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THE SELECTIVE ACTION OF β-ADRENOCEPTOR BLOCKING DRUGS AND THE NATURE OF β_1 and β_2 ADRENOCEPTORS

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1 Purified membranes retaining a catecholamine responsive adenylate cyclase have been prepared from rabbit heart, lung and (pseudo-pregnant) uterus.

2 These preparations have the characteristics of plasma membranes and both heart and lung respond to β-adrenoceptor agonists in the order:

(±)-isoprenaline > (−)-noradrenaline > (−)-adrenaline > (+)-isoprenaline > salbutamol.

The sensitivity of the adenylate cyclase to β-adrenoceptor stimulation is improved by pre-treatment of the animals with reserpine and syrosingopine.

3 Dose-ratios for several concentrations of propranolol (non-selective β-adrenoceptor blocker), practolol and atenolol (cardio-selective β-adrenoceptor blockers) have been measured on all three membrane preparations. Schild plots of log (dose ratio −1) vs. log dose were virtually coincident for heart and lung with a dissociation constant (K_b) for propranolol very close to the pharmacological value. The ratio of K_b values was 0.65 for practolol and 1.23 for atenolol compared with pharmacological cardio-selectivity ratios (measured on isolated atria and tracheal chain) of 67.6 and 110 respectively. The uterus/heart K_b ratio was 51.5 for atenolol. Inhibition of the uterus by practolol gave a Schild plot with slope significantly less than 1, indicating a different mechanism of action from the heart.

4 K_b values obtained by measuring adenylate cyclase stimulation in chopped tissue (including preparations of bronchial tree and alveolar tissue as well as whole lung) resembled the membrane values rather than those found in whole organs.

5 The results show that the pharmacological selectivity of practolol and atenolol is maintained at the receptor-adenylate cyclase level, at least as far as heart and uterus are concerned, though the smaller selectivity ratios in the biochemical system suggest that receptor difference is not the only factor and that phase distribution of the drug may also be important. Membranes prepared from whole lung show an overall β_1 response which may simply reflect the predominance of β_1 cell types containing β_1-adrenoceptors over bronchial smooth muscle.

Introduction

The sub-division of β-adrenoceptor proposed by Lands, Arnold, McAuliff, Luduena & Brown (1967) was based on the varying responses of fat tissue, heart, lung and blood vessels to a number of agonists. The lipolytic response correlated well with cardiac stimulation and was classed as β_1, whereas bronchodilatation and vasodepression correlated with neither of these and were classed together as β_2. The implication was that these represented distinct receptor sub types and support for this concept was increased by the discovery of practolol (Dunlop & Shanks, 1968), a β-adrenoceptor blocking drug which has selective actions in the heart and has proved useful in the clinic for its ability to control cardiac dysrhythmias in asthmatic patients without producing bronchoconstriction (Van Durme, Dossaert, Vermeire & Pannier, 1973). A number of other considerations may affect selectivity, however, besides a true difference in receptor. Metabolism and lipid solubility may affect the gross distribution of the active species and diffusion barriers at the micro-environmental level may act to produce different concentrations of drug around identical receptors. Some attempts have already been made to characterize the β-adrenoceptors in biochemical terms and it now appears to be well established that responses to β-adrenoceptor stimulation are mediated in some way through the cyclic AMP system: β-adrenoceptor agonists are known to stimulate adenylate cyclase in cell-free preparations and their action can be blocked

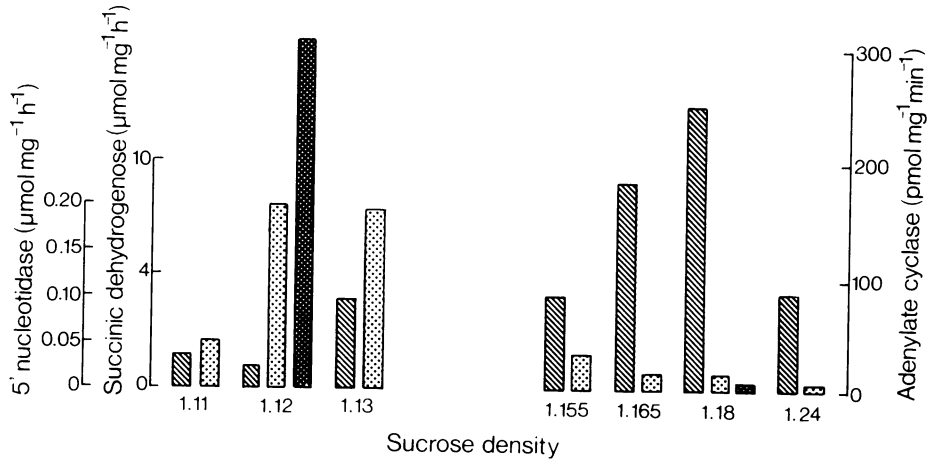

Figure 1 Enzyme activity of sucrose density gradient fractions from rabbit ventricle. Diagonally-hatched columns = succinic dehydrogenase, dotted columns = 5'nucleotidase, cross-hatched columns = adenylate cyclase.

competitively by β-adrenoceptor antagonists. This subject is reviewed by Robison, Butcher & Sutherland (1971). The cyclic AMP response has been used by Burges & Blackburn (1972) to examine the actions of selective agonists and blockers on homogenates of heart and lung and by Mayer (1972) for a similar study on heart and liver. Both these authors concluded that selective agonist and antagonist actions were still apparent at the tissue homogenate level. The purpose of the present study was to prepare more highly purified and characterized membrane fractions with β-adrenoceptor properties from β_1 and β_2 tissues and to make a quantitative study of their response to selective and non-selective β-adrenoceptor blocking drugs.

