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α_1-Adrenoceptor function and autoradiographic distribution in human asthmatic lung

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1 The autoradiographic distribution of α_1-adrenoceptors was investigated in non-diseased and asthmatic human lung by use of [^{3}H]-prazosin (H-PZ). To validate binding and autoradiographic methods, H-PZ binding was also measured in rat heart.

2 Significant levels of specific H-PZ binding were detected in sections of rat heart. This binding was associated with a single class of non-interacting sites of high affinity (dissociation constant, $K_d = 1.17 \pm 0.26$ nM). The maximum binding capacity (B_{max}) was 59.5 ± 4.5 fmol mg^{-1} protein.

3 In sharp contrast, very low levels of specific H-PZ binding were found in both human non-diseased and asthmatic bronchus, although a high level of binding of [^{125}I]-iodocyanopindolol (I-CYP, 50 pM) to β-adrenoceptors was detected in these airways. Furthermore, very low levels of autoradiographic grains representing specific H-PZ binding were found in all airway structures in human non-diseased or asthmatic lung parenchyma.

4 Consistent with these data, the α-adrenoceptor agonist phenylephrine failed to induce significant increases in tone in bronchi isolated from either non-diseased or asthmatic human lung. Results indicate that asthma does not involve significant increases in airway α_1-adrenoceptor function.

Introduction

It has been proposed that asthma is associated with enhanced α-adrenoceptor activity (Szentivanyi, 1980). This theory, was supported by some studies suggesting that α_1-adrenoceptor agonists such as phenylephrine and methoxamine, induced significant bronchoconstriction in asthmatics (Patel & Kerr, 1973; Snashall *et al.*, 1978; Black *et al.*, 1982; 1984), although others have produced evidence to the contrary (Thomson *et al.*, 1982). Some evidence suggests that α-adrenoceptor antagonists may be therapeutically beneficial in asthma (Griffith *et al.*, 1972; Patel & Kerr, 1975), while other studies do not support this concept (Ind & Dollery, 1983; Utting, 1979; Svedmyr, 1984; Baudouin *et al.*, 1988). Clearly, the role of α-adrenoceptors in airway smooth muscle function is controversial.

Several studies have demonstrated that α_1-adrenoceptor-mediated contractile responses in human isolated bronchial preparations are small and only evident after β-adrenoceptor blockade (Mathe *et al.*, 1971; Goldie *et al.*, 1984; 1985). However, greater α_1-adrenoceptor activity may reside at the level of bronchiolar airways (Barnes *et al.*, 1983a). Furthermore, significant α-adrenoceptor activity has been associated with increases in submucosal gland secretion (Peatfield & Richardson, 1982; Phipps *et al.*, 1982). Few studies have investigated α_2-adrenoceptor function in the lung. Barnes *et al.* (1983b) demonstrated α_2-adrenoceptor-mediated contraction of canine tracheal smooth muscle. However, this was not observed in human bronchus, although α_2-adrenoceptor-mediated attenuation of excitatory nerve transmission was documented (Grundstrom & Andersson, 1985). This is perhaps consistent with the fact that the α_2-selective agonist clonidine reduced bronchial obstruction in asthma (Lindgren *et al.*, 1986). Thus we were particularly interested in the possibility that the up-regulation of airway α_1-adrenoceptors is involved in asthma. To this end, we have examined the autoradiographic localization and distribution of specific binding sites for the α_1-selective radioligand [^{3}H]-prazosin (H-PZ) in human non-diseased and asthmatic lung parenchyma and bronchus. We have also investigated

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the functional effects of the α_1-selective agonist phenylephrine in human bronchi, to ascertain the likely physiological significance of such receptors.

Methods

Radioligand binding and autoradiography

Tissue preparation Samples of macroscopically normal lung were obtained from 3 victims of cardiovascular or automobile accidents with a mean subject age of 47.0 ± 17.4 years and a mean post-mortem age of 5.2 ± 2.0 h. Samples of asthmatic lung were also obtained from 3 severely asthmatic individuals. These subjects died rapidly before medical assistance arrived. Subject 1 was a 63 year old female (lung post-mortem age = 7 h) who used a Ventolin inhaler regularly and who died in respiratory failure. Subject 2 was a 62 year old male (lung post-mortem age = 4 h) asthmatic with chronic obstructive lung disease, for whom medication consisted of Ventolin, Moduretic, Minipress and Zyloprim. This subject died following a myocardial infarction. Subject 3 was a 60 year old male asthmatic (lung post-mortem age = 7.5 h) and who died following a coronary occlusion and who regularly used a Ventolin inhaler and oral theophylline.

Human bronchi (2–3 mm i.d.) were dissected free of parenchymal tissue and visible blood vessels and placed in aluminium foil trays containing Macrodex (6% dextran in 5% glucose). Parenchymal tissue was inflated by bronchial instillation of OCT embedding medium diluted 1:4 with 0.9% w/v NaCl solution (saline). Samples of rat ventricle were obtained from male Wistar rats (300–500 g) and placed in aluminium foil trays containing Macrodex (6% dextran in 5% glucose). All tissue samples were snap frozen in isopentane which had been quenched with liquid nitrogen. Tissue samples were then stored at −75°C until required. Serial frozen tissue sections (10 μm, autoradiographs; 16 μm, binding experiments) were cut at −30°C, mounted and thawed onto glass slides covered with gelatin. Sections were stored at −75°C for up to 2 weeks before use without loss of radioligand binding capacity.

