Br. J. Pharmacol. (1990), 99, 136–144

Distribution of β_1- and β_2-adrenoceptors in mouse trachea and lung: a quantitative autoradiographic study

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1 Binding and quantitative autoradiography were used to detect [^{125}I]-iodocyanopindolol (I-CYP) associated with β_1- and β_2-adrenoceptors in mouse tracheal epithelium and airway smooth muscle as well as in lung parenchymal tissue.

2 Specific I-CYP binding to slide-mounted tissue sections of both trachea and parenchyma was of high affinity (K_D = 49.0 pM, n = 3, trachea; K_D = 118.9 pM, n = 3, parenchyma) and saturable, involving single populations of non-interacting binding sites (Hill coefficient nH = 1.00 ± 0.02, trachea; nH = 0.99 ± 0.03, parenchyma).

3 Direct measurement of tissue radioactivity also showed that specific I-CYP binding was competitively inhibited in the presence of the β-adrenoceptor antagonists (−)-propranolol (non-selective), CGP 20712A (β_1-selective) and ICI 118,551 (β_2-selective). Analysis of the competition binding curves for the two selective antagonists revealed mixed populations of β_1- and β_2-adrenoceptors in the approximate proportions 33% and 67% respectively in mouse trachea and 28% and 72% respectively in mouse lung parenchyma.

4 Densities of autoradiographic grains derived from specific I-CYP binding to alveolar wall tissue and to tracheal epithelium and airway smooth muscle were quantified by a computer-assisted image analysis system, which allowed the construction of competition binding curves in the presence of the selective β-adrenoceptor antagonists CGP 20712A and ICI 118,551. Analysis of these data demonstrated that in alveolar wall, β_1- and β_2-adrenoceptors co-existed in the proportions 18% and 82%, respectively.

5 Quantitative autoradiographic analyses also showed that β_1- and β_2-adrenoceptors were differentially distributed in tracheal epithelium and airway smooth muscle. The β_2-adrenoceptor subtype accounted for 71% of all β-adrenoceptors in epithelium. Conversely, β_1-adrenoceptors which mediate relaxant responses of mouse trachea to β-adrenoceptor agonists (Henry & Goldie, 1990), accounted for 69% of all β-adrenoceptors in the airway smooth muscle.

Introduction

Respiratory tract viral infections have been shown to provoke asthma attacks (Minor *et al.*, 1976; Gump *et al.*, 1976). Furthermore, bronchial hyperreactivity to various airway spasmogenic stimuli, which is a characteristic feature of asthma (Boushey *et al.*, 1980), has also been demonstrated following respiratory viral infection in man (Empey *et al.*, 1976; Little *et al.*, 1978; Aquilina *et al.*, 1980). This may be due, in part, to airway inflammation and epithelial damage (Laitinen *et al.*, 1985). However, it is also known that bronchoconstriction can be precipitated in many asthmatics following exposure to β-adrenoceptor antagonists including propranolol (Richardson & Sterling, 1969; Bernecker & Roetscher, 1970; Schwartz *et al.*, 1980). It is possible that viruses which cause respiratory infections, also induce bronchial β-adrenoceptor hypofunction, which in asthmatics might result in bronchoconstriction. This concept is supported by evidence for viral recognition of and binding to cell surface β-adrenoceptors (Co *et al.*, 1985). The mouse has been successfully used in studies of viruses causing respiratory tract infections in man, since similar infections result in both hosts in response to some of these viruses (Kilbourne, 1987).

Receptor binding studies with membrane preparations have shown that β_1- and β_2-adrenoceptors can co-exist in central and peripheral airways (Rugg *et al.*, 1978; Carswell & Nahorski, 1983). Furthermore, identification of the cellular locations of the β-adrenoceptor subtypes is now possible by the use of autoradiography. High numbers of β-adrenoceptors have been detected in guinea-pig trachea (Goldie *et al.*, 1986a,c) and human bronchus (Spina *et al.*, 1989a,b), as well as in lung parenchyma from several species, by light microscopic autoradiography (Barnes *et al.*, 1982a; Finkel *et al.*, 1984; Carstairs *et al.*, 1985; Goldie *et al.*, 1986b; Spina *et al.*, 1989a), although mouse trachea and lung parenchyma have not previously been studied. In general, these studies have shown that the greatest densities of β-adrenoceptors are found in alveolar septae. Studies using this approach have proposed the coexistence of β_1- and β_2-adrenoceptors in alveolar wall and submucosal glands (Carstairs *et al.*, 1985; Goldie *et al.*, 1986b). To study further the distribution and relative densities of β_1- and β_2-adrenoceptors within airway structures, we have developed a quantitative autoradiographic technique which utilizes an image analysis system in conjunction with a grain counting algorithm (Reep & Creegan, 1988).

Before interactions between respiratory viruses and mouse respiratory tract β-adrenoceptors can be appropriately assessed, some of the characteristics of β-adrenoceptors in this tissue, including density, distribution and subtype proportions, must be determined. In the present study, we have combined radioligand binding and quantitative autoradiographic techniques to determine the distribution and relative densities of β_1- and β_2-adrenoceptors within mouse tracheal structures, including smooth muscle and epithelium. In addition, we have described β-adrenoceptor populations in mouse lung parenchyma.

Methods

Male mice of the CBA/CaH strain (8–10 weeks of age) were anaesthetised with ether and killed by cervical dislocation and exsanguination.

Tissue preparation

The thorax was opened and the lungs instilled *in situ* via the trachea with OCT embedding medium (Tissue-Tek), diluted 1 in 4 with 0.9% w/v NaCl solution. After occlusion of the

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trachea, the heart and lungs were excised from the chest cavity and placed in Macrodex (6% dextran 70 in 5% glucose; Pharmacia). Lung parenchymal tissue was dissected free of the heart, submerged in Macrodex and frozen by immersion in isopentane, quenched with liquid nitrogen. In a separate group of mice killed as described above, the trachea was dissected free from surrounding tissue and placed in Krebs-bicarbonate solution containing (mM): NaCl 117, KCl 5.36, $NaHCO_3$ 25.0, KH_2PO_4 1.03, $MgSO_4.7H_2O$ 0.57, $CaCl_2.2H_2O$ 2.5 and glucose 11.1 at room temperature. Tracheal segments (4 mm in length) from six mice were submerged in Macrodex and frozen as for parenchyma. Serial transverse frozen sections (10 μm) were cut from all tissue blocks at −20°C and thaw-mounted onto gelatin/chrome alum-coated glass slides.