Methods

Plasma membrane preparation: continuous density gradients

New Zealand white rabbits (2–2.5 kg) were dosed 22 h before death with syrosingopine (4 mg/kg s.c.) followed 16 h before death with reserpine (4.5 mg/kg s.c.). For uterus preparations, females had previously been ovariectomized bilaterally under halothane anaesthesia and allowed to recover for one week. They were then dosed with oestradiol benzoate (4 μg/kg s.c. in arachis oil) for five consecutive days followed by 5 daily doses of oestradiol benzoate (0.4 μg/kg s.c.) and progesterone (2 mg/kg s.c. suspended in arachis oil).

After killing the animals by a sharp blow at the back of the skull, the tissues required were quickly excised. The ventricle was dissected free of excess fat, large vessels and atria and the lung freed from the trachea. The uterus was cut open longitudinally and the internal mucosal layer scraped off with a glass microscope slide. The tissues were then placed in cold sucrose buffer (mM): tris pH 7.4(5), $MgSO_4$(0.5), EGTA(1) in sucrose solution sp.gr. 1.04. All sucrose solutions are referred to by specific gravity at 20°C: refractive index was measured with an Abbé refractometer and the corresponding specific gravity obtained from tables. All subsequent stages of the preparation were at 4°C.

Tissue (4 g) was chopped with scissors into 150 ml of the sucrose buffer (sp.gr. 1.04) and homogenized with a Braun multimix blender for 4 periods of 5 s each at speed 1, then given 5 strokes with an air driven teflon glass homogenizer. The slurry was centrifuged for 10 min at 400 *g* in an MSE 18 centrifuge. The supernatant was put through 3 layers of cheesecloth and centrifuged at 177,000 *g* for 40 minutes.

The pellet was resuspended in 10 ml (ventricle or uterus) or 20 ml (lung) of sucrose buffer (sp.gr. 1.04). This homogenate (3 ml) was layered on to continuous sucrose density gradients from sp.gr. 1.09 to sp.gr. 1.16 in SW 25.1 rotor tubes and centrifuged at 25,000 rev/min for 135 minutes. The gradients were then pumped off through a u.v. monitor set at 280 nm, and collected in fractions.

Enzyme marker and electron micrograph studies

Pellets prepared from the density gradient fractions were fixed in 2.5% glutaraldehyde and postfixed in Caulfields fixative (giving 1% OsO_4 final) for electron microscopy. Succinate dehydrogenase was estimated by the method of Ganser & Forte (1973). The 5'

nucleotidase assay was modified from Avruch & Wallach (1971). The OD_{280} profiles show the lung and heart fragments to have slightly different sedimentation characteristics. Electron micrographs and enzyme marker studies confirmed that the plasma membrane rich fraction was a fairly sharp band at sp.gr. 1.115 to sp.gr. 1.12 for heart and a more diffuse band at sp.gr. 1.13 to sp.gr. 1.15 in lung. These membranes form vesicles about 1 μm diameter and little whole mitochondrial contamination is seen. The histogram in Figure 1 shows the enzyme marker distribution in one of the ventricle continuous density gradient separations.

These findings led to the routine use of sucrose step gradients to prepare quantities of plasma membranes: sp.gr. 1.09 on sp.gr. 1.136 for heart and uterus and sp.gr. 1.11 on sp.gr. 1.16 for lung.

For preparations in Krebs-Ringer buffers the tissue was homogenized or chopped in a sucrose buffer (sp.gr. 1.04) containing (mM): NaCl 122, KCl 3, KH_2PO_4 0.4, $MgSO_4$ 1.2, $NaHCO_3$ 24, EGTA 0.1, theophylline 9, ICI 63,197 1.5 (a potent phosphodiesterase inhibitor—Somerville, 1973), gassed for 30 min beforehand with 95% O_2 and 5% CO_2. This buffer was also used in the density gradient steps with the appropriate concentration of sucrose and 1.5 mM NaATP in most cases. Calcium was not included due to its inhibitory effects on adenylate cyclase (Birnbaumer, 1973).

Chopped tissue preparations

The tissue was excised and chopped by several passes on a McIlwain tissue chopper set at 0.5 mm, and suspended in 10 volumes of either 40 mM tris-HCl pH 7.4 containing (mM) $MgSO_4$ 6, ICI 63,197 1.5, theophylline 9, EGTA 0.1, sucrose (sp.gr. 1.04) or the Krebs-Ringer buffer as stated above. When trachea was used, individual rings were suspended in buffer in some cases; in others, the entire smooth muscle band was dissected free of cartilage and homogenized in the medium.

Protein estimation

The recovery of protein at the various stages of a preparation starting from 1 g (wet wt.) of ventricle was as follows (protein estimated by the method of Lowry, Rosebrough, Farr & Randall (1951), using bovine serum albumin as standard). Homogenates layered on to gradients totalled 14 mg; of this, the plasma membrane rich step gradient interface contained 2 mg, while the mitochondrial band from the continuous gradients contained 1 mg.

Adenylate cyclase incubations

In chopped tissue all the enzyme systems were already present to convert 5'AMP to ATP. The cyclase incubation therefore, relied solely on endogenous ATP as substrate. In the membrane preparations exogenous ATP was added with an ATP regenerating system.