Radioligand binding Slide-mounted sections (16 μm) of rat ventricle were incubated at 22°C for 5–60 min in Tris-HCl buffer (170 mM, pH 7.6) which contained [^{3}H]-prazosin (H-PZ; 79 Ci mmol^{-1}, 2 nM, Amersham) and the protease inhibitor phenylmethylsulphonylfluoride (PMSF, 10 μM). In another series of experiments, sections of rat ventricle were co-incubated with sections of human lung parenchyma at 22°C for 25 min in Tris-HCl buffer containing H-PZ (0.25–9 nM) and PMSF (10 μM). Specific binding of H-PZ to α_1-adrenoceptors was defined as that which was displaced by 10 μM phentolamine. Sections incubated with H-PZ were washed at 4°C in radioligand-free buffer for 1 min and again for 2 × 15 min periods, then rapidly rinsed in distilled water and wiped from slides with glass fibre filter paper (Whatman, GF/A). Tissue radioactivity was measured in a Packard liquid scintillation counter (Model B2450). The protein content of sections from each tissue block was estimated by the method of Lowry *et al.* (1951).

Autoradiography To determine the tissue distribution of α_1-adrenoceptors, slide-mounted sections (10 μm) of human non-diseased and asthmatic lung parenchyma or bronchus were incubated with H-PZ (1 nM) for 25 min in the absence or presence of 10 μM phentolamine. These preparations were washed as described for binding studies. Sections were then rapidly dried under a stream of cold dry air. Emulsion-coated (type 0) coverslips coated with NTB-3 nuclear track emulsion (Eastman Kodak Co., Rochester, N.Y.) were attached to one end of these tissue slides with cyanoacrylate adhesive and stored in desiccated light-tight X-ray cassettes at 4°C for 70 days. Autoradiographs were developed in Dektol (Kodak; diluted 1:1 with distilled water) for 3 min, rinsed in 1% acetic acid containing 2.5% Hypam hardener (Ilford) and fixed for 3 min with Hypam Rapidfix (Ilford; diluted 1:4 with distilled water) containing 2.5% Hypam hardener. Tissue sections were then lightly stained with Gill's haematoxylin for 30 s, dehydrated in graded solutions of ethanol, cleared in xylene and the coverslips re-apposed to the slides by tissue mounting in DePeX medium (BDH). Autoradiographs were viewed with a Zeiss III photomicroscope under light and dark-field illumination.

For comparative purposes, the distribution of β-adrenoceptors was also determined in slide-mounted sections of asthmatic bronchus using [^{125}I]-iodocyanopindolol (I-CYP, 50 pM) as previously described (Goldie *et al.*, 1986a).

Organ bath experiments

The mean ± s.e.mean subject age and post-mortem age of lung samples obtained from individuals with severe bronchial asthma were 29.2 ± 10.1 years and 10.5 ± 2.2 h ($n = 4$), respectively. Similarly, non-diseased lung was obtained from individuals with a mean subject age and post-mortem age of 44.4 ± 12.5 years and 8.4 ± 1.2 h ($n = 5$), respectively. There was no significant difference between non-diseased and asthmatic groups with respect to either the mean age of the subjects or the post-