Binding experiments

Slide-mounted tissue sections were incubated with the β-adrenoceptor radioligand (−)-[^{125}I]-iodocyanopindolol (I-CYP) in Tris-HCl buffer (170 mM, pH 7.6, 22°C) containing the protease inhibitor phenylmethylsulphonyl fluoride (10 μM). Non-specific binding was estimated in the presence of 1 μM (−)-propranolol. After incubation periods of 120 min (trachea) or 150 min (lung parenchyma), which were found to be optimal for these tissues, tissue sections were washed for 2 × 15 min in Tris-HCl buffer to remove unbound I-CYP. Sections were wiped onto GF/A glass-fibre filter paper (Whatman) and counted in a Packard gamma counter. The concentration-dependence of I-CYP binding to mouse tracheal and lung parenchymal sections was determined at 10, 20, 40, 80, 120, 180 and 240 pM. In these experiments, total and non-specific binding were estimated in mouse trachea in tissue sections from 18 mice (3 blocks) and in mouse parenchyma by triplicate determinations from 3 mouse lungs. Specific binding for trachea was expressed as fmol per tracheal section and as fmol mg^{-1} protein for lung parenchyma. Protein levels were estimated by the Hartree (1972) modification of the Lowry method.

To characterize mouse tracheal and peripheral lung β-adrenoceptor subtypes, tissue sections were incubated with 70 pM I-CYP in the presence and absence of the competitive β-adrenoceptor antagonists (−)-propranolol (nonselective, 0.1 nM–100 μM), CGP 20712A (β_1-adrenoceptor selective, 0.05 nM–100 μM) or ICI 118,551 (β_2-adrenoceptor selective, 0.05 nM–100 μM).

Autoradiography

Slide-mounted tissue sections (prepared as for binding studies) were incubated at 22°C for 120 or 150 min in Tris-HCl buffer (170 mM, pH 7.6) containing 70 pM I-CYP in the presence or absence of ICI 118,551 (0.1 nM–2 μM) or CGP 20712A (0.1 nM–20 μM). Non-specific binding was determined by 1 μM (−)-propranolol. For trachea, 18 slides prepared in triplicate (i.e. 54 slides) containing tissue from 6 different mice were prepared, 12 of which were exposed to selected concentrations of ICI 118,551 or CGP 20712A, while 6 were used to determine specific binding. For lung parenchyma, 18 slides were prepared in duplicate (i.e. 36 slides) using tissue from 3 mice. Of these duplicate slides, 12 were exposed to selected concentrations of ICI 118,551 or CGP 20712A and 6 were used to determine specific binding. All slides were washed for 2 × 15 min in Tris-HCl buffer, briefly rinsed in distilled H_2O and sections rapidly dried by a stream of cold, dry air. Emulsion-coated coverslips (Kodak NTB-2) were attached with cyanoacrylate adhesive to one end of the slides and the preparations exposed for 24 h (lung parenchyma) or 90 h (trachea) at 4°C. The emulsion was developed (Kodak Dektol; 1:1, 3 min), rinsed briefly in dilute acetic acid (2%) containing 2.5% hardener (Ilford Hypam Hardener) and fixed (Ilford Hypam; 1:4, 2.5 min). Tissue sections were stained with haematoxylin, dehydrated in ethanol, cleared in xylene and mounted (DePeX; BDH) for light microscopy.

Quantitation of autoradiographic data Quantitative autoradiography was used to determine the relative proportions of β_1- and β_2-adrenoceptors located over tracheal epithelium, airway smooth muscle and over alveolar wall tissue. Autoradiographic slides were viewed by a charge coupled device solid state black and white video camera (National) attached to an Olympus BH-2 photomicroscope with an oil immersion objective lens (× 100, Zeiss PlanApo). The video signal was processed by a Data Translation DT-2803 video digitizer board running in an IBM AT compatible microcomputer. Video digitization gave an image with 256 × 256 pixel resolution, with each pixel having a grey scale of 0–63 units. The software of the MD-20 Image-analysis system (Flinders Imaging, Adelaide, Australia) controlled image capture, display, editing, feature selection, thresholding, measurement and storage. Stored images were downloaded onto the hard disc by use of a memory resident programme and autoradiographic grains then detected and counted by a series of assembler algorithms based on the method of Reep & Creegan (1988). These algorithms detect silver grains by a series of tests which involved nearest-neighbourhood analyses, prospective grain centre detection, contrast and artefact detection.

In these experiments, 9 fields were viewed from each tracheal section; 5 over epithelium (mean area = 1200 μm^2) and 4 over smooth muscle (mean area = 3000 μm^2). Thus, approximately 3000 fields were analysed (9 fields from each of 6 tracheal ring sections on 54 slides). For lung parenchyma 5 separate fields over alveolar wall were viewed from each lung section (mean field area = 2800 μm^2). Thus, approximately 540 fields were analysed (5 fields from each of 36 slides from 3 mice). The emulsion background grain density was determined in 2 fields for each slide and measured over non-tissue within the lumen of a major non-cartilaginous airway. Grain densities were expressed as grains 1000 μm^{-2}.

Drugs

Drugs used were; (−)-[^{125}I]-iodocyanopindolol (Amersham), ICI 118,551 (erythro-DL-1-(7-methylindan-4-yloxyl)-3-(isopropylaminobutan-2-ol hydrochloride), (−)-propranolol hydrochloride (Imperial Chemical Industries), CGP 20712A (2-hydroxy-5-(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)-1H-imidazole-2-yl)-phenoxy)propyl)amino)ethoxy)-benzamide monomethanesulphonate), phentolamine mesylate (Ciba-Geigy Pharmaceuticals, Basel); (−)-isoprenaline hydrochloride, 5-hydroxytryptamine creatinine sulphate (Sigma).

Analysis of results

Estimates of the dissociation constant (K_D) and maximum binding capacity (B_{max}) of specific I-CYP binding were obtained by non-linear least squares regression analysis of data fitted to a single site model, by the computer programme MLAB (N.I.H., U.S.A.). Estimates were also obtained by Scatchard analysis. The nature of the binding was determined by Hill analysis. In competition binding experiments, parameters describing the competition of drugs with specific I-CYP binding at 1 site (inhibitor constant, K_i) and at 2 sites (inhibitor constants at β_1- and β_2-adrenoceptors, $K_i\beta_1$, $K_i\beta_2$; relative proportions of β_1- and β_2-adrenoceptors, $\%\beta_1$ and $\%\beta_2$) were estimated by non-linear regression analysis of data fitted to 1- and 2-site binding models respectively. An overall estimate of the relative proportions of β_1- and β_2-adrenoceptors within a tissue compartment was derived by averaging the respective estimates of $\%\beta_1$ and $\%\beta_2$ obtained with the selective competitors CGP 20712A and ICI 118,551.