Chopped tissue incubation method

Agonist (100 μl) and antagonist (100 μl) at the appropriate concentrations were mixed and preheated to 30°C in glass test tubes. The incubation was started by adding 1 ml of chopped tissue taken from a suspension which was constantly stirred at 0°C. The incubation tubes were shaken for 10 min in a waterbath set at 30°C and the reaction stopped by adding 2 NHCl (50 μl), then heated at 95°C for 10 min to extract the cyclic AMP. After adding 10 ml water and homogenizing the mixture for 10 s with a Polytron homogenizer, a 50 μl aliquot was taken for protein estimation. A 500 μl aliquot of the homogenate was neutralized to pH 7.4 with 50 μl tris (110 mM) and heated to 95°C for 7 min to precipitate the protein. After centrifuging in an Eppendorf bench centrifuge for 2 min, 50 μl of supernatant was taken for the cyclic AMP assay.

Membrane incubation method

Incubation medium (200 μl 40 mM tris-HCl pH 7.4, $MgSO_4$ 6 mM, EGTA 0.1 mM, theophylline 9 mM, ICI 63,197 1.5 mM, tris ATP 1.8 mM, creatine phosphate 20 mM, 200 μg/ml creatine kinase) was added to 20 μl agonist and 20 μl antagonist at the appropriate concentrations and preheated to 30°C. The drug concentrations were randomized to minimize intra-assay variations. The reaction was started by adding 20 μl membrane suspension (3 μg lung protein or 7 μg heart protein) and incubated at 30°C for 10 min; 100 μl of 160 mM HCl was added to stop the reaction and then heated at 95°C for 6 minutes. Tris buffer (100 μl of 200 mM) was added to adjust to pH 7.4 followed by heating at 95°C for 7 minutes. After centrifuging, an aliquot of supernatant was taken for the cyclic AMP assay.

Where preparations were made in Krebs-Ringer buffer, incubations were done in Krebs-Ringer, 1.8 mM NaATP and the ATP regenerating system, and only 60 μl of 200 mM tris was required for neutralization. A zero time control was necessary to compensate the cyclic AMP assay standard curve for possible interferences from tissue or medium: HCl was added to the incubation medium which was heated to 95°C before addition of tissue or membrane.

All incubations were at 30°C and at this temperature cyclic AMP production was linear with time, at least up to 10 min, and also with protein concentration.

Cyclic AMP assay

Cyclic AMP was assayed by the method of Brown, Alban, Ekins, Sgherzi & Tampion (1971) using commercial binding protein (B.D.H. Ltd., Poole, Dorset). Samples were processed in batches of 12 every 3 min in order to standardize time of contact with charcoal. Isotope counting was done by a Philips Automatic Liquid Scintillation Analyser connected on-line with a NOVA 1220 computer.

Tissue interference with the assay was eliminated by the use of zero time controls in the blanks and standard curves as described above. The use of perchloric acid for extraction of cyclic AMP and of glycyl-glycine buffers also produced interference, as did the $Ba(OH)_2/ZnSO_4$ treatment of Krishna, Weiss & Brodie (1968) when ATP was present at 1 mM or more. Crude beef adrenal binding protein had some adenylate cyclase activity which gave rise to cyclic AMP with ATP from the incubation medium, but the commercial preparation from B.D.H. was satisfactory in this respect.

Materials

Propranolol (Inderal), practolol (Eraldin), atenolol (Tenormin) and halothane (Fluothane) were obtained from ICI Ltd. The other drugs and materials were obtained from various commercial sources.

Results

Fractions isolated from sucrose density gradients of heart and lung as described above appear to have the characteristics of plasma membranes. When examined by electron microscopy they contain a large proportion of sealed vesicles with some larger membrane fragments and minimal contamination by mitochondria. They have a high content of adenylate cyclase and 5'nucleotidase and only a small amount of succinic dehydrogenase, whereas later fractions from the gradient (see Figure 1) have high succinic dehydrogenase and low cyclase and 5'nucleotidase activity. These later fractions are seen in the electron microscope to consist almost entirely of mitochondria.

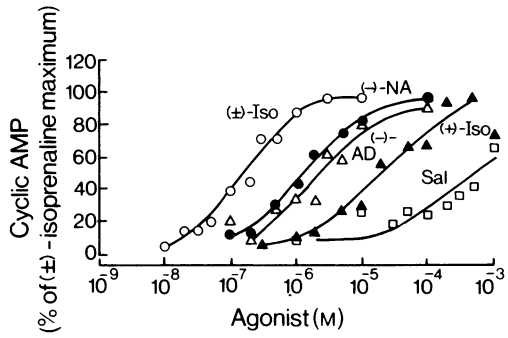

Figure 2 Heart membranes: increase in cyclic AMP in response to (isoprenaline ((±)-Iso), ±)-Iso, (−)-noradrenaline ((−)-NA), (−)-adrenaline, ((−)-AD), (+)-isoprenaline ((+)-Iso) and salbutamol (Sal). ((±)-Isoprenaline maximum taken as 100%). Ka values, with number of experiments in brackets were: (±)-Iso 1.55×10^{-7} (7), (−)-NA 1.23×10^{-6} (3), (−)-AD 1.9×10^{-6} (1), (+)-Iso 1.82×10^{-5} (1) and Sal 1.54×10^{-4} (3).