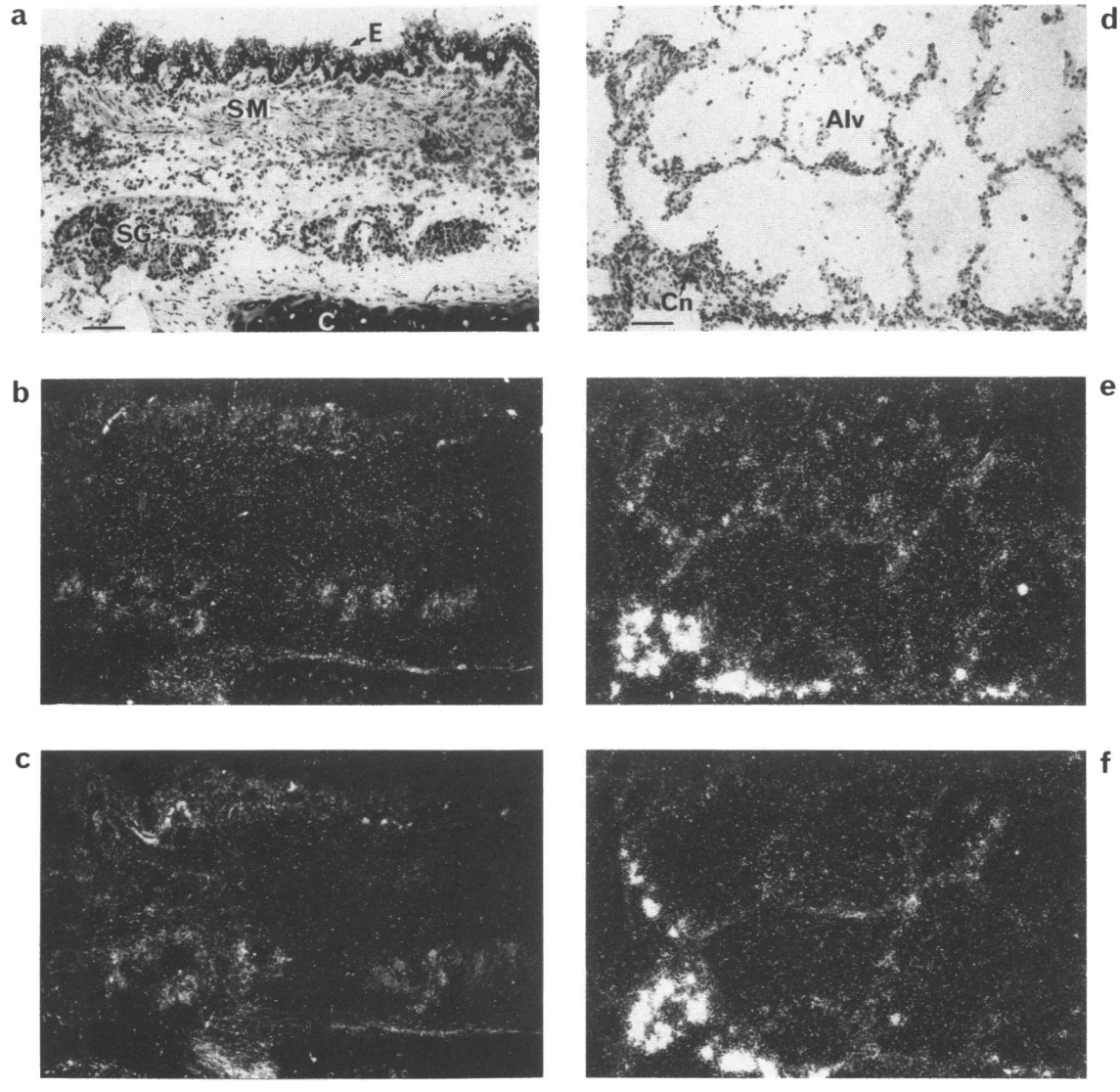

Figure 1 Photomicrographs of 10 μm thaw-mounted frozen sections of human non-diseased bronchus (a–c) and lung parenchyma (d–f). Light-field photomicrograph of (a) human bronchus showing the epithelium (E), smooth muscle (SM), submucosal glands (SG) and cartilage (C); (d) lung parenchyma showing alveoli (Alv), some of which contain carbon particles (Cn). (b and e) Dark-field photomicrographs of the above sections showing the distribution and localization of autoradiographic grains derived from [^{3}H]-prazosin (H-PZ, 1 nM) binding. (c and f) Dark-field photomicrograph showing the distribution of non-specific autoradiograph grains in respective serial sections incubated with H-PZ (1 nM) and phentolamine (10 μM). Bar = 100 μm.

mortem age of the lung samples ($P > 0.05$, non-paired t test).

Bronchial spiral preparations were dissected from both human non-diseased and asthmatic lung and suspended in organ baths as previously described (Goldie *et al.*, 1986b). All preparations were left to equilibrate for 2.5 h at 37°C in Krebs solution gassed with 5% CO_2 in oxygen before any pharmacological testing was attempted. Cumulative concentration-effect curves to carbachol were constructed in all