Results

Binding experiments

Assessment of specific I-CYP binding to β-adrenoceptors Total I-CYP binding to mouse trachea and lung parenchyma consisted of specific binding to β-adrenoceptors in addition to binding to non-β-adrenoceptor sites (i.e. non-specific binding). Non-specific I-CYP binding must be accurately assessed and subtracted from total binding to derive specific β-adrenoceptor-associated binding. Figure 1 shows that (−)-propranolol caused concentration-dependent inhibition of total I-CYP (70 pM) binding in both mouse trachea and lung parenchyma. In mouse trachea, (−)-propranolol caused biphasic inhibition of total I-CYP binding, with the first phase plateauing at 1 μM. At this concentration, total binding was reduced by 54.4 ± 2.0% in mouse trachea and 94.7 ± 0.1% in lung parenchyma. Similar relative levels of inhibition were produced in the presence of 200 μM (−)-isoprenaline (Figure 1).

First phase binding competition data (0.1 nM–1 μM) were fitted best to a 1-site binding model for both tissues (trachea: nH = 0.95 ± 0.09, K_i = 2.7 nM (95% confidence limits 1.45–4.95), n = 4; lung parenchyma: nH = 0.92 ± 0.05, K_i = 2.0 nM (1.9–2.2), n = 3). These data indicate that the first phase of (−)-propranolol-induced inhibition of total I-CYP binding involved β-adrenoceptors and at 1 μM, (−)-propranolol caused maximal selective inhibition of this specific binding. (−)-Propranolol (1 μM) was used thereafter in all experiments to determine specific I-CYP binding. Neither the first nor the second phase of (−)-propranolol-induced inhibition of total I-CYP binding involved competition at receptors for 5-hydroxytryptamine, since 10 μM 5-hydroxytryptamine failed to alter both total and non-specific (i.e. with 1 μM (−)-propranolol) I-CYP binding in both tissues. Conversely, phentolamine (10 μM) inhibited both total and non-specific I-CYP binding without reducing specific I-CYP binding to β-adrenoceptors. Thus, the second phase of (−)-propranolol-sensitive I-CYP binding most likely involved displacement of I-CYP from non-specific lipophilic sites (Rademaker *et al.*, 1985).

Trachea Specific and non-specific binding of I-CYP to slide-mounted mouse tracheal sections is shown in Figure 2a. Specific I-CYP binding was saturable, involving a population of noninteracting sites (Hill coefficient, nH = 1.00 ± 0.02, n = 3 experiments). Specific binding data were analysed by both a nonlinear curve-fitting algorithm and the Scatchard transformation (Figure 2b). These analyses provided similar estimates for the dissociation constant for I-CYP (K_D = 49.0 pM; 95% confidence limits, 18.0–133.0 pM and 50.5 pM; 30.0–84.9 pM, respectively) and revealed similar densities of binding sites (B_{max} = 0.022 ± 0.003 fmol per tracheal section and 0.022 ± 0.002 fmol per tracheal section, respectively).

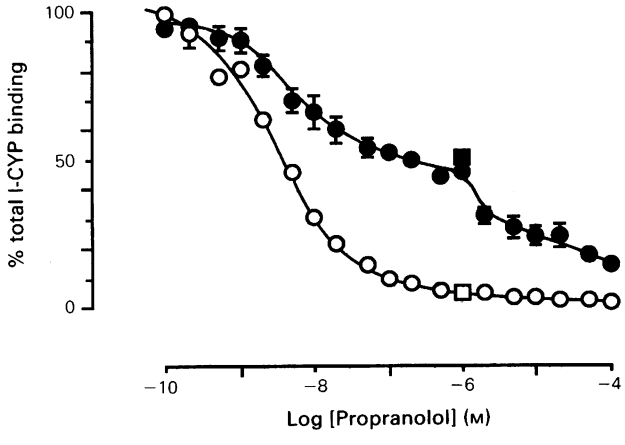

Figure 1 Inhibition of total [^{125}I]-iodocyanopindolol (I-CYP) (70 pM) binding by (−)-propranolol (non-selective) in 10 μm frozen sections of mouse trachea (●) and mouse lung parenchyma (○). Each data point represents the mean from 3–4 experiments conducted in triplicate. Vertical lines show s.e.mean (when larger than the symbol). For comparison, inhibition of total I-CYP (70 pM) binding by 200 μM (−)-isoprenaline in similar sections of mouse trachea (■) and lung parenchyma (□) is also shown.

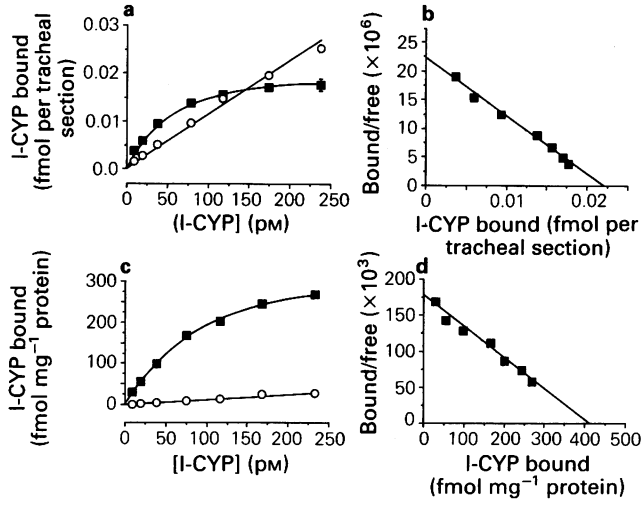

Figure 2 Concentration-dependence of [^{125}I]-iodocyanopindolol (I-CYP) binding to 10 μm transverse frozen sections of (a) mouse trachea and (c) mouse lung parenchymal tissue showing specific (■) and non-specific (○) binding. Each point represents the mean and s.e.mean of data from 3 experiments conducted in triplicate. Scatchard analysis of specific I-CYP binding for (b) mouse trachea and (d) mouse lung parenchyma.