Uterus preparations were not characterized in as much detail as heart and lung but they were high in adenylate cyclase and electron micrographs showed membrane vesicles free of mitochondria.

Table 1 shows that pre-treatment of the rabbit with reserpine and syrosingopine increased both the basal adenylate cyclase levels and the stimulation by β-adrenoceptor agonist and sodium fluoride.

The adenylate cyclase from heart and lung responded well to hormone stimulation with a maximum increase of about 100% above basal activity. Uterus showed between 50 and 100% stimulation. Dose-response curves to (±)-isoprenaline, (−)-noradrenaline, (−)-adrenaline, (+)-isoprenaline and salbutamol, together with K_a values are shown in Figures 2 and 3 for heart and lung. Since the basal level and degree of stimulation varied from one membrane preparation to another, it was necessary to express the cyclic AMP formed as a percentage of the maximum obtained with isoprenaline on the same preparation, after deducting the basal level, in order to

Table 1 Effect of reserpine-treatment on rabbit ventricle adenylate cyclase levels

	Preparation I (*pmol cyclic AMP per mg protein/min*)		*Preparation II* (*pmol cyclic AMP/min* / *μmol/h 5'nucleotidase*)	
	without reserpine	*with reserpine*	*without reserpine*	*with reserpine*
Basal	113	134	194	451
+ Isoprenaline (10^{-4} M)	219	310	310	821
+ NaF (10^{-4} M)	285	477	521	1679

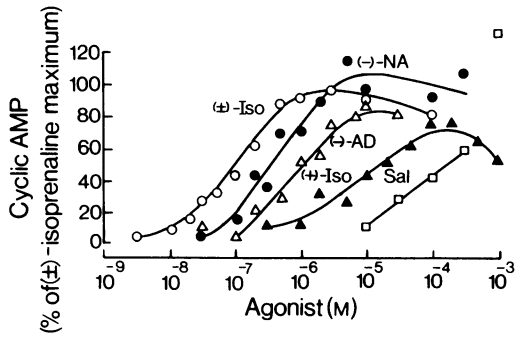

Figure 3 Lung membranes: increase in cyclic AMP in response to (±)-isoprenaline ((±)-Iso, (−)-noradrenaline ((−)-NA, (−)-adrenaline ((−)-AD), (+)-isoprenaline ((+)-Iso) and salbutamol (Sal). ((±)-Isoprenaline maximum taken as 100%) Ka values, with number of experiments in brackets were: (±)-Iso 9.7×10^{-8} (6), (−)-NA 4.9×10^{-7} (5), (−)-AD 8.3×10^{-7} (1), (+)-Iso 6.95×10^{-6} (1) and Sal 6.94×10^{-5} (4).

combine the results of several experiments. The K_a was taken as the concentration necessary to produce 50% of the maximal stimulation obtainable with that agonist. The values for salbutamol are estimates only since at 10^{-3} M there was consistently an abnormally high stimulation, suggesting that a secondary effect was coming into play and making it impossible to determine a true maximum.

All agonists are more active on the lung than the heart, but the heart/lung ratios (Table 2) are very similar in all cases, suggesting that they are all acting on the same receptors. It is notable that salbutamol shows only a two-fold preference for the lung (similar to the other agonists) as compared with the 155-fold difference found between guinea-pig trachea and atria (Giles, Williams & Finkel, 1973).

To check whether some of the difference in K_a values between isoprenaline and noradrenaline could be explained by the presence of a specific uptake mechanism for the natural catecholamine, the stimulation by both agonists was measured in the presence and absence of desmethylimipramine (DMI), a potent blocker of amine uptake mechanisms. Heart membranes prepared with and without reserpinization of the animal were used since reserpinization might be expected to block the storage sites and hence reduce any effect of DMI. The results (Table 3) show no significant effect either of DMI or of reserpinization and we conclude that there is no specific uptake mechanism for noradrenaline in our heart membrane preparations.

The response of the uterus to sympathomimetic agents varies with the state of oestrus and to obtain consistent responses to β-adrenoceptor stimulation it was found essential to make the animal pseudo-pregnant by ovariectomy followed by oestradiol and progesterone treatment as described above. Even so the dose-response curves for uterus showed more scatter than those for other tissues.

Table 2 Comparison of agonist affinities in heart and lung membranes

Agonist	*Ka heart/Ka lung*
(±)-Isoprenaline	1.60
(−)-Noradrenaline	2.51
(−)-Adrenaline	2.29
(+)-Isoprenaline	2.62
Salbutamol	2.22

Table 3 Effect of desmethylimipramine (DMI, 1 μg/ml) on adenylate cyclase activity of heart membranes prepared from reserpinized and non-reserpinized animals. For incubation conditions see text.

		cyclic AMP (pmol ± s.e. mean)	
		without reserpine	*with reserpine*
Noradrenaline	3×10^{-7} M	0.37 ± 0.01	0.33 ± 0.01
	„ + DMI	0.36 ± 0.01	0.30 ± 0.01
	3×10^{-6} M	0.35 ± 0.01	0.42 ± 0.03
	„ + DMI	0.42 ± 0.03	0.43 ± 0.04
Isoprenaline	3×10^{-8} M	0.31 ± 0.04	0.25 ± 0.03
	„ + DMI	0.30 ± 0.01	0.26 ± 0.02
	3×10^{-6} M	0.46 ± 0.01	0.36 ± 0.02
	„ + DMI	0.43 ± 0.01	0.33 ± 0.02
	10^{-4} M	0.48 ± 0.02	
Control		0.29 ± 0.01	0.16 ± 0.01
	„ + DMI	0.28 ± 0.01	0.16 ± 0.01