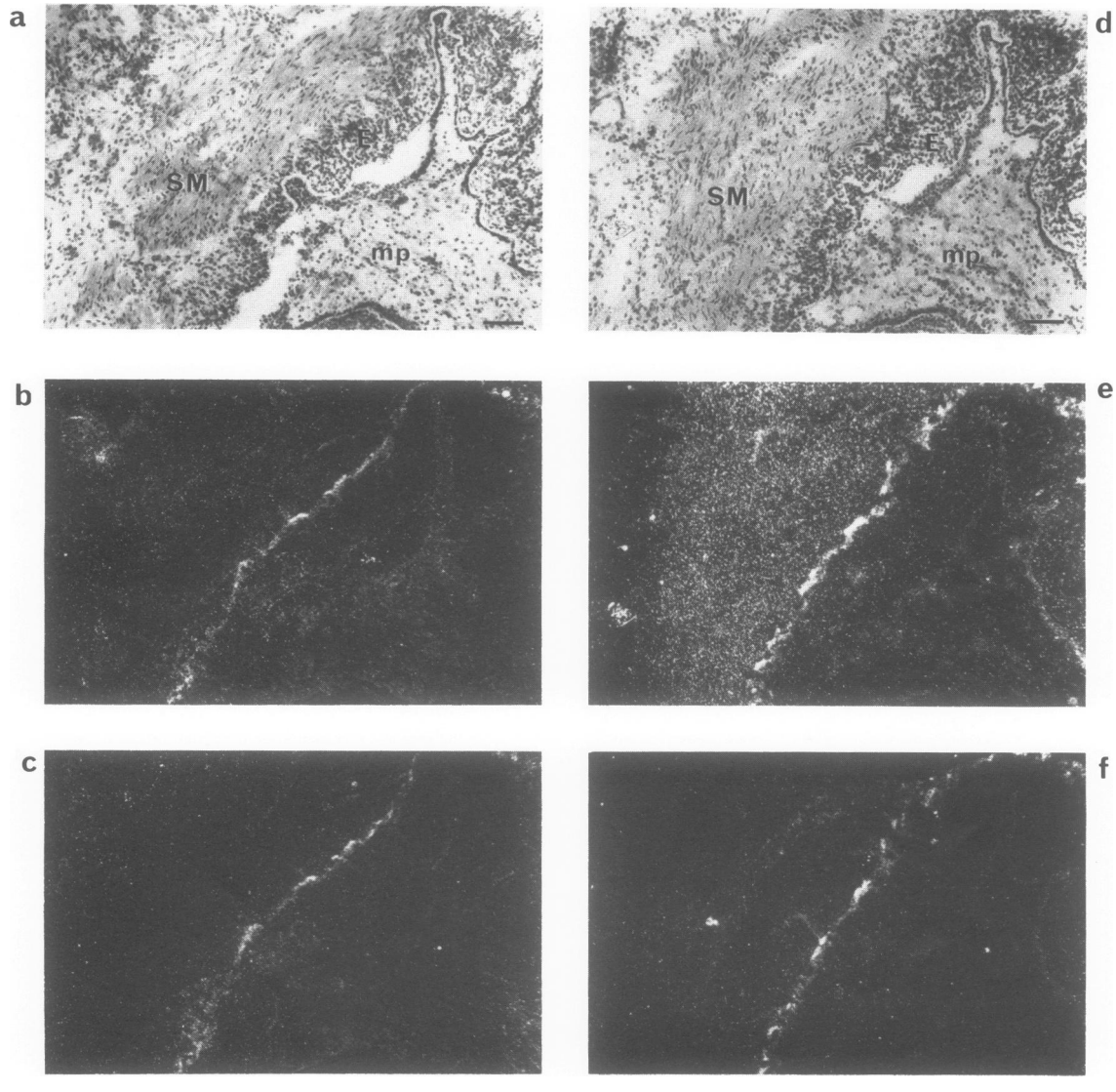

Figure 2 Photomicrographs of 10 μm thaw-mounted frozen sections of human asthmatic bronchus. (a, d) Light-field photomicrographs of 2 serial sections of human asthmatic bronchus showing smooth muscle (SM), damaged epithelium (E) and a luminal mucous plug (mp) infiltrated with cells. Dark-field photomicrographs of the above sections showing the distribution and localization of autoradiographic grains derived from (b) [^{3}H]-prazosin (H-PZ, 1 nM) and (e) [^{125}I]-iodocyanopindolol (I-CYP; 50 pM) binding. Dark-field photomicrograph showing the distribution of non-specific autoradiographic grains in respective serial sections incubated with (c) H-PZ (1nM) and phentolamine (10 μM) or (f) I-CYP (50 pM) and isoprenaline (200 μM). An artifact seen as bright areas associated with the epithelial basement membrane appears in each dark-field photomicrograph. Bar = 100 μm.

preparations used. Cumulative concentration-effect curves were also produced for (±)-phenylephrine in the absence or presence of propranolol (0.5 μM), in bronchial preparations with spontaneous or carbachol-induced tone. The concentration of carbachol used to induce tone was that concentration producing 50% (EC_{50}) of the maximum response to this agonist, as determined from the first cumulative concentration-effect curve. Preparations were washed at the end of each curve by 3 complete

changes of Krebs solution and an interval of 1 h allowed between curves.

Drugs and solutions

Carbamylcholine chloride, (±)-isoprenaline hydrochloride, (±)-phenylephrine hydrochloride, (±)-propranolol hydrochloride, theophylline (Sigma); (±)-phentolamine mesylate (Ciba); PMSF (phenylmethylsulphonylfluoride, Calbiochem). PMSF was freshly prepared in absolute ethanol. [^{3}H]-prazosin, 79 Ci mmol^{-1}; [^{125}I]-iodocyanopindolol, 2000 Ci mmol^{-1} (Amersham). The Krebs solution used throughout the study had the following composition (mM): NaCl 117.6, KCl 5.4, $NaHCO_3$ 25, KH_2PO_4 1.03, $MgSO_4$ 0.57, D-glucose 11.1 and $CaCl_2$ 2.5.

Analysis of results

All numerical results were expressed as mean ± s.e.mean. Parameters describing the concentration-dependence of H-PZ binding (dissociation constant, K_d; maximum binding capacity, B_{max}) were estimated by non-linear least squares regression analysis of data fitted to a one site binding model, by use of the computer programme MLAB (N.I.H., U.S.A.). Scatchard analysis was used to confirm these results and the nature of binding was described using Hill analysis. The probability (P) of differences between mean values was determined by use of Student's two tailed, non-paired t test and was considered significant if $P < 0.05$.