Specific I-CYP binding was also displaced in the presence of the competitive β-adrenoceptor antagonists ICI 118,551 and CGP 20712A. Competition binding data in trachea for these agents are shown in Figure 3a and Table 1. Competition binding curves were characterized by low nH values and were fitted best to a 2-site binding model. Analyses of the competition binding curves for the β_2-adrenoceptor-selective antagonist ICI 118,551 indicated that 58% of the β-adrenoceptors were of the β_2-subtype and 42% were of the β_1-subtype. Similarly, analyses of the competition binding data for the β_1-adrenoceptor selective antagonist CGP 20712A revealed a minor population of β_1-adrenoceptors (24%, on average). Thus, binding studies indicate that β_1- and β_2-adrenoceptors co-existed in mouse trachea approximately in the ratio 1:2.

Peripheral lung Specific I-CYP binding in mouse lung parenchyma reached equilibrium between 120 and 180 min. Subsequent binding experiments were performed with an incubation time of 150 min. Specific binding of I-CYP was saturable involving a population of non-interacting sites (nH = 0.99 ± 0.03; n = 3 experiments). Specific binding data (Figure 2c) were analysed by non-linear least squares regression and the Scatchard transformation (Figure 2d), providing estimates of the dissociation constant (K_D) of 118.9 pM (95% confidence limits, 43–326) and 122.5 pM (71–211), respectively. These methods also provided similar estimates of maximum binding capacity (B_{max} = 412 ± 19 fmol mg^{-1} protein and 417 ± 34 fmol mg^{-1} protein, respectively).

Specific binding of I-CYP (70 pM) was displaced in the presence of the competitive β-adrenoceptor antagonists CGP 20712A (β_1-selective) and ICI 118,551 (β_2-selective) (Figure 3b). These competition binding curves were characterized by low nH values and were fitted best to a 2-site binding model, indicating the presence of both β_1- and β_2-adrenoceptors in mouse peripheral lung. Computer analysis of displacement data for CGP 20712A indicated the presence of β_1- and β_2-adrenoceptors in the proportions 27.1 ± 1.7% and 72.9 ± 1.7% respectively (n = 5). A similar assessment of ICI 118,551 displacement data indicated that the proportions were

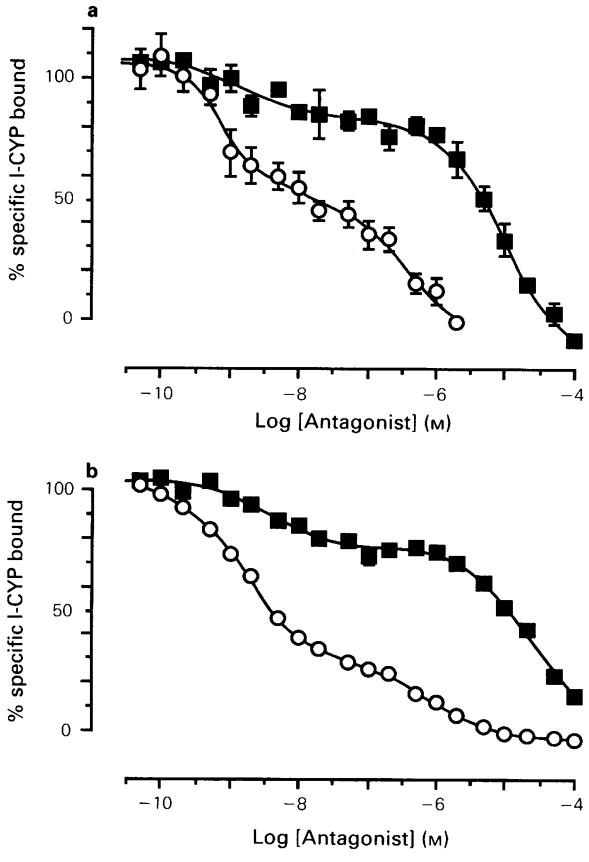

Figure 3 Displacement of specific [^{125}I]-iodocyanopindolol (I-CYP) (70 pM) binding from 10 μm frozen sections of (a) mouse trachea and (b) mouse lung parenchyma by ICI 118,551 (β_2-adrenoceptor selective antagonist; ○) and CGP 20712A (β_1-adrenoceptor selective antagonist; ■). Each point represents the mean from 4–5 experiments conducted in triplicate. Vertical lines show s.e.mean; for mouse lung parenchyma, error bars fall within the bounds of the symbol locating means for all data points, except one. These data were fitted best to a 2-site binding model.

28.9 ± 1.4% (β_1) and 71.1 ± 1.4% (Table 2). Thus on average, the proportions of β_1- and β_2-adrenoceptors in mouse lung were 28% and 72% respectively.

Quantitative autoradiography

Trachea As shown in Figure 4, significant densities of autoradiographic grains were located over tracheal epithelium and smooth muscle. The mean density of grains located over epithelium was 154 ± 4 grains 1000 μm^{-2}, 73.7% of which were associated with β-adrenoceptors as determined in the presence of 1 μM (−)-propranolol. Smooth muscle contained a greater density of grains (270 ± 6 grains 1000 μm^{-2}), although only 44.1% was specific binding to β-adrenoceptors. Overall, the density of grains specifically associated with β-adrenoceptors was similar in epithelium and smooth muscle (115 and 119 grains 1000 μm^{-2} respectively). The background density of