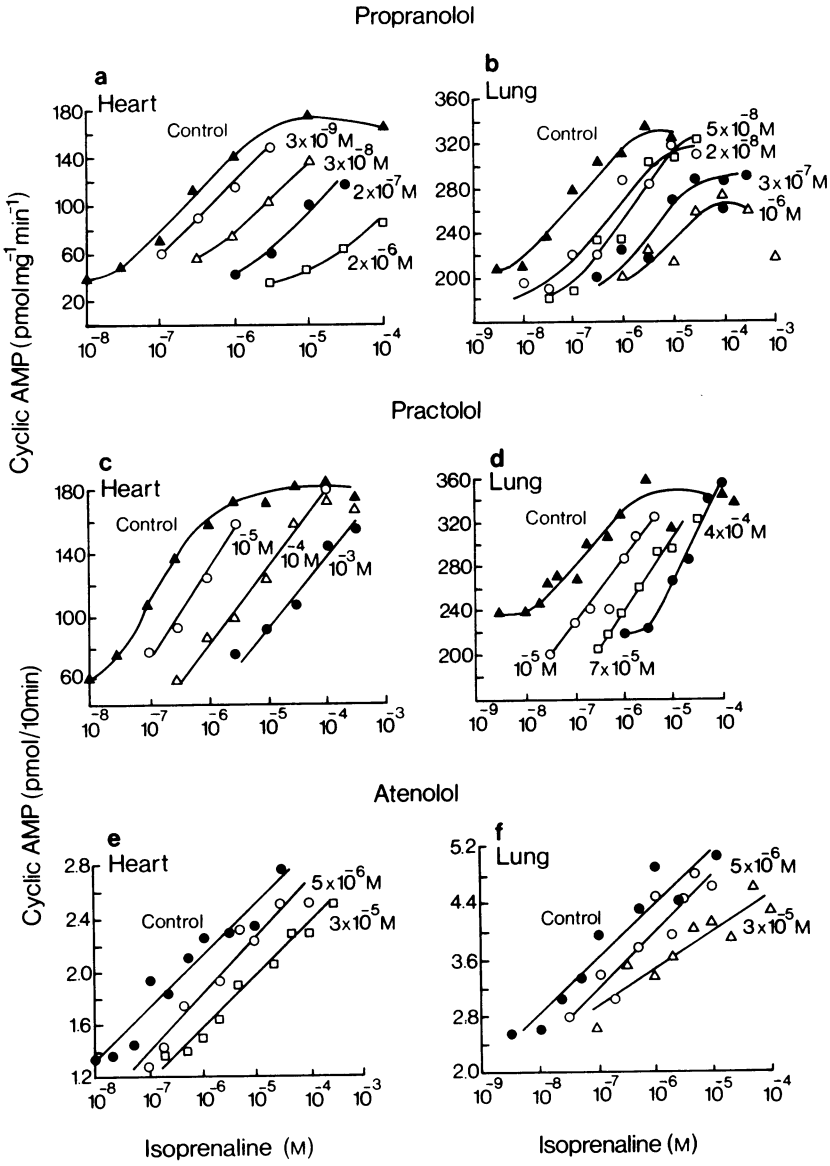

Figure 4 (±)-Isoprenaline dose response curves to (±)-isoprenaline alone (control) and in the presence of varying amounts of propranolol (a, b), practolol (c, d) and atenolol (e, f), using heart and lung membrane preparations.

Figures 4 and 5 show the shift of the dose-response curves to isoprenaline by varying concentrations of propranolol, atenolol and practolol for heart, lung and uterus, and Figure 6 shows the corresponding Schild plots (log (dose ratio −1) vs. log (dose)) derived from several such experiments for each drug. Similar plots for noradrenaline with propranolol and practolol are given in Figure 7 (for heart and lung only). These figures show the original uncorrected data; in order to obtain the best values for the dissociation constants (K_b) a statistical analysis was carried out. A standard parallel line assay procedure was used to fit parallel lines to the straight portions of the dose-response curves (determined visually) and an analysis of variance indicated that the parallel line model fitted the isoprenaline data adequately, though some deviations

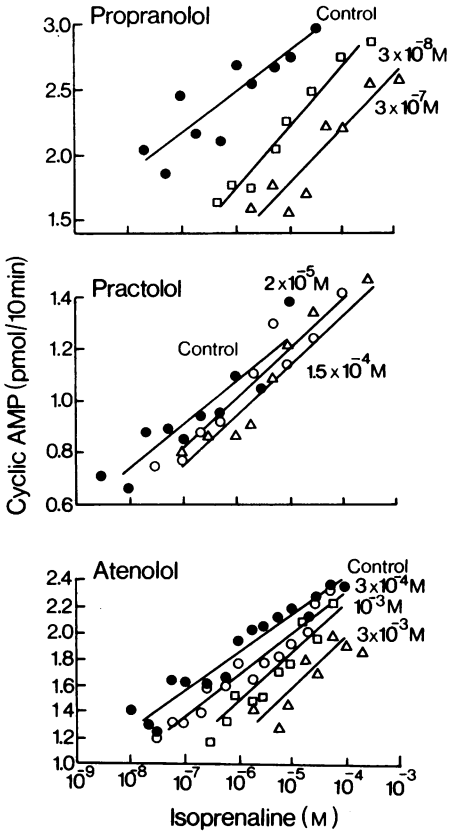

Figure 5 (±)-Isoprenaline dose-response curves to (±)-isoprenaline alone (control) and in the presence of propranolol, practolol and atenolol, using uterus membrane preparations.