Results

Characteristics of [^{3}H]-prazosin binding

Incubation conditions appropriate for the detection of specific H-PZ binding were determined from studies using rat heart which is known to contain a significant population of α_1-adrenoceptors (Guicherey & Meyer, 1981). The specific binding of H-PZ (2 nM) in rat heart sections reached equilibrium after approximately 25 min, and was saturable and involved a single population of non-interacting, high affinity sites (Hill coefficient, nH = 1.007 ± 0.124). Non-linear regression analysis of the specific binding data using a one binding site model yielded a maximum binding capacity (B_{max}) of 59.5 ± 4.5 fmol mg^{-1} protein and a dissociation constant (K_d) of 1.17 ± 0.26 nM (where the errors were estimated directly from non-linear regression analysis of data from one heart). Specific binding accounted for 74% of total binding at a H-PZ concentration of 0.25 nM and 60% at a H-PZ concentration of 3.5 nM.

In sharp contrast, no evidence of a time-related increase in H-PZ (2 nM) binding or of binding saturability was obtained in tissue specimens from 3 samples of human non-diseased lung. Similarly, no significant levels of specific H-PZ binding were detected in tissue sections from asthmatic lung samples.

Autoradiography

The distribution of autoradiographic grains derived from H-PZ (1 nM) binding was assessed in human lung tissue in an attempt to identify locations of small populations of α_1-adrenoceptors, which might not be detected in binding studies. However, only very low levels of specific H-PZ binding were observed in specimens of human non-diseased bronchus and lung parenchyma (Figure 1) and asthmatic bronchus (Figure 2) and lung parenchyma. Some areas of peripheral lung tissue contained black deposits resembling carbon particles or tar (Figures 1d), presumably derived from atmospheric pollutants including cigarette smoke. These areas are associated with high levels of non-specific binding (Figures 1f). Specific grain densities over smooth muscle and submucosal glands in bronchi from both human non-diseased and asthmatic lung were also very low. In contrast to the low levels of specific H-PZ binding in asthmatic bronchus, this tissue contained high levels of specific I-CYP binding which represents β-adrenoceptors (Figure 2).

Organ bath experiments

Phenylephrine caused partial concentration-dependent relaxation in 10 of 11 bronchial preparations from human non-diseased lung with spontaneously developed (Figure 3a) or carbachol-induced tone (Figure 3b), while 1 preparation failed to respond to phenylephrine but contracted in response to carbachol. In contrast, in the presence of the β-adrenoceptor antagonist propranolol (0.5 μM), phenylephrine caused small concentration-dependent increases in tone in 6 of 10 bronchial preparations from 5 separate non-diseased lung samples (Figure 3c). Maximal phenylephrine-induced contractions amounted to 10, 11, 12, 13, 18 and 24% of the respective maximum contractile response to carbachol.

In one bronchial preparation from an asthmatic lung sample, phenylephrine caused an increase in basal tone equivalent to 14% of the maximal contractile response to carbachol. Conversely, concentration-dependent relaxation was obtained in 2 of 4 bronchial preparations with spontaneous tone from 3 asthmatic lungs (Figure 3d). The 2 bronchial