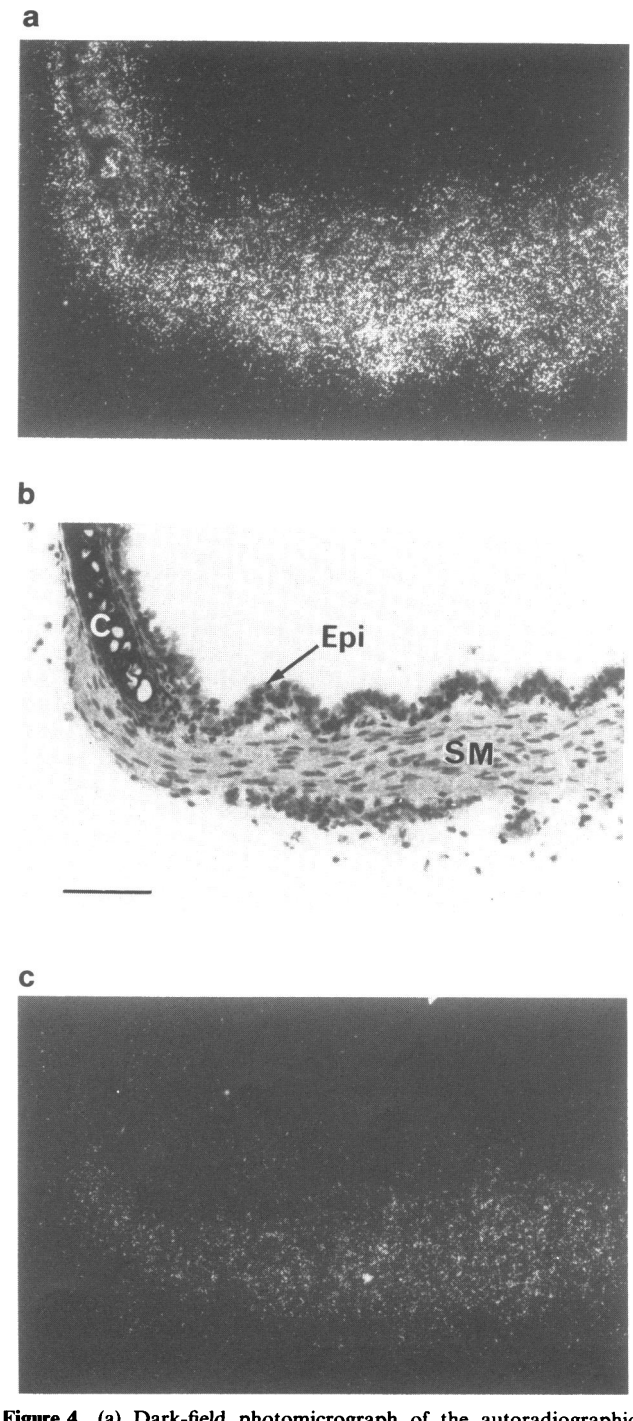

Figure 4 (a) Dark-field photomicrograph of the autoradiographic distribution of total binding sites for [^{125}I]-iodocyanopindolol (I-CYP) (70 pM) in 10 μm frozen transverse sections of mouse trachea. (b) Light-field photomicrograph of the above section showing the epithelium (Epi), airway smooth muscle (SM) and cartilage (C). Bar = 100 μm. (c) Dark-field photomicrograph showing the distribution of I-CYP binding sites in the presence of 1 μM (−)-propranolol, i.e. non-specific binding.

Table 1 Binding parameter estimates obtained from analyses of competition binding curves between [^{125}I]-iodocyanopindolol (I-CYP) and the subtype-selective β-adrenoceptor antagonists CGP 20712A and ICI 118,551 in mouse trachea

	nH	$pK_i\beta_1$	$pK_i\beta_2$	$\%\beta_1$	$\%\beta_2$
CGP 20712A	0.31 ± 0.02	9.55 ± 0.21	5.58 ± 0.08	23.5 ± 4.4	76.5 ± 4.4
ICI 118,551	0.53 ± 0.05	6.83 ± 0.14	9.38 ± 0.21	42.5 ± 4.9	57.5 ± 4.9
			Average = 33		67

Values shown are the means ± s.e.mean of 4 separate determinations conducted in triplicate. Shown are pseudo Hill coefficients (nH), −log (inhibitor constants) at β_1- and β_2-adrenoceptor binding sites ($pK_i\beta_1$, $pK_i\beta_2$) and the percentage of β_1- and β_2-adrenoceptor binding sites in mouse tracheal sections ($\%\beta_1$, $\%\beta_2$).

Table 2 Binding parameter estimates obtained from analysis of competition binding curves between [^{125}I]-iodocyanopindolol (I-CYP) (70 pM) and the β-adrenoceptor-selective antagonists CGP 20712A and ICI 118,551 in mouse lung parenchyma

	$pK_i\beta_1$	$pK_i\beta_2$	$\%\beta_1$	$\%\beta_2$
Tissue binding¶ ($n = 5$)				
CGP 20712A	8.74 ± 0.07	4.89 ± 0.01	27.1 ± 1.7	72.9 ± 1.7
ICI 118,551	6.46 ± 0.04	9.03 ± 0.04	28.9 ± 1.4	71.1 ± 1.4
		Average = 28		72
Autoradiographic† *grain counting* ($n = 3$)				
CGP 20712A	9.03 ± 0.77	4.95 ± 0.14	20.0 ± 2.4	80.0 ± 2.4
ICI 118,551	6.23 ± 0.13	9.13 ± 0.13	16.2 ± 2.1	83.8 ± 2.1
		Average = 18.1		81.9

Results are expressed as means ± s.e.mean fron n mouse lungs. Shown are −log (inhibitor constants) at β_1- and β_2-adrenoceptors ($pK_i\beta_1$, $pK_i\beta_2$) and the % of β_1- and β_2-adrenoceptors ($\%\beta_1$, $\%\beta_2$). These data were derived from specific [^{125}I]-iodocyanopindolol (I-CYP) binding to ¶total lung tissue using mouse lung sections and from the †quantitation of autoradiographic grains derived from specific I-CYP binding associated only with alveolar wall tissue.

autoradiographic grains, determined over non-tissue, was approximately 9.1 grains 1000 μm^{-2}.

Specific grain densities over tracheal epithelium and smooth muscle were markedly reduced in the presence of the selective β-adrenoceptor antagonists ICI 118,551 and CGP 20712A. Competition binding curves for I-CYP with ICI 118,551 and CGP 20712A, constructed with autoradiographic grain counting data (Figure 5), gave psuedo Hill values (nH) significantly less than 1 and were described best by fitting to a 2-site model. These results suggest the presence of both β_1- and β_2-adrenoceptors within both the epithelium and smooth muscle. Further analyses of these competition binding curves clearly indicated a differential distribution of β_1- and β_2-adrenoceptors within the epithelium and smooth muscle. In epithelium about 70.9% of β-adrenoceptors were of the β_2-subtype, whereas β_1-adrenoceptors predominated in smooth muscle (69%). Estimates of $pK_i\beta_1$ and $pK_i\beta_2$ for ICI 118,551 were similar in smooth muscle (6.94, 9.60, respectively) and epithelium (6.67, 9.28, respectively). Furthermore, these estimates were comparable to those obtained for ICI 118,551 in binding studies with whole tracheal sections (6.83, 9.38; Table 1). For CGP 20712A, the $pK_i\beta_1$ and $pK_i\beta_2$ values obtained were 9.17 and 5.77 in smooth muscle and 8.19 and 5.93 in epithelium, respectively.