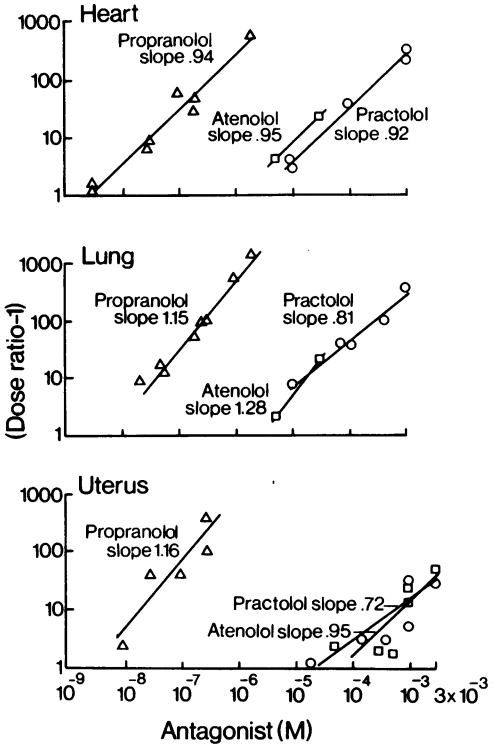

Figure 6 Schild plots for propranolol (△), practolol (○) and atenolol (□) in heart, lung and uterus membranes, using isoprenaline as agonist.

from parallelism were apparent in the noradrenaline experiments. The dose-ratio and an estimate of the variance of ln (dose-ratio) were obtained by standard techniques. Occasionally a vertical shift in the dose-response curve was apparent between different concentrations of blocking agent. In these cases the readings were reduced to a common baseline by subtracting the difference between the mean for antagonist only and the control mean.

The dose-ratio (r) obtained in this way were analysed by a weighted least squares analysis, regressing $\ln(r-1)$ upon $\log B$ (where B = antagonist concentration). The weight attached to each value of $\ln(r-1)$ was inversely related to its estimated variance as approximated by the expression:

$$\text{Var}\,(\ln(r-1) \simeq \left(\frac{r}{r-1}\right)^2 \text{var}\,(\ln r)$$

According to theory (Schild, 1957) simple competitive antagonism should result in a straight line of slope 1 and an intercept with the x axis of $-\log K_b$, corresponding to the equation:

$$\log(r-1) = \log B - \log K_b$$

In no case (other than practolol and the uterus, which is discussed below) was the slope of the Schild plot significantly different from 1, so a line of slope 1 was used to calculate the K_b values, which are shown in Table 4. Uncorrected Schild plots are shown in Figures 6 and 7. Since the data for whole lung and heart revealed no significant degree of cardio-selectivity, we endeavoured to separate the bronchial tree from the spongy tissue of the lung by mechanical combing and examined the responses of both fractions to isoprenaline and atenolol. We also used preparations of tracheal rings and homogenates of tracheal smooth muscle. The Schild plots of Figure 8 show that the dose-ratios for both fractions of lung fall on the same line as for whole lung, while the response of the trachea is intermediate between lung and uterus. Histological examination of these tissues showed that the proportion of smooth muscle in the 'bronchial tree' fraction was very small and not too different from the 'spongy tissue' and that the smooth muscle of the

