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β-Adrenoceptor heterogeneity in guinea-pig airways: comparison of functional and receptor labelling studies

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1 The distribution of β-adrenoceptor subtypes in guinea-pig airways has been studied by radioligand binding assays and analysis of mechanical responses.

2 Binding studies with the ligands [^{3}H]-dihydroalprenolol and [^{125}I]-cyanopindolol, revealed that β-adrenoceptors were unevenly distributed throughout the airways with the highest density located in the parenchyma and the lowest density in the trachea.

3 The relative proportion of $\beta_1 : \beta_2$-adrenoceptor binding sites was assessed by computer-assisted analysis of the inhibition curves generated by selective agents. It was virtually identical in each region and in the order of 15 : 85%.

4 β-Adrenoceptor agonists caused concentration-dependent relaxations of both tracheal spirals and parenchymal lung strips. This response appeared to be mediated by both β_1- and β_2-adrenoceptors in tracheal spirals as the pA_2 value for the β_1-selective antagonist, atenolol, varied depending upon which agonist was used, and, in the presence of the β_2-adrenoceptor antagonist ICI 118,551, noradrenaline and isoprenaline produced biphasic concentration-effect curves. In parenchymal lung strips only the one subtype was involved as antagonist pA_2 values were not dependent on the agonist used and the properties were consistent with those expected for a β_2-adrenoceptor.

Introduction

The sub-classification of β-adrenoceptors into β_1- and β_2-subtypes was first suggested by Lands, Arnold, McAuliff, Luduena & Brown (1967) on the basis of relative responses to sympathomimetic amines in different tissues, and led to the receptors of adipose tissue being classified as β_1 and those in bronchial and vascular smooth muscle as β_2. Further support for this hypothesis has come from the use of antagonists which are selective for each subtype. Using these selective drugs Carlsson, Ablad, Brandstrom & Carlsson (1972) proposed that both β_1- and β_2-adrenoceptors may co-exist within the same organ or tissue and, under certain circumstances, may serve the same function. This has been clearly demonstrated for catecholamine-induced relaxation in guinea-pig tracheal smooth muscle by several studies, including those of Zaagsma, Oudhof, Van der Heijden & Plantje (1979) and O'Donnell & Wanstall (1979), which have shown that both β_1- and β_2-adrenoceptors mediate the relaxation. However, it should be noted that Zaagsma *et al.* (1979) showed that this heterogeneity is not present in guinea-pig parenchymal lung strips where the relaxant response appears to be mediated solely by the β_2-adrenoceptor.

Considerable support for β-adrenoceptor heterogeneity and for the co-existence of β_1- and β_2-adrenoceptors in the same tissue has come from radioligand receptor binding studies (Nahorski, 1981). Using this approach, experiments in our laboratory (Rugg, Barnett & Nahorski, 1978) first revealed that in the rat both β_1- and β_2-adrenoceptors are present in peripheral lung tissue. This would at first sight appear to conflict with the evidence from functional studies in the guinea-pig. However, it remained possible that the differences relate to species variation. Also, since binding studies are performed on homogenates of tissue, the heterogeneity found in these studies might relate to β_1- and β_2-adrenoceptors being located on functionally distinct cell types, with β_1-receptors not being involved in smooth muscle relaxation. In the present study, the distribution of β-adrenoceptor subtypes has been determined by comparing results from radioligand binding assays with functional studies in different regions of guinea-pig airways. Some of

these results have been presented to the British Pharmacological Society (Carswell & Nahorski, 1982a).

Methods

Male guinea-pigs (250–300 g) were killed by a blow to the head and exsanguinated.

Membrane preparation

The trachea and lungs were dissected on ice to give three separate preparations of parenchyma, bronchi and trachea, as described previously (Carswell & Nahorski, 1982b). Each preparation was homogenized in 30 vols. of ice-cold 50 mM Tris-HCl buffer (pH 7.8) with a Polytron homogenizer at setting 5 for 2×10 s bursts. Homogenates were then filtered through a double layer of cheesecloth and centrifuged at 48,000 *g* for 15 min at 4°C in a Sorvall RC5 refrigerated centrifuge. Each pellet was washed three times by rehomogenization and centrifugation, followed by resuspension in buffer and stored at −40°C. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as standard.