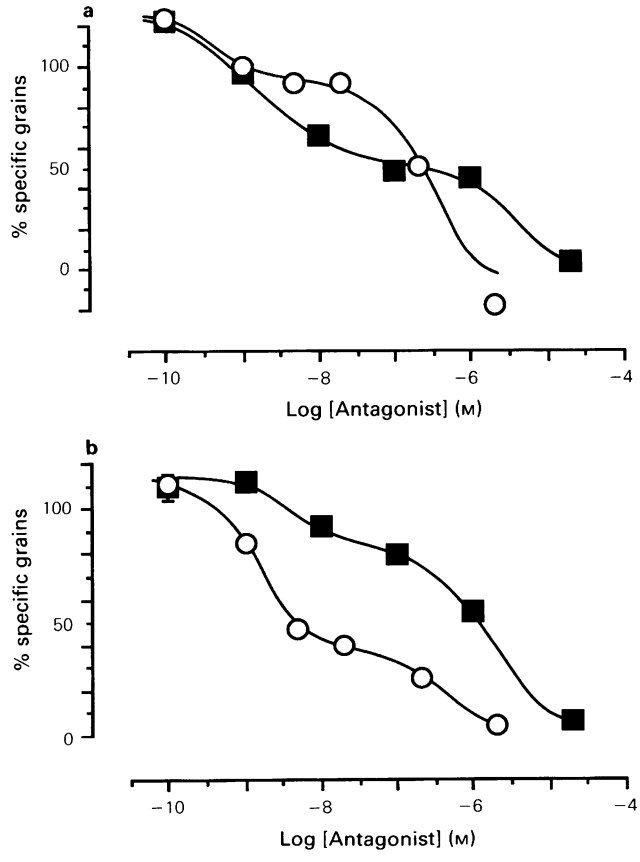

Figure 5 Competition binding curves derived from autoradiographic data describing the displacement of specific [^{125}I]-iodocyanopindolol (I-CYP) binding located over (a) tracheal smooth muscle and (b) tracheal epithelium, by ICI 118,551 (○) and CGP 20712A (■). Each data point is the mean and s.e.mean from 6 animals. These data were fitted best to a 2-site binding model.

Peripheral lung Figure 6a shows that high densities of β-adrenoceptors were present throughout the mouse lung parenchyma. The highest density of specific I-CYP binding sites was associated with alveolar septae, with much lower densities associated with other airway structures and blood vessels. The mean density of autoradiographic grains derived from I-CYP (70 pM) binding over alveolar wall tissue was 92.5 ± 8.8 grains 1000 μm^{-2}, 98% of which were apparently associated with β-adrenoceptors. The background density of autoradiographic grains over non-tissue was 13.7 ± 0.8 grains 1000 μm^{-2}. Labelling over alveolar walls was dense and uniform, giving no indication that I-CYP was specifically bound to particular cell types. The effects of the selective β-adrenoceptor antagonists ICI 118,551 and CGP 20712A on the specific I-CYP (70 pM) grain density over alveolar septae are shown in Figure 7b and c respectively. ICI 118,551 (50 nM) reduced specific binding by 84.7 ± 0.9% ($n = 3$), while CGP 20712A (20 nM) caused an 11.6 ± 1.4% ($n = 3$) reduction. Non-specific binding determined in the presence of 1 μM (−)-propranolol accounted for only 1.9 ± 0.2% of total I-CYP binding (Figure 7d). These data indicate the co-existence of both β_1- and β_2-adrenoceptors in alveolar wall tissue of mouse lung parenchyma.

Competition binding curves for the selective β-adrenoceptor antagonists ICI 118,551 and CGP 20712A were also constructed from quantitative autoradiographic data in an attempt to assess accurately the proportions of β_1- and β_2-adrenoceptors present in mouse lung alveolar septae (Figure 8). Data shown in Figure 8 were also fitted best to a 2-site binding model. The mean proportions of β_1- and β_2-adrenoceptors present in the alveolar septae were assessed from competition binding (grain counting) curves constructed for tissue from three different mice, providing estimates of 18.1 ± 1.6% and 81.9 ± 1.6%, respectively (Table 2). These data closely resemble those from competition binding experiments (Figure 3b) which indicate the proportions of β-adrenoceptor subtypes in total lung parenchyma.

