of the airway smooth muscle rather than to alterations in extrinsic systems controlling airway calibre. It may be that the airway response to carbachol, histamine and other spasmogens in man is governed by several factors, including basal airway tone, the integrity of the airway epithelium (Flavaham *et al.*, 1985; Barnes *et al.*, 1985; Goldie *et al.*, 1986) as well as the sensitivity of the airway smooth muscle. Our data are consistent with similar results in isolated trachea from guinea-pigs with experimentally-induced asthma (Souhrada, 1978). Thus, the non-specific bronchial hyperreactivity of asthma does not appear to involve primarily changes in the contractile properties of airways smooth muscle per se.

Szentivanyi (1968, 1980) has suggested that reduced airways β-adrenoceptor function, with increased α-adrenoceptor function, is central to the causation of bronchial asthma. However, we have previously shown that bronchial α-adrenoceptors mediating increased airway tone was of little functional significance in preparations of either non-diseased or asthmatic bronchi (Goldie *et al.*, 1985). These data are at odds with the concept of increased bronchial α-adrenoceptor function in asthma (Szentivanyi, 1980) but are consistent with the failure of the α-adrenoceptor agonist phenylephrine to alter airflow resistance in asthmatics after β-adrenoceptor blockade (Thomson *et al.*, 1982).

The relaxant potency of theophylline was similar in non-diseased and asthmatic bronchi. This indicates that the integrity of intracellular processes mediating relaxation in both non-diseased and asthmatic human isolated bronchi was intact even though responsiveness to some β-adrenoceptor agonists was reduced in diseased preparations.

The attenuated bronchial sensitivity of human asthmatic bronchi to some β-adrenoceptor agonists, clearly demonstrates β-adrenoceptor dysfunction. The fact that the potency of different β-adrenoceptor agonists was reduced to different extents, even in bronchi from the same asthmatic lung, was to be expected assuming that some of these agonists have greater efficacies at β_2-adrenoceptors than others. Kenakin (1984) has shown that the potencies of agonists with the greatest efficacies will be more severely reduced than those with lesser efficacies, under conditions of reduced stimulus-response coupling. Such a state could result following a reduction in β-adrenoceptor number in human asthmatic

bronchial smooth muscle. We did not design the present experiments to compare β-adrenoceptor agonist efficacies in non-diseased and asthmatic bronchi. However, our data clearly show that salbutamol was a partial relaxant while isoprenaline and fenoterol had greater efficacies. Preliminary unpublished data also suggest that rimiterol and terbutaline have lesser efficacies than either isoprenaline or fenoterol in human isolated bronchi.

It could be argued that the lesser sensitivity of asthmatic bronchi to carbachol as well as to isoprenaline, fenoterol and terbutaline compared with that in non-diseased bronchi, resulted from the very different conditions within the lungs of the two groups prior to death, resulting in a non-specific attenuation of airway responsiveness to drugs. However, the similarities in the potencies of histamine, rimiterol, salbutamol and theophylline in bronchi from asthmatic and non-diseased lung indicates that there were no significant deleterious effects on smooth muscle function in asthmatic bronchi or that the deleterious changes were reversed in the organ bath.

The relative influence of β-adrenoceptor agonist therapy and the disease state on responses to β-adrenoceptor agonists could not be determined in this study. However, recent findings suggest that, in contrast to β-adrenoceptors in leukocytes from asthmatics, β-adrenoceptors in asthmatic airways do not become significantly desensitized after regular exposure to long acting β-adrenoceptor agonists (Harvey & Tattersfield, 1982; Tashkin *et al.*, 1982). Reduced responsiveness to β-adrenoceptor agonists in bronchi from asthmatic subjects 1 and 2, who had received little or no β-adrenoceptor agonist therapy, appears to have resulted from a reduction in the number of functional airways β-adrenoceptors associated with the disease state. Brooks *et al.* (1979) have shown that lymphocyte β-adrenoceptor density decreased in direct proportion to the severity of airways obstruction in asthma patients not receiving β-adrenoceptor agonist therapy.

The results of studies with β-adrenoceptor blockers in non-asthmatic subjects also argue against β-adrenoceptor hypofunction as the cause of asthma (Zaid & Beall, 1966; Townley *et al.*, 1976). Tattersfield *et al.* (1983) has shown that airways responsiveness to inhaled or intravenous salbutamol is similar in healthy subjects and mild asthmatics with relatively normal lung function, indicating that β-adrenoceptor dysfunction was not the cause of asthma. This result contrasts with the present *in vitro* data in bronchial preparations from severe asthmatics. Nevertheless, our data could be reconciled with those of Tattersfield *et al.* (1983) if β-adrenoceptor hypofunction was a result but not a cause of severe asthma. If this were so, then mild asthmatics could show airways sensitivity to β-adrenoceptor agonists within the normal range. In severe asthma, bronchial β-adrenoceptors would be down-regulated as a result of the disease. Such hypofunction might prove critical to the maintenance of adequate airways calibre.

Although results from the present study were limited by the obvious difficulties involved in obtaining samples of airways tissues from asthmatic lung, this work adds considerable weight to the concept of reduced bronchial β-adrenoceptor function in severe asthma. Furthermore, this study has clearly shown that airway smooth muscle from asthmatic lung is not intrinsically hyperresponsive to spasmogens.

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