Radioligand binding assays

Binding studies were performed with either tritiated dihydroalprenolol ([^{3}H]-DHA) or iodinated cyanopindolol ([^{125}I]-Cyp) in a final incubation volume of 250 µl with all constituents made up in 50 mM Tris-HCl, pH 7.8. For agonists the buffer also contained 0.1% ascorbate as an antioxidant.

Membranes were incubated with [^{3}H]-DHA to equilibrium (22°C, 30 min) and then terminated by addition of 1 ml ice-cold buffer and collection on Whatman GF/B filters under a vacuum pressure of about 500 mmHg. The filters were rapidly washed with 3×5 ml of buffer and the radioactivity on filters was extracted into 4 ml of Fisofluor '1' scintillation fluid and counted in a Packard liquid scintillation spectrometer at an efficiency of about 40%.

Incubations with [^{125}I]-Cyp were carried out at 37°C for 50 min then terminated by the addition of 1 ml ice-cold buffer and collection on Whatman GF/B filters under a low vacuum pressure of 250 mmHg. The filters were then washed with 1×30 ml buffer and radioactivity on the filters counted in an Ames Gammacord II gamma-counter.

Functional studies

Parenchymal lung strips measuring approximately $30 \times 3 \times 3$ mm were cut from the peripheral edge of the lower lobes of the lung and placed in ice-cold Krebs-Ringer. The trachea from the same animal was kept overnight in Krebs-Ringer at 4°C, gassed with 95% O_2 and 5% CO_2, and then cut into a spiral the next day and divided into two strips of equal length (trachea kept overnight in this way responded to β-agonists in the same way as fresh tissue).

Paired preparations were suspended in two 50 ml organ baths which contained Krebs-Ringer solution at 37°C and continuously gassed with 95% O_2 and 5% CO_2. The solution also contained desmethylimipramine 1×10^{-6} M to block neuronal catecholamine uptake and phenoxybenzamine 5×10^{-6} M was added directly to the baths to block α-adrenoceptors and extra-neuronal agonist uptake, then washed out after 30 min. An initial tension of 250 mg was applied to parenchymal lung strips and 5 g to tracheal spirals, throughout an initial equilibrium period of 1 h. During this time the preparations were rinsed with fresh solution every 20 min.

Concentration-effect (C/E) curves to agonists were constructed by use of a cumulative dosing technique (Van Rossum, 1963). The effect of antagonists was studied by adding the antagonist to one strip with the other serving as a control. A 60 min equilibrium period was allowed for each antagonist before the C/E curve was repeated on both the control and treated strips.

Responses were measured isotonically by Washington Type TII isotonic lever transducers, and recorded on a Washington oscillograph. pD_2 values ($-\log EC_{50}$) for agonists were estimated by analysing the C/E curves with a computer-assisted curve fitting procedure (De Lean, Munson & Rodbard, 1978).

Table 1 Distribution of β-adrenoceptors in guinea-pig airways identified by [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) and [^{125}I]-cyanopindolol ([^{125}I]-Cyp)

	B_{max}[^{3}H]-DHA (fmol mg^{-1} protein)	B_{max} [^{125}I]-Cyp (fmol mg^{-1} protein)	K_D [^{3}H]-DHA (nM)	K_D [^{125}I]-Cyp (pM)
Trachea	46 ± 4	59 ± 13	0.14 ± 0.04	16.0 ± 3.2
Bronchi	196 ± 14	125 ± 12	0.21 ± 0.03	23.1 ± 1.4
Parenchyma	366 ± 31	400 ± 25	0.20 ± 0.03	21.2 ± 2.6

B_{max} values were estimated from Scatchard plots and K_D values from Hill plots. Values are the mean + s.e.mean of three separate determinations

Table 2 K_i values of selective antagonists for β_1- and β_2-adrenoceptors and the proportions of each receptor in the trachea, bronchi and parenchyma, from radioligand binding displacement studies