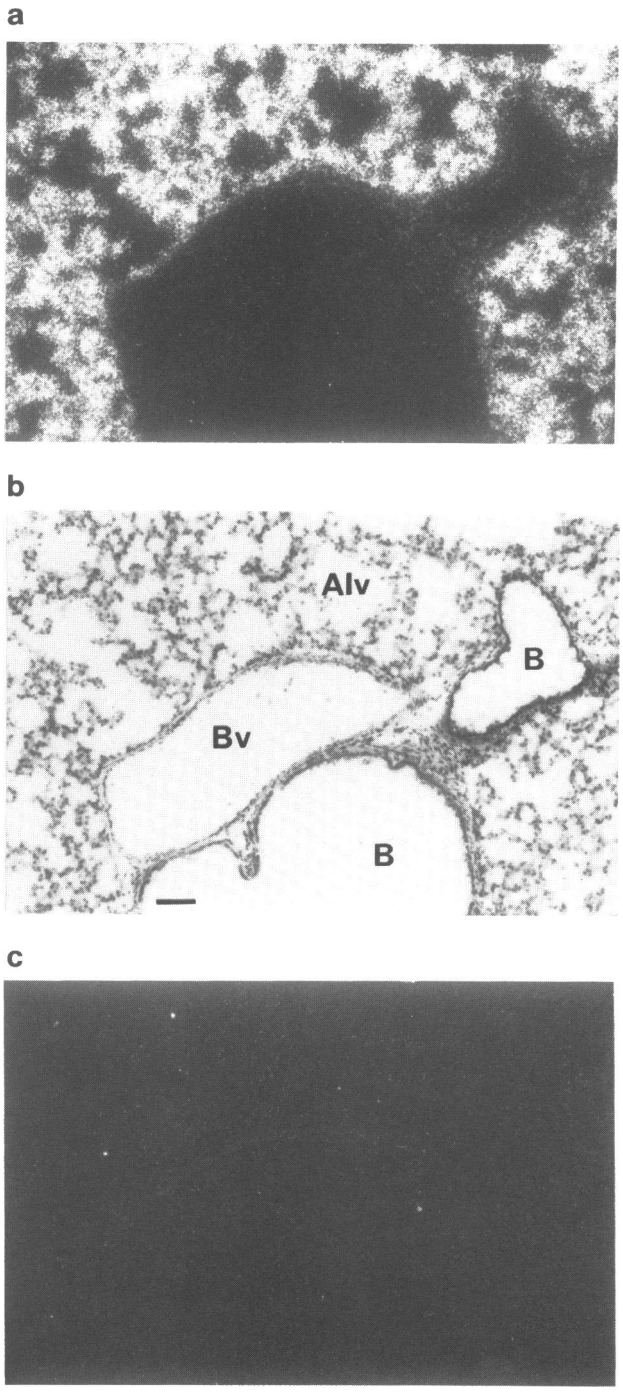

Figure 6 (a) Dark-field photomicrograph of a 10 μm frozen section of mouse lung parenchyma showing the distribution and localization of autoradiographic grains derived from [^{125}I]-iodocyanopindolol (I-CYP) (70 pM) binding. (b) Bright-field photomicrograph of the above section. B = bronchiole (non-cartilaginous airway), Bv = blood vessel, Alv = alveolus. Bar = 100 μm. (c) Dark-field photomicrograph showing the distribution of non-specific autoradiographic grains in the next serial section incubated with I-CYP (70 pM) and (−)-propranolol (1 μM).

Discussion

The competitive displacement of I-CYP binding to slide-mounted mouse tracheal and lung parenchymal sections by (−)-propranolol identified single populations of non-interacting binding sites in these tissues, characteristic of β-adrenoceptors. I-CYP binding to β-adrenoceptors was competitively inhibited by the selective β-adrenoceptor antagonists, CGP 20712A (β_1-adrenoceptor selective) and ICI 118,551 (β_2-adrenoceptor selective) and analyses of the respective competition binding curves revealed the co-existence of significant populations of β_1- and β_2-adrenoceptors. On average, transverse sections of mouse trachea contained approximately twice as many β_2-adrenoceptors as β_1-adrenoceptors. However, as the mouse trachea contains a number of different cell types including smooth muscle and epithelium, the relative proportions of β_1- and β_2-adrenoceptors determined in whole tracheal sections may bear little relation to the relative densities and proportions of β-adrenoceptors present in such cell types. Quantitative autoradiographic techniques were used to establish the relative densities and distribution of β-adrenoceptors in these distinct tracheal structures.

Autoradiography showed the density of β-adrenoceptors to be significantly higher in tracheal smooth muscle and epithelium than in cartilage. Similar findings have been obtained in studies of central airways preparations from man (Spina *et al.*, 1989a,b), pig (Goldie *et al.*, 1986b) and guinea-pig (Goldie *et al.*, 1986a,c). Moreover, although the densities of β-adrenoceptors observed in tracheal smooth muscle and epithelium were similar, quantitative autoradiography revealed a significant difference in the relative proportions of β_1- and β_2-adrenoceptors between the two tracheal structures. Whereas the epithelium contained mostly β_2-adrenoceptors (71%), smooth muscle contained predominantly β_1-adrenoceptors (69%). The concentration of β_1-adrenoceptors in smooth muscle is consistent with our recent findings that this β-adrenoceptor subtype played the predominant role in mediating smooth muscle relaxation in mouse isolated trachea (Henry & Goldie, 1989). Functional studies with mouse isolated trachea also demonstrated a very minor role with respect to tracheal relaxation, for the smaller population (31%) of β_2-adrenoceptors (Henry & Goldie, 1989). Some of these β_2-adrenoceptors may also mediate metabolic effects of β-agonists.

Functional studies and quantitative autoradiographic analyses of human isolated bronchus have shown that β-adrenoceptor agonists induce relaxation of bronchial smooth muscle via activation of an apparently homogeneous population of β_2-adrenoceptors (Zaagsma *et al.*, 1983; Goldie *et al.*, 1984; Carstairs *et al.*, 1985). There is no evidence of β_1-adrenoceptors co-existing with β_2-adrenoceptors in human bronchial smooth muscle. Conversely, quantitative autoradiography in human alveolar wall and submucosal gland tissue indicated the existence of both β-adrenoceptor subtypes in these tissues (Carstairs *et al.*, 1985), although it is not clear whether these β-adrenoceptor subtypes were located on the same cell types or mediated the same function. A number of functional and receptor binding studies suggest β_1- and β_2-adrenoceptors co-existed within the smooth muscle of guinea-pig and dog trachea and mediated the same spasmolytic functions (Barnes *et al.*, 1982b; Carswell & Nahorski, 1983).

Morphologically, mouse epithelium is a heterogeneous single layer of cells consisting primarily of ciliated cells and non-ciliated Clara-like secretory cells resting on a basement membrane (Pack *et al.*, 1980). The relative densities and proportions of β_1- and β_2-adrenoceptors were not determined in these individual cell types. However, it is likely that both cell types contained β-adrenoceptors since β-adrenoceptor agonists have been shown to cause increased ciliary beat frequency (Verdugo *et al.*, 1980; Lopez-Vidriero *et al.*, 1985) and stimulation of secretion from Clara cells (Massaro *et al.*, 1981).

Binding studies revealed high densities of both β_1- and β_2-adrenoceptors within the peripheral lung of the mouse in the proportions 28% and 72% respectively. Analyses of competition binding curves constructed from autoradiographic data established that the great majority of these receptors was associated with alveolar septae (Table 2). However, it is not known whether different alveolar cells expressed only one or both β-adrenoceptor subtypes. Guinea-pig lung parenchymal membranes also possessed both β_1- and β_2-adrenoceptors in