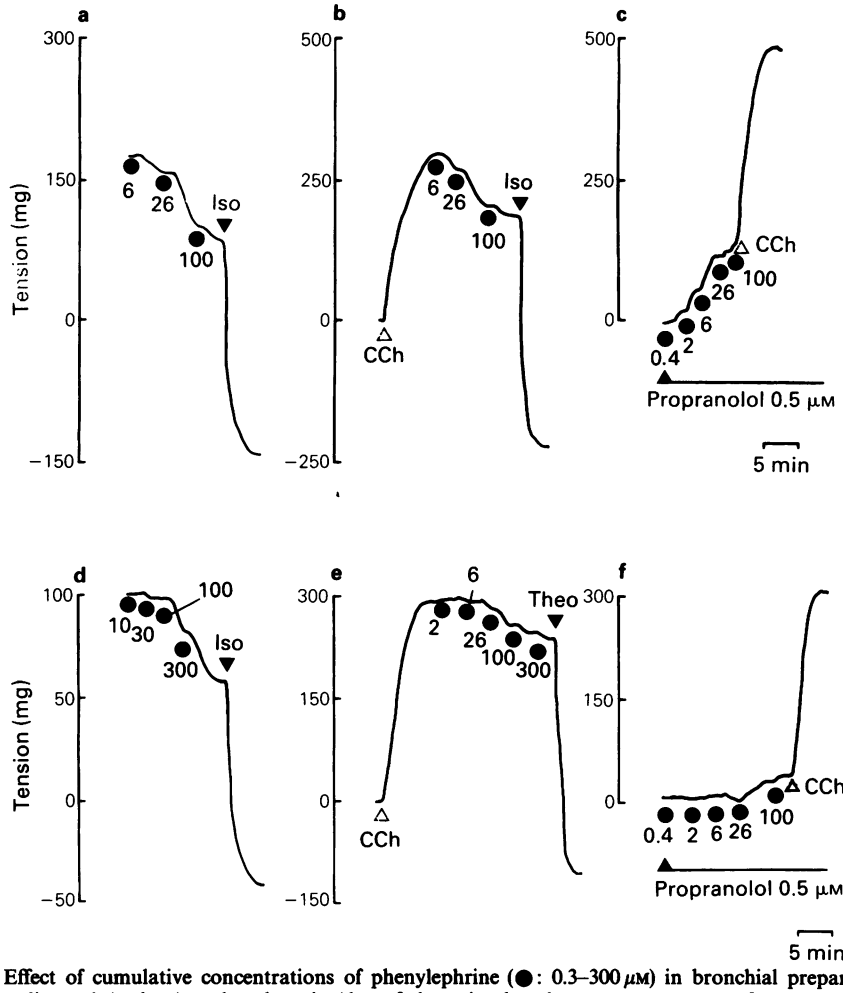

Figure 3 Effect of cumulative concentrations of phenylephrine (●: 0.3–300 μM) in bronchial preparations from human non-diseased (a, b, c) and asthmatic (d, e, f) lung in the absence or presence of propranolol (0.5 μM). CCh = carbachol (0.3–1 μM). Iso = isoprenaline (6.4 μM). Theo = theophylline (1 mM).

preparations which were unresponsive to phenylephrine did contract in response to carbachol and relaxed in response to isoprenaline. Phenylephrine also caused concentration-dependent relaxation in each of 5 bronchial preparations with carbachol-induced tone from 4 asthmatic lung samples (Figure 3e). Even in the presence of propranolol (0.5 μM), these preparations failed to contract in response to phenylephrine (Figure 3f).

Discussion

After β-adrenoceptor blockade, adrenaline, noradrenaline or phenylephrine-induced stimulation of human pulmonary α-adrenoceptors has been shown to cause vascular smooth muscle contraction (Goldie *et al.*, 1982), submucosal gland secretion (Phipps *et al.*, 1982), inhibition of excitatory neurotransmission (Grundstrom & Andersson, 1985; Andersson *et al.*, 1986) and weak contractions of bronchial smooth muscle (Goldie *et al.*, 1984; 1985). While the physiological significance of airway smooth muscle α-adrenoceptor function in healthy lung is uncertain, some studies suggest that α_1-adrenoceptor function is enhanced in asthma (Black *et al.*, 1982; 1984; Szentivanyi *et al.*, 1984), perhaps as a result of the presence of mediators including histamine (Kneussel & Richardson, 1978). However, we were unable to demonstrate such enhancement in the presence of

histamine (Goldie *et al.*, 1985). Other studies have also failed to show increased α-adrenoceptor activity in asthmatic compared to non-asthmatic bronchi (Svedmyr, 1984; Goldie *et al.*, 1988; Barnes, 1986).

Samples of post-mortem asthmatic human lung are obtained very infrequently. The need to obtain approximately age-matched samples of non-diseased and asthmatic human lung within 11 h post-mortem in order to conduct appropriate comparisons of both functional and autoradiographic data, further reduces the number of suitable tissue samples. Despite these constraints, it is clear from the present study that asthma does not involve significantly increased airway α_1-adrenoceptor function. Indeed, phenylephrine caused only small contractions of bronchi from both non-diseased and asthmatic lung. Furthermore, in all but one case, weak contractions were only elicited after propranolol-induced blockade of β-adrenoceptors. In the absence of propranolol, phenylephrine caused β-adrenoceptor-mediated partial relaxation of these bronchi, which emphasizes the relative insignificance of α_1-adrenoceptor function in human bronchial smooth muscle.

Autoradiographic experiments were conducted in order that populations of α-adrenoceptors present throughout the lung including airway smooth muscle could be examined. Consistent with previous findings (Sholtz *et al.*, 1980; Guicherey & Meyer, 1981; Muntz *et al.*, 1986), high levels of specific H-PZ binding to sections of rat heart were detected in binding analyses.

In sharp contrast, very low levels of specific H-PZ binding were detected in association wtih airway structures within human lung parenchyma and bronchi. Interestingly, bronchiolar airways in ferret lung (Barnes *et al.*, 1983a) have been shown to contain significant numbers of α_1-adrenoceptors. This suggests that great care must be taken when extending conclusions drawn from studies using animal lung to human airways. However, it is noteworthy that there was no evidence of significant numbers of bronchiolar α_1-adrenoceptors in rat lung (Xue *et al.*, 1983). Conversely, high levels of binding of I-CYP to β-adrenoceptors were detected in various structures within bronchi from both non-diseased and asthmatic lung.