	Trachea		*Bronchi*		*Parenchyma*	
Drug	β_1	β_2	β_1	β_2	β_1	β_2
Atenolol	4.6×10^{-8}	2.2×10^{-5}	4.6×10^{-8}	1.1×10^{-5}	1.8×10^{-8}	5.5×10^{-6}
	15.6%	84.4%	14.7%	85.3%	16.8%	83.2%
ICI 118,551	2.0×10^{-7}	6.5×10^{-9}	4.0×10^{-7}	2.9×10^{-9}	2.0×10^{-7}	6.3×10^{-9}
	15.3%	84.7%	9.6%	90.4%	12.8%	87.2%

The means of three separate inhibition curves were analysed using a computer-assisted iterative curve-fitting procedure. K_i values (molar) were calculated using the equation $K_i = IC_{50}/1 + L/K_D$ and the percentages indicate the proportions of β_1- and β_2-receptors. The above data were obtained using [^{125}I]-cyanopindolol, but virtually identical results were also obtained with [^{3}H]-dihydroalprenolol

For strips treated with antagonists, the response is expressed as % control maximum response and apparent pA_2 values were calculated from a Schild plot (Arunlakshana & Schild, 1959) of log (dose ratio − 1) vs log [antagonist] by linear regression analysis.

Drugs used were (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, (−)-isoprenaline bitartrate (Sigma Chemical Co.); terbutaline sulphate (Astra); (−)-timolol hydrochloride (Leo Laboratores); atenolol hydrochloride and ICI 118,551 erythro-DL-1 (7-methylindan-4-yloxyl-3-isopropylaminbutan-2-ol, ICI Pharmaceuticals); desmethylimipramine (Geigy Pharmaceuticals) and phenoxybenzamine (Smith Kline & French). (−)-[^{3}H]-dihydroalprenolol (103 Ci/mmol) and (±)-[^{125}]-cyanopindolol (2200 Ci/mmol) were both obtained from Amersham International. The iodinated ligand was only used within its half-life (60 days).

Results

Radioligand binding studies

Specific binding, defined as the binding displaceable by 200 μM (−)-isoprenaline (Nahorski & Richardson, 1979) of [^{3}H]-DHA and [^{125}I]-Cyp, to homogenates of guinea-pig airways was saturable, produced linear Scatchard plots and had Hill coefficients of close to unity. The dissociation constant (K_D) for both radioligands was virtually identical between each area (Table 1), but although both ligands labelled a virtually identical number of sites within each area, there were significant regional differences in B_{max} values. The highest density of β-adrenoceptors was found in the parenchyma, with the lowest density in the trachea (Table 1).

Binding of both ligands was displaced stereoselectively by the (+)- and (−)-isomers of propranolol in

Table 3 pA_2 values and slopes of Schild plots of timolol, atenolol and ICI 118,551 for the antagonism of β-adrenoceptor mediated relaxation of guinea-pig tracheal spirals and lung strips

		Timolol (non-selective)		*Atenolol* (β_1-selective)		*ICI 118,551* (β_2-selective)	
	pD_2*	pA_2	*Slope*	pA_2	*Slope*	pA_2	*Slope*
Tracheal spirals							
Iso	8.80	8.98	1.04	5.06	0.96	—	—
	(±0.39)	(±0.15)	(±0.09)	(±0.07)	(±0.13)		
NA	7.80	9.20	0.91	6.96	0.60	—	—
	(±0.21)	(±0.09)	(±0.02)	(±0.06)	(±0.01)		
Ter	6.8	9.10	1.13	5.01	0.92	—	—
	(±0.4)	(±0.12)	(±0.11)	(±0.06)	(±0.05)		
Parenchymal lung strips							
Iso	8.13	9.25	1.07	5.50	1.10	8.45	1.11
	(±0.11)	(±0.13)	(±0.01)	(±0.10)	(±0.08)	(±0.06)	(±0.04)
NA	5.43	9.38	1.07	6.06	0.56	8.27	0.74
	(±0.15)	(±0.13)	(±0.08)	(±0.06)	(±0.11)	(±0.01)	(±0.04)
Ter	7.08	9.30	1.10	5.5	1.06	8.26	0.86
	(±0.10)	(±0.35)	(±0.19)	(±0.15)	(±0.12)	(±0.04)	(±0.03)

