2-[^{125}I]iodomelatonin binding sites in both chick forebrain and retina exhibit a high affinity for melatonin and much lower affinity for *N*-acetyl-5-HT (Table 2). Melatonin and *N*-acetyl-5-HT have approximately equal affinities at this site in hamster hypothalamus and testes. Melatonin itself is 4–8 fold more potent in the chick than in the hamster, arguing a species difference between the sites. Prazosin has much higher affinity for this site in hamster tissues than in chick tissues (Table 2 and Fig. 1). While the central and peripheral 2-[^{125}I]iodomelatonin sites seem similar within the hamster or within the chick, a species difference exists between the hamster and chick. It remains to be seen if functional differences exist across species.

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A study of β-adrenoceptors in rat lung parenchymal strip

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Abstract—The aim of the present study was to characterize the β-adrenoceptor population in rat lung strip. For this purpose, Schild plots were obtained for the β-adrenoceptor antagonists atenolol (β_1-selective), butoxamine (β_2-selective) and propranolol (non-selective), using three different agonists: isoprenaline (non-selective), salbutamol (β_2-selective) and noradrenaline (β_1-selective). The slopes of these Schild plots were close to the theoretical value of unity, and pA_2 values determined with isoprenaline, salbutamol and noradrenaline as agonists were: for propranolol, 7·86 ± 0·22, 7·72 ± 0·15 and 7·89 ± 0·23; for atenolol, 5·19 ± 0·05, 5·33 ± 0·07 and 5·47 ± 0·22 and for butoxamine, 6·31 ± 0·11, 6·34 ± 0·03 and 5·99 ± 0·23, respectively. These data suggest that pharmacological responses of rat isolated lung strip to β-adrenoceptor agents are mediated by a homogeneous population of β_2-adrenoceptors, although the presence of a minor population of β_1-adrenoceptors undetected by the agonists used cannot be excluded.

We have previously studied the inhibitory effects of sympathomimetic amines on preparations of bathed as well as superfused lung strips of rat and the results obtained indicated that responses are mediated predominantly by β_2-adrenoceptors (Candenas et al 1986). β_2-adrenoceptors have also been found to be the only, or the predominant, β-adrenoceptor subtype involved in responses of lung strip preparations of other animal species, such as cat (Lulich et al 1976), pig (Goldie et al 1982) or guinea-pig (Carswell & Nahorski 1983). In rabbit lung strip, however, β_1-adrenoceptors seem to predominate (Chand & Deroth 1979).

To investigate further the β-adrenoceptor population within the rat lung strip, we have examined the antagonism by propranolol, atenolol and butoxamine of the effects of isoprenaline, salbutamol and noradrenaline, using the method described by O'Donnell & Wanstall (1979, 1981).

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Materials and Methods

Male Wistar rats, 200–260 g were killed by a blow on the head and exanguinated by sectioning of the carotid arteries. The lungs were removed and placed in modified Krebs-Henseleit solution maintained at 37°C. Strips of tissue approximately 10 mm in length and 2 mm in width, were cut from the centre of the lobe according to Lulich et al (1976). Strips were always dissected from the centre of the lobe since strips from the peripheral margin did not maintain a regular tone and did not show consistent pharmacological responses. The strips were mounted under a tension of 1 g in a 30 mL organ bath containing modified Krebs-Hanseleit solution that was maintained at 37°C and gassed continuously with a mixture of 95% O_2 and 5% CO_2. The composition of the physiological solution was (mM): NaCl 118·4; KCl 4·7; $CaCl_2$ 2·5; $NaHCO_3$ 25·0; KH_2PO_4 1·2; dextrose 11·1 and EDTA 0·04. The solution was adjusted to a pH between 7·2 and 7·4 with NaMOPS (morpholinopropane-sulphonic acid titrated with NaOH). The strips were allowed to rest for 1 h during which time the physiological solution was changed at 10 min intervals and tension was periodically readjusted to 1 g. Changes in tension was measured isometrically using a Gould Statham UC2 transducer and recorded on a HP 680M via an HP 8805C carrier amplifier.

Agonists were added to the bath in a cumulative manner, to obtain concentration-response curves. Only two curves to any given agonist were determined in any single experiment and in these conditions, preparations did not lose sensitivity. Hence, it was not necessary to introduce correction factors. An iterative computerized procedure (Basulto et al 1978) was applied to fit individual concentration-response curves and to calculate the maximum effect (E_{max}) and the concentration producing 50% of this maximum (EC50).