While the present study shows that α_1-adrenoceptor activity in healthy and asthmatic human airway structures is low, these findings are contrary to results from some studies using homogenized membrane preparations from human non-diseased (Szentivanyi *et al.*, 1979; Barnes *et al.*, 1980b), asthmatic (Szentivanyi *et al.*, 1979) and bronchitic lung (Barnes *et al.*, 1980b) and in guinea-pig lung (Barnes *et al.*, 1979; 1980a). The detection of low but significant numbers of α_1-adrenoceptors in these investigations may reflect the much higher protein content of membrane preparation aliquots compared with that in lung tissue sections. Importantly, however, the number of α_1-adrenoceptors detected in these membrane preparations was at least 5–10 fold lower than the number of β-adrenoceptors present. While the possibility of some post-mortem fall out of α_1-adrenoceptors in airway smooth muscle cannot be excluded, we have previously demonstrated powerful α_1-adrenoceptor-mediator contraction of human pulmonary artery obtained up to 14 h after death (Goldie *et al.*, 1982). Thus it seems more likely that the number of α_1-adrenoceptors in human bronchi are too low to be readily detected by radioligand binding or light microscopic autoradiography.

The present study has clearly demonstrated the absence of significant numbers of airway α_1-adrenoceptors in human lung. These results are in line with studies reporting only weak α-agonist-induced bronchoconstriction (Thomson *et al.*, 1982) and disappointing therapeutic efficacy with α-adrenoceptor antagonists in asthma (Svedmyr, 1984).

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References

ANDERSSON, R.G.G., GRUNDSTROM, N. & LINDGREN, B.R. (1986). Alpha$_2$-adrenoceptors and asthma. *Trends Pharmacol. Sci.*, **7**, 177–178.

BARNES, P.J. (1986). Neural control of human airways in health and disease. *Am. Rev. Resp. Dis.*, **134**, 1289–1314.

BARNES, P.J., BASBAUM, C.B., NADEL, J.A. & ROBERTS, J.M. (1983a). Pulmonary α-adrenoceptors: Autoradiographic localization using [^{3}H]-prazosin. *Eur. J. Pharmacol.*, **88**, 57–62.

BARNES, P.J., DOLLERY, C.T. & MACDERMOT, J. (1980a). Increased pulmonary α-adrenergic and reduced β-adrenergic receptors in experimental asthma. *Nature*, **285**, 569–571.

BARNES, P.J., KARLINER, J.S. & DOLLERY, C.T. (1980b). Human lung adrenoceptors studied by radioligand binding. *Clin. Sci.*, **58**, 457–461.

BARNES, P.J., KARLINER, J.S., HAMILTON, C.A. & DOLLERY, C.T. (1979). Demonstration of α_1-adrenoceptors in guinea-pig lung using [^{3}H]-prazosin. *Life Sci.*, **25**, 1207–1214.

BARNES, P.J., SKOOGH, B-E., NADEL, J.A. & ROBERTS, J.M. (1983b). Postsynaptic alpha$_2$-adrenoceptors predominate over alpha$_1$-adrenoceptors in canine tracheal smooth muscle and mediate neuronal and hormonal alpha-adrenergic contraction. *Mol. Pharmacol.*, **23**, 570–575.

BAUDOUIN, S.V., AITMAN, T.J. & JOHNSON, A.J. (1988). Prazosin in the treatment of chronic asthma. *Thorax*, **43**, 385–387.

BLACK, J.L., SALOME, C.M., YAN, K. & SHAW, J. (1982). Comparison between airways response to an alpha-adrenoceptor agonist and histamine in asthmatic and non-asthmatic subjects. *Br. J. Clin. Pharmacol.*, **14**, 464–466.

BLACK, J.L., SALOME, C.M., YAN, K. & SHAW, J. (1984). The action of prazosin and propylene glycol on methoxamine-induced bronchoconstriction in asthmatic subjects. *Br. J. Clin. Pharmacol.*, **18**, 349–353.

GOLDIE, R.G., LULICH, K.M. & PATERSON, J.W. (1985). Bronchial α-adrenoceptor function in asthma. *Trends Pharmacol. Sci.*, **6**, 469–472.

GOLDIE, R.G., LULICH, K.M., SPINA, D. & PATERSON, J.W. (1988). Role of alpha-adrenoceptors in the lung. In *Focus on Pulmonary Pharmacology and Toxicology*, Vol. 1, ed. Hollinger, M.A. pp. 91–110. Boca Raton: CRC Press Inc.

GOLDIE, R.G., PATERSON, J.W. & WALE, J.L. (1982). Pharmacological responses of human and porcine lung parenchyma, bronchus and pulmonary artery. *Br. J. Pharmacol.*, **76**, 515–521.

GOLDIE, R.G., PATERSON, J.W., SPINA, D. & WALE, J.L. (1984). Classification of β-adrenoceptors in human bronchus. *Br. J. Pharmacol.*, **81**, 611–615.

GOLDIE, R.G., PAPADIMITRIOU, J.M., PATERSON, J.W., RIGBY, P.J. & SPINA, D. (1986a). Autoradiographic localization of β-adrenoceptors in pig lung using [^{125}I]-iodocyanopindolol. *Br. J. Pharmacol.*, **88**, 621–628.