Cumulative agonist concentration-effect curves were performed in the absence and presence of increasing concentrations of antagonist as described in Methods. Values represent the mean ± s.e.mean, $n = 3$ (*$n = 12$)

all regions, with the (−)-isomer being approximately 100 times more potent than the (+)-isomer (data not shown), and was also displaced by timolol, another non-selective β-antagonist. These drugs all produced monophasic displacement curves and had slope factors close to unity. The selective antagonists, atenolol (β_1-selective; Barrett, 1977) and ICI 118,551 (β_2-selective; Bilski, Dorries, Fitzgerald, Jessup, Tucker & Wale, 1980) however, generated displacement curves deviating from law of mass action behaviour. Analysis of these curves by a computer-assisted curve-fitting procedure (Dickinson, Richardson & Nahorski, 1981) to a two-site model, indicated that they represented displacement of radioligand from a population of β-adrenoceptors which consisted of approximately 85% of the β_2 subtype and 15% of the β_1 subtype. The relative proportion of these two subtypes was very similar between the different regions (Table 2).

Functional studies

Both tracheal spirals and parenchymal lung strips relaxed in response to the agonists isoprenaline (Iso, non-selective), noradrenaline (NA, β_1-selective) and terbutaline (Ter, β_2-selective). In the trachea, the rank order of potency for these agonists was Iso > NA > Ter, but in parenchymal lung strips Ter was more potent than NA (Table 3).

The non-selective antagonist, timolol, produced parallel shifts to the right of the C/E curves for all three agonists, in both tracheal spirals and parenchymal lung strips. Schild plots obtained from these curves had slopes close to unity and the pA_2 value was the same irrespective of the preparation or which agonist was used (Table 3).

In the presence of the β_1-selective antagonist atenolol, agonist C/E curves were again shifted to the right, but when NA was used as the agonist, the size of the shift in the trachea was much greater than the shift produced by the same concentration of atenolol in the lung strip (Figure 1). This resulted in the apparent inhibitory potency of atenolol being 100 × greater when the agonist was NA than when either Iso or Ter were used in tracheal spirals, but only a 3 fold difference in parenchymal lung strips (Table 3). The slopes of the Schild plots for the antagonism of NA by atenolol were also significantly different from unity.

Pretreatment of tracheal spirals with the highly selective β_2-antagonist, ICI 118,551, transformed the sigmoidal C/E curves for both NA (Figure 2) and Iso (not shown) into biphasic curves. Low concentrations (2×10^{-8} M) of this antagonist produced shifts to the right of the upper portion of the C/E curve and had no effect on the lower portion of the curve. At higher concentrations of ICI 118,551 (2×10^{-7} M), the whole C/E curve was displaced to the right. In parenchymal lung strips, however, the same concentrations of ICI 118,551 produced parallel shifts to the right of the complete C/E curve for all three agonists, giving Schild plots that had slopes of close to unity and similar pA_2 values (Table 3).