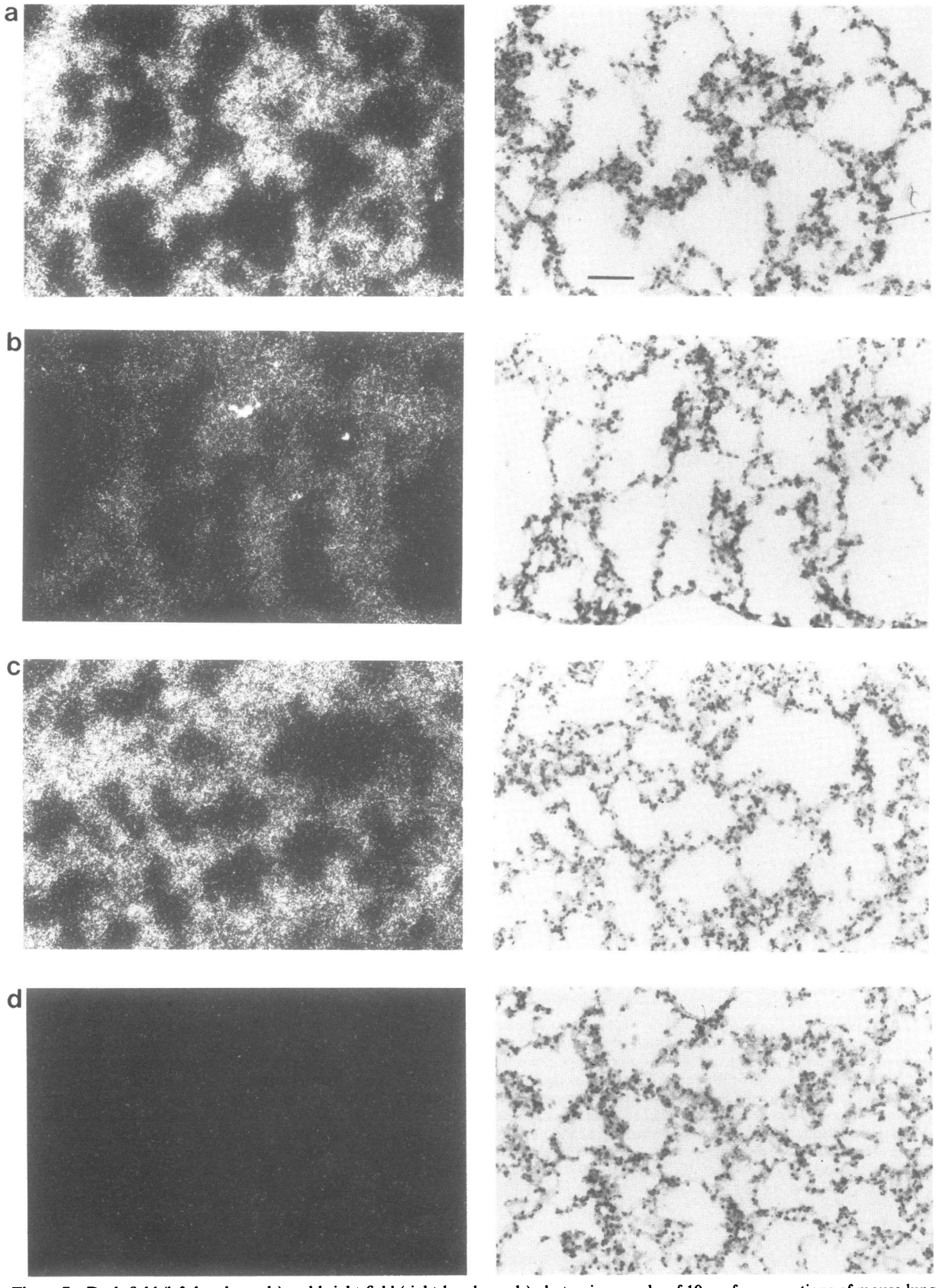

Figure 7 Dark-field (left-hand panels) and bright-field (right-hand panels) photomicrographs of 10 μm frozen sections of mouse lung parenchyma showing the localization of autoradiographic grains derived from [^{125}I]-iodocyanopindolol (I-CYP) (70 pM) to alveolar wall tissue in the absence (a) or presence of (b) ICI 118,551 (50 nM), (c) CGP 20712A (20 nM), or (d) (−)-propranolol (1 μM). Bar = 100 μm.

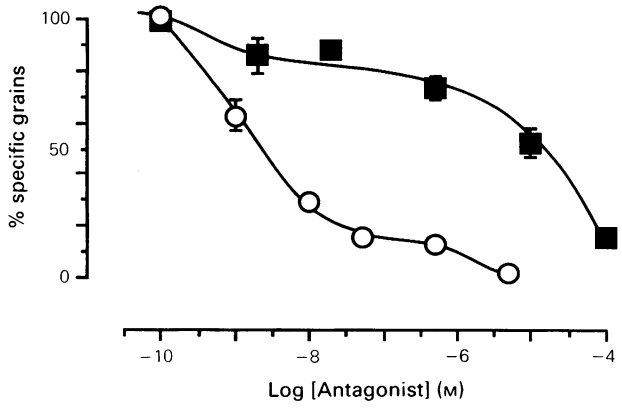

Figure 8 Displacement by CGP 20712A (β_1-selective) (■) and ICI 118,551 (β_2-selective) (○) of specific [^{125}I]-iodocyanopindolol (I-CYP) (70 pM) binding as assessed by the quantitation of autoradiographic grains associated with lung alveolar wall tissue in 10 μm frozen sections of mouse lung parenchymal tissue. Each point represents the mean of data from 3 mice obtained in 5 fields in duplicate. Vertical lines show s.e.mean (when larger than size of symbol). These data were fitted best to a 2-site binding model.

the approximate proportions 20% and 80% respectively (Engel *et al.*, 1981; Carswell & Nahorski, 1983). Similar values were obtained in hamster (Benovic *et al.*, 1983), rat (Minneman *et al.*, 1979; Dickinson *et al.*, 1981) and human lung membranes (Brown *et al.*, 1985). In contrast, β_1-adrenoceptors predominated in rabbit lung with estimates varying between 60% (Rugg *et al.*, 1978; Barnett *et al.*, 1978) and 80% (Dickinson *et al.*, 1981; Brodde *et al.*, 1983).

As the populations of β-adrenoceptors throughout the respiratory tree of the mouse have been characterized, potential interactions between these sites and viruses causing respiratory tract infections, both in man and in the mouse and their likely significance to human asthma can now be evaluated.

This research was supported by grants from the National Health & Medical Research Council of Australia and the Clive and Vera Ramaciotti Foundations (Australia). The authors also thank Imperial Chemical Industries (Australia) for their gifts of (−)-propranolol hydrochloride and ICI 118,551 and Ciba-Geigy Pharmaceuticals for their gift of CGP 20712A.

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(Received April 18, 1989
Revised August 7, 1989
Accepted September 1, 1989)