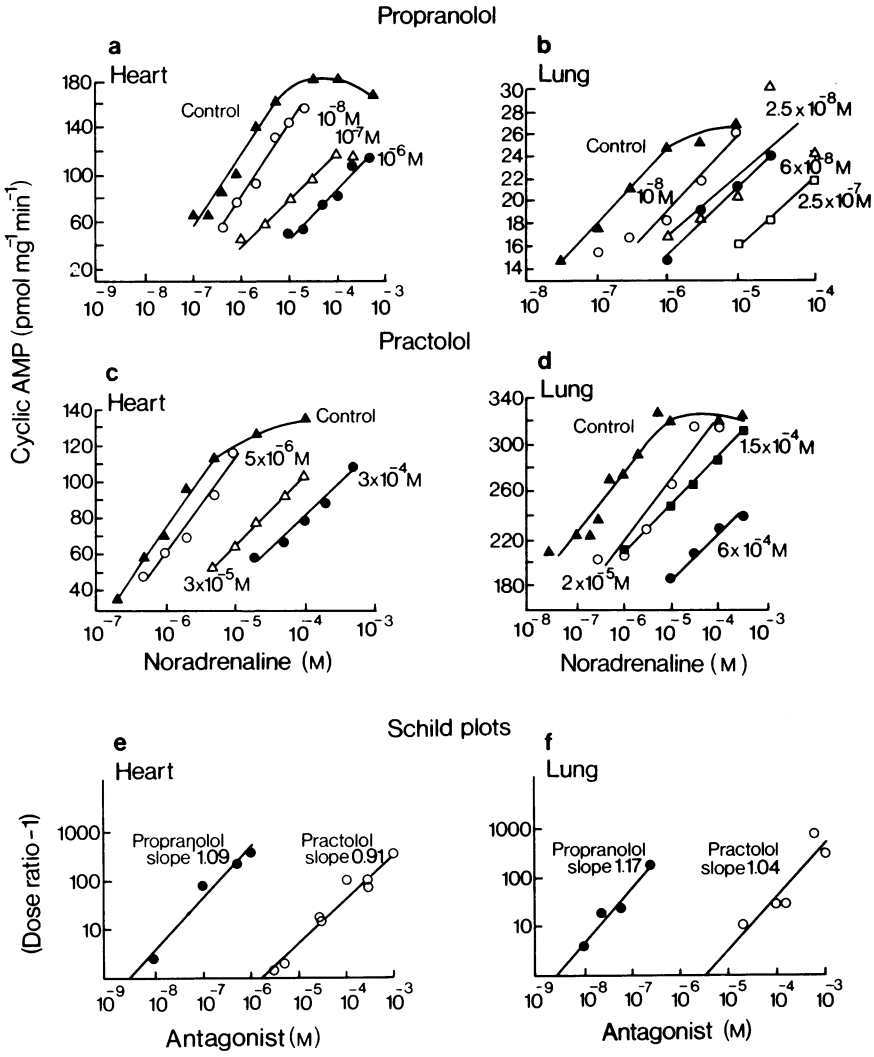

Figure 7 (–)-Noradrenaline dose response curves to noradrenaline alone (control) and in the presence of varying amounts of propranolol (a, b) and practolol (c, d) together with Schild plots (e, f), using heart and lung membrane preparations.

trachea still retained its inner lining of epithelial cells so that the possibility of a mixed response existed. The situation in uterus is quite otherwise and there is clear demonstration of a difference in dissociation constant (Figures 6 and 8, Table 4). The slope of the Schild plot for practolol is significantly less than 1, which suggests either that the inhibition is not strictly competitive or that the interaction of practolol with the uterus receptor is not 1:1—in both cases reinforcing the conclusion that the uterus receptor is different from the heart.

Some experiments were also carried out with chopped tissue, and at the same time the effects of preparing tissues in Krebs-Ringer buffer instead of the tris buffer which had been used in all previous experiments was compared, since a number of reports in the literature suggest that tris may have harmful effects on membranes and even result in the loss of hormone stimulation from brain cyclase preparations (Chasin, Mamrak & Samaniego, 1974). Figures 9 and 10 show dose-response curves to isoprenaline in chopped heart and lung (prepared in Krebs-Ringer) in the presence and absence of 10^{-4} M practolol, and Table 5 summarizes the K_a values for isoprenaline and K_b values for practolol obtained in two experiments

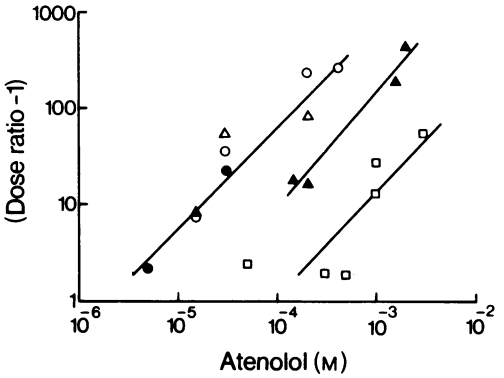

Figure 8 Schild plots for atenolol in trachea (▲), uterus (□) and various lung preparations using isoprenaline as agonist. (●), whole lung membranes; (○), homogenate of lung spongy tissue and (△) homogenate of lung bronchial tree.

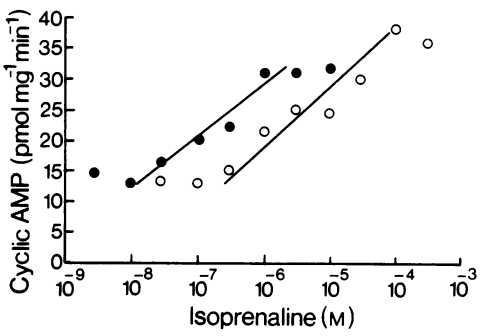

Figure 9 Chopped heart tissue (in Krebs-Ringer). Dose-response curves to isoprenaline alone(●) and in the presence of 10^{-4} M practolol (○).

with tissues prepared in Krebs-Ringer and one in which tris buffer was used. The results show that tris is without important effect and that the K_a values obtained with chopped tissue preparations do not differ from those obtained with membranes.

Similar K_a and K_b values were obtained from freshly prepared membranes and from membranes stored for some time in liquid nitrogen. Taken together these factors lead us to believe that the β-adrenoceptors and their linkage to adenylate cyclase have not been significantly perturbed in the process of membrane preparation and storage, and are, therefore, representative of β-adrenoceptors in the whole organ.

Discussion

The cardioselectivity of practolol and atenolol in isolated organs has been clearly demonstrated by Barrett, Carter, Fitzgerald & Le Count (1973) and

Table 4 Antagonist K values (mM) determined on membranes. (Calculated from corrected data and Schild plots of slope 1—see text).