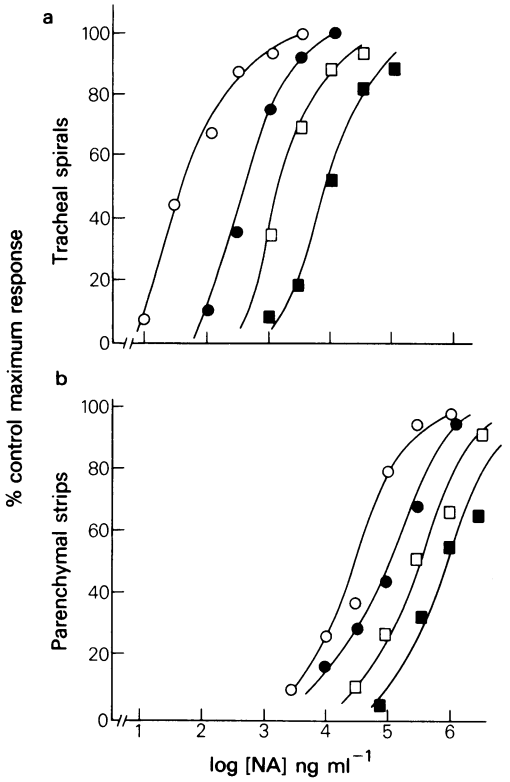

Figure 1 Antagonism of noradrenaline (NA) by the β_1-selective antagonist, atenolol. NA concentration-effect curves were produced as described in the Methods, in the presence of increasing concentrations of atenolol, with 1 h equilibration time between each curve. The data represent a single experiment only on (a) tracheal spiral and (b) parenchymal strip of guinea-pig, but similar results were obtained on at least two separate occasions. (○) Control; (●) 2×10^{-6} M; (□) 2×10^{-5} M; (■) 2×10^{-4} M atenolol.

Discussion

Substantial recent evidence from both functional and receptor binding studies suggests that both β-adrenoceptor subtypes can co-exist in tissues such as heart (Carlsson *et al.*, 1972; Hedberg, Minneman & Molinoff, 1980), lung (Furchgott, 1976; Rugg *et al.*, 1978; Zaagsma *et al.*, 1979; O'Donnell & Wanstall,

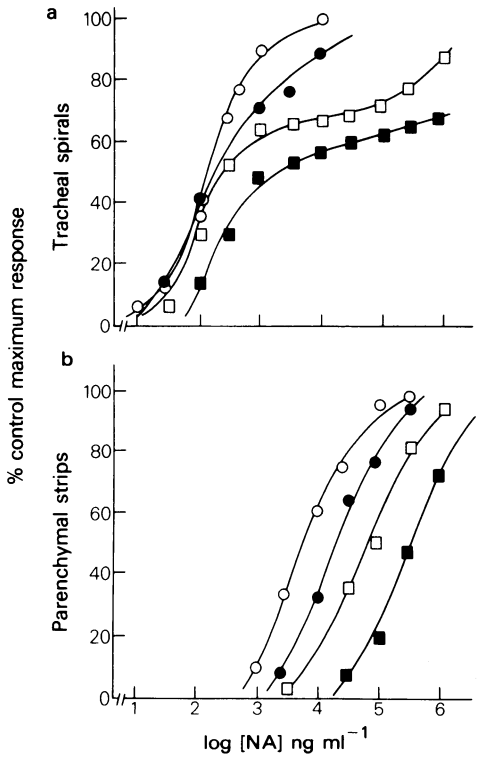

Figure 2 Antagonism of noradrenaline (NA) by the β_2-selective antagonist, ICI 118,551. Concentration-effect curves for NA were produced as described in the Methods, in the presence of increasing concentrations of ICI 118,551, with 1 h equilibration time between each curve. The data represent a single experiment on (a) tracheal spiral and (b) parenchymal strip of guinea-pig, but similar results were obtained on at least two separate occasions. (○) Control; (●) 2×10^{-8} M; (□) 5×10^{-8} M; and (■) 2×10^{-7} M ICI 118,551.

1979), rat jugular vein (Cohen & Wiley, 1978) or cat nictitating membrane (Varma & Nickerson, 1981). However, there is no reason to expect that the direct identification of both β-adrenoceptor subtypes in a tissue should necessarily be accompanied by functional heterogeneity and there are good examples of this kind of discrepancy in guinea-pig atria (O'Donnell & Wanstall, 1979; Hedberg *et al.*, 1980) and in the present study, at least in the case of peripheral lung tissue. It is very likely that β-receptor heterogeneity identified in binding studies may merely reflect the heterogeneous cellular nature of the tissue in question. Indeed, it is only with functional receptor heterogeneity that one can suggest that since both receptors mediate the same function, they may co-exist on the same cell.