When antagonists were used, and after the dose-response curve to one agonist on three strips from the same animal had been established, a single and different concentration of an antagonist was added to each tissue, and after a 30 min

incubation, the dose-response curve was repeated on these strips. Values of concentration-ratios (CR) were then calculated from the EC50 in the presence of the antagonist, divided by the EC50 in the absence of the antagonist. Schild plots (Arunlakshana & Schild 1959) of log (CR-1) versus log of the molar concentration of the antagonist, log (B), were constructed and pA_2 values determined by regression analysis of individual data points (Snedecor & Cochran 1967). A mean Schild plot and a mean pA_2 value ± s.e. were then calculated for each agonist-antagonist combination by using all the individual data points from the different animals for the regression analysis. If the mean Schild regression slope is not significantly different from unity, pK_B values (−log of the equilibrium dissociation constant of the antagonist, K_B) calculated from the equation: mean pK_B = mean (log(CR-1) − log(B)) must be close to the mean pA_2 ± s.e. calculated graphically, providing confirmation that the antagonism is competitive.

All experiments were carried out in the absence of catecholamine uptake inhibitors since in a preliminary set of experiments, the negative log EC50 values calculated for isoprenaline, salbutamol and noradrenaline in the presence of cocaine and corticosterone (10^{-5} M, 30 min contact time) did not differ significantly from the values obtained when sites of uptake were not blocked. α-adrenoceptors were blocked with phentolamine (10^{-5} or 10^{-4} M) only when noradrenaline was used as agonist since in the absence of an α-adrenoceptor antagonist, noradrenaline elicited contractile responses.

Drugs used were: (±)-isoprenaline sulphate (Sigma); salbutamol (Sigma); (−)-noradrenaline L-tartrate (Merck); propranolol hydrochloride (Sigma); butoxamine hydrochloride (Welcome); atenolol (ICI) and phentolamine mesylate (Sigma).

Fresh drug solutions were prepared and kept ice-cold during the course of each experiment. Solutions of sympathomimetic amines were stabilized with ascorbic acid (40 μg mL^{-1}).

Drug concentrations are expressed for the free acid or base as final molar bath concentrations. Mean values are quoted together with the error of the mean (s.e. mean). Comparisons of the data have been made using a Student's *t*-test at a 5% significance level. The significance of any deviation from unity of the slopes of Schild plots has been calculated acording to Snedecor & Cochran (1967).

Results

Loaded rat lung strip preparations responded regularly to β-adrenoceptor agonists and dose-related relaxations were obtained with isoprenaline, salbutamol and noradrenaline (in the presence of phentolamine 10^{-5} M).

In experiments in which antagonists were used, propranolol (non-selective β-adrenoceptor antagonist), butoxamine (β_2-selective antagonist) and atenolol (β_1-selective antagonist) caused concentration-dependent shifts to the right of the concentration-response curves for the three agonists. The location of the Schild plots for these antagonists did not vary with the agonist used (Fig. 1). The slopes of these plots together with their standard errors (s.e.) are shown in Table 1. None of these slopes were significantly different from unity, which indicates competitive antagonism by the three antagonists of the agonists used. Mean pA_2 values obtained by extrapolation of individual Schild plots to log (CR-1) = 0 were close to mean pK_B values and no statistical differences between these values using different agonists were found (Table 2). This provides further confirmation that the antagonism was competitive.

Table 1. Slopes (±s.e. of the slopes) of the Schild plots for propranolol, atenolol and butoxamine with isoprenaline, salbutamol or noradrenaline as agonists.

	Agonist		
Antagonist	Isoprenaline	Salbutamol	Noradrenaline
Propranolol	−0·99 ± 0·16 (15)	−1·04 ± 0·13 (18)	−0·91 ± 0·11 (18)
Atenolol	−1·18 ± 0·08 (12)	−1·04 ± 0·10 (15)	−1·03 ± 0·02 (12)
Butoxamine	−0·91 ± 0·07 (12)	−1·07 ± 0·04 (15)	−1·06 ± 0·08 (18)

Number of data points are given in parentheses.

As can be observed, the pA_2 values obtained for propranolol were similar for each of the agonists used, and the pA_2 values for butoxamine and atenolol were within the range of previous estimates of pA_2 for these antagonists when acting on β_2-adrenoceptors but not on β_1-adrenoceptors.