			K_b (nM ± s.e. mean)	K_b relative to heart
a. Isoprenaline as agonist				
	Propranolol	Heart	3.4 ± 0.5	1
		Lung	2.83 ± 0.32	0.85
		Uterus	1.5 ± 0.6	0.45
	Practolol	Heart	3200 ± 400	1
		Lung	2100 ± 390	0.65
		Uterus*	(54,000)	(17)
	Atenolol	Heart	1300 ± 600	1
		Lung	1600 ± 400	1.23
		Uterus	67,000 ± 20,000	51.5
b. Noradrenaline as agonist				
	Propranolol	Heart	2.3 ± 0.5	1
		Lung	1.7 ± 0.3	0.74
	Practolol	Heart	2600 ± 300	1
		Lung	2200 ± 700	0.84

* Schild plot slope significantly < 1

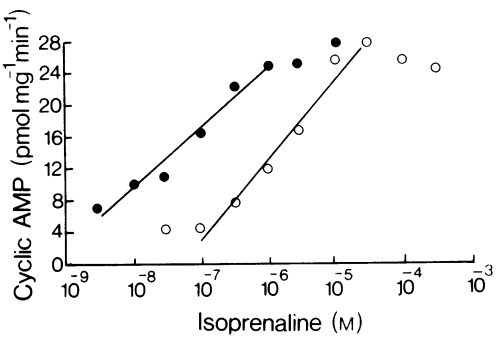

Figure 10 Chopped lung tissue (in Krebs-Ringer). Dose-response curves to isoprenaline alone (●) and in the presence of 10^{-4} M practolol (○).

Table 6 shows the K_b values for atrium and trachea derived from these authors' pA2 values (in some cases updated to include more recent experiments from the same laboratory—personal communication from Mr J. Carter and Dr J. Wale). Selectivity could be due to differences in one or more of the following factors: 1 the β-adrenoceptors; 2. non-specific binding of the drug; 3. the presence of diffusion barriers to the β-adrenoceptor sites.

Factors 2 and 3 could act in the whole organ to reduce the effective concentration of a drug at the β-adrenoceptor of one tissue relative to the other and make it more difficult for the drug to reach a true equilibrium with the receptor. Our expectation was that these factors would be considerably reduced in membrane preparations and hence that K_b values obtained with these preparations would be a truer measure of receptor interaction. In the event, the similarity of values obtained for practolol in both membranes and chopped tissue suggests that there is no question of equilibration in the tissue being incomplete as compared with the membrane. The fact that the K_b for propranolol measured on the cyclic AMP response of heart membranes ($3.4 \pm 0.5 \times 10^{-9}$ M) is very close to that measured on the chronotropic response of whole atrium ($4.9 \pm 0.61 \times 10^{-9}$M) reinforces the view, expressed above, that the receptors have not been greatly altered during preparation of the membranes. We have, therefore, to look elsewhere for an explanation of the lack of selectivity shown by practolol and atenolol in heart v. lung membranes and the most likely reason lies in the multiplicity of cell types to be found in lung. Histological examination shows a relatively small proportion of bronchial smooth muscle cells and if these were sensitive to β_2-adrenoceptor stimulation it is conceivable that their response in terms of cyclic AMP could be swamped by a large excess of β_1 responding cells (possibly secretory). Neither mechanical combing of the tissue nor density gradient centrifugation of the membranes appear to produce any separation of these types. Further work on characterizing the adrenergic response of the cell types involved is in progress but in the meantime we have to say that the predominant response of the lung to adrenoceptor agonists, in terms of cyclic AMP, is β_1, though this is not necessarily true of bronchial smooth muscle. The contrary results found by other authors (Burges & Blackburn, 1972; Lefkowitz, 1975) can be traced to the

Table 5 Comparison of K_a values for isoprenaline and K_b for practolol obtained with chopped tissue in tris buffer (1 expt.) and Ringer (2 expts.). For details see text.

	Buffer	*Ka for Isoprenaline (μM)*	*Kb for Practolol (μM)*
Heart	*Tris*	0.24	4.2
	Ringer (1)	0.103	2.0
	Ringer (2)	0.11	3.3
Lung	*Tris*	0.035	4.3
	Ringer (1)	0.084	5.0
	Ringer (2)	0.056	

Table 6 K_b values (nM ± s.e. mean) determined on isolated tissues. Isoprenaline used as agonist. (Data adapted from Barrett *et al.* (1973) updated by personal communications from Mr J. Carter and Dr J. Wale)

	*Atrium**	*Trachea†*	*Ratio Trachea/Atrium*
Propranolol	4.9 ± 0.61	21.5 ± 6.0	4.4
Practolol	324 ± 7	24,320 ± 4180	75.1
Atenolol	53.7 ± 14	6090 ± 810	113.4

* Spontaneous beating rate of guinea-pig right atrium
† Relaxation of guinea-pig tracheal smooth muscle

inaccuracies of comparing ED_{50} values for antagonists. These values, estimated from dose-response curves at a single, fixed, agonist concentration, do not allow for differences in agonist K_a between tissues and are subject to considerable error depending on the agonist concentration chosen. The use of dissociation constants calculated from Schild plots, as in the present study, is free from these disadvantages.

The same two compounds, practolol and atenolol, show a much lower potency on uterus membranes and we conclude that this represents a real difference in receptor type. The intermediate position of the trachea presumably indicates a mixed β_1- and β_2-adrenoceptor population. The selectivity ratios found in membranes, however, are considerably lower than those found in the whole organ system (Tables 4 and 6) and experiments with a wider series of compounds (Coleman & Somerville, in preparation) show that the difference varies in a systematic manner with partition coefficient. This suggests that different receptor types alone are not sufficient to account for selectivity, but that distribution of the drug in the micro-environment of the receptor may also have a part to play.

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