GOLDIE, R.G., SPINA, D., HENRY, P.J., LULICH, K.M. & PATERSON, J.W. (1986b). *In vitro* responsiveness of human asthmatic bronchus to carbachol, histamine, β-adrenoceptor agonists and theophylline. *Br. J. Clin. Pharmacol.*, **22**, 669–676.

GRIFFITH, J.P., KAMBUROFF, P.L. & PRIME, F.J. (1972). Thymoxamine amd airways obstruction. *Lancet*, **i**, 1288.

GRUNDSTROM, N. & ANDERSSON, R.G.G. (1985). Inhibition of the cholinergic neurotransmission in human airways via prejunctional alpha-2-adrenoceptors. *Acta Physiol. Scand.*, **125**, 512–517.

GUICHEREY, P. & MEYER, P. (1981). Binding of [^{3}H]-prazosin and [^{3}H]-dihydroergocryptine to rat cardiac α-adrenoceptors. *Br. J. Pharmacol.*, **73**, 33–39.

IND, P.W. & DOLLERY, C.T. (1983). Pulmonary adrenoceptors and asthma. *Agents Actions*, (Suppl. **13**), 213–244.

KNEUSSL, M.P. & RICHARDSON, J.B. (1978). Alpha-adrenergic receptors in human and canine tracheal smooth muscle. *J. Appl. Physiol.*, **45**, 307–311.

LINDGREN, B.R., EKSTROM, T. & ANDERSSON, R.G.G. (1986). The effect of inhaled clonidine in patients with asthma. *Am. Rev. Resp. Dis.*, **134**, 266–269.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. & RANDELL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

MATHE, A.A., ASTROM, A. & PERSSON, N-A. (1971). Some bronchoconstriction and bronchodilating responses of human isolated bronchi; evidence for the existence of alpha-adrenoceptors. *J. Pharm. Pharmacol.*, **23**, 905–910.

MUNTZ, K.H., GARCIA, C. & HAGLER, H.K. (1986). α_1-Receptor localization on rat heart and kidney using autoradiography. *Am. J. Physiol.*, **249**, H512–H519.

PATEL, K.R. & KERR, J.W. (1973). The airways response to phenylephrine after blockade of alpha and beta receptors in extrinsic asthma. *Clin. Allergy*, **3**, 439–448.

PATEL, K.R. & KERR, J.W. (1975). Effect of alpha receptor blocking drug, thymoxamine, on allergen induced bronchoconstriction in extrinsic asthma. *Clin. Allergy*, **5**, 311–316.

PEATFIELD, A.C. & RICHARDSON, P.S. (1982). The control of mucin secretion into the lumen of the cat trachea by α- and β-adrenoceptors and their relative involvement during sympathetic nerve stimulation. *Eur. J. Pharmacol.*, **81**, 617–626.

PHIPPS, R.J., WILLIAMS, I.P., RICHARDSON, P.S., PELL, J., PACK, R.J. & WRIGHT, N. (1982). Sympathetic drugs stimulate the output of secretory glycoprotein from human bronchi *in vitro*. *Clin. Sci.*, **63**, 23–28.

SCHOLTZ, H. (1980). Effects of beta and alpha-adrenoceptor activators and adrenergic releasing agents in the mechanical activity of the heart. In *Adrenergic Activators and Inhibitors* Pt 1, ed. Szekeres, L. pp. 651–712. New York: Springer-Verlag.

SNASHALL, P.D., BOOTHER, F.A. & STERLING, G.M. (1978). The effect of alpha-adrenoceptor stimulation on the airways of normal and asthmatic man. *Clin. Sci. Molec. Med.*, **54**, 283–289.

SVEDMYR, N. (1984). Szentivanyi's hypothesis of asthma. *Eur. J. Resp. Dis.*, (Suppl. 136), **65**, 59–65.

SZENTIVANYI, A. (1980). The radioligand binding approach in the study of lymphocytic adrenoceptors and the constitutional basis of atopy. *J. Allergy Clin. Immunol.*, **65**, 5–11.

SZENTIVANYI, A., HEIM, O. & SCHULTZE, P. (1979). Changes in adrenoceptor densities in membranes of lung tissue and lymphocytes from patients with atopic disease. *Ann. New York Acad. Sci.*, **332**, 295–298.

SZENTIVANYI, A., POLSON, J.B. & SZENTIVANYI, J. (1984). Issues of adrenoceptor behaviour in respiratory and cutaneous disorders of atopic allergy. *Trends Pharmacol. Sci.*, **5**, 280–282.

THOMSON, N.C., DANIEL, E.E. & HARGREAVE, F.E. (1982). role of smooth muscle alpha$_1$-receptors in nonspecific bronchial responsiveness in asthma. *Am. Rev. Resp. Dis.*, **126**, 521–525.

UTTING, J.A. (1979). Alpha-adrenergic blockade in severe asthma. *Br. J. Dis. Chest*, **73**, 317–318.

XUE, Q-F., MAURER, R. & ENGEL, G. (1983). Selective distribution of beta- and alpha$_1$-adrenoceptors in rat lung visualized by autoradiography. *Arch. Int. Pharmacodyn. Ther.*, **266**, 308–314.

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