The present study confirms and extends earlier studies (Zaagsma *et al.*, 1979; O'Donnell & Wanstall, 1979) that suggest that relaxation of tracheal smooth muscle of the guinea-pig can be mediated by β_1- and β_2-adrenoceptors whereas in the finer peripheral airways, a homogeneous β_2 population mediates this function. The use of both selective agonists and antagonists has been critical here. Thus, the non-selective antagonist, timolol, had very similar affinity irrespective of the agonists in both areas, but with the selective antagonists, atenolol and ICI 118,551, this was only true in peripheral lung strips. In tracheal spirals the apparent affinity for atenolol was dependent on the agonist used; atenolol was 100 × more potent with NA than with Iso and Ter as agonists. NA therefore probably produces relaxant responses by stimulating different receptors from those stimulated by the other agonists and, as the slope of the Schild plot was much less than unity, more than one type of receptor could be involved. In the presence of atenolol, higher concentrations of NA are required to produce a response and as its selectivity for β_1-receptors (from ligand binding studies) is only around 30 fold, this agonist may also stimulate β_2-receptors. This could result in an apparent decrease in the potency of atenolol at higher NA concentrations (where the latter acts at β_2-receptors) and thus produce a Schild plot with a regression line of low slope. In parenchymal strips the low value of the slope for atenolol with NA may, in part, be due to difficulties in accurately measuring the C/E curve for NA in this tissue owing to its low potency; and it should be noted that the low slope will increase the error in estimating the pA_2 value.

The involvement of both β-adrenoceptor subtypes in the response of the trachea received support from the studies with the highly β_2-selective antagonist, ICI 118,551, which produced biphasic C/E curves with both Iso and NA. These data confirm and extend those of Zaagsma, Van der Heijden, Van der Schaar & Bank (1983) with this antagonist. The findings may be explained by ICI 118,551 producing a greater inhibitory effect at β_2-receptors than β_1-receptors, thereby increasing the apparent selectivity of NA for β_1-receptors and making Iso a β_1-selective agonist. The opposite is true for atenolol, which will have a greater inhibitory effect at β_1-receptors than β_2-receptors, thus reducing the selectivity of NA for β_1-receptors and explaining why biphasic curves to NA were not seen in the presence of atenolol. The apparent parallel shifts in the C/E curves for Iso in the presence of atenolol are, however, more difficult to explain, but they may reflect some functional interaction between the two subtypes whereby β_2-receptors have the capacity to produce the full maximum response when β_1-receptors are inhibited, but β_1-receptors alone cannot produce the full maximum response. From the curves for NA in the presence of

ICI 118,551 it appears that β_1-receptors may only be able to produce 50–60% of the maximum response, but this appears to vary from animal to animal (unpublished observations, see also Furchgott & Wakade, 1976).

Whatever the relationship between the two subtypes, it is clear that whilst both β_1 and β_2-receptors mediate the relaxant response to catecholamines in the trachea, only the β_2-subtype appears to be involved in the response of parenchymal lung strips. This is particularly intriguing since these strips possess several potential contractile elements including bronchiolar and vascular smooth muscle (Lulich, Mitchell & Sparrow, 1976) and alveolar interstitial myofibroblasts (Kapanci, Assimacopoulos, Irle, Zwahlen & Gabbiani, 1974). The distribution of functional β_1-adrenoceptors appears to correspond to the density of noradrenergic innervation in the airways (O'Donnell, Saar & Wood, 1978) and thus tends to support the concept of Ariens & Simonis (1976) that β_1-receptors respond primarily to noradrenaline released from sympathetic nerve endings, whereas β_2 sites are humoral receptors responding mainly to circulating adrenaline. However, the direct identification of β-adrenoceptors using ligand binding techniques suggests that the relative proportion of β_1-adrenoceptors is similar throughout the airways. If we are to accept that these β_1 sites mediate functions other than smooth muscle relaxation, we may have to consider that they could respond to NA which may overflow from sympathetic nerves that innervate pulmonary blood vessels (see Ainsworth, Garland & Payne, 1982). Clearly the most urgent objective is to obtain much more precise information on the cellular localisation of β-adrenoceptor subtypes in the airways. The recent use of light microscope autoradiography in ferret lung with [^{3}H]-dihydroalprenolol (Barnes, Basbaum, Nadel & Roberts, 1982) has revealed that high densities of β-adrenoceptors are associated with alveoli and airway smooth muscle, particularly the smooth muscle of the smaller airways (bronchioles). It will be important now to extend this approach to examine receptor subtypes in the airways of several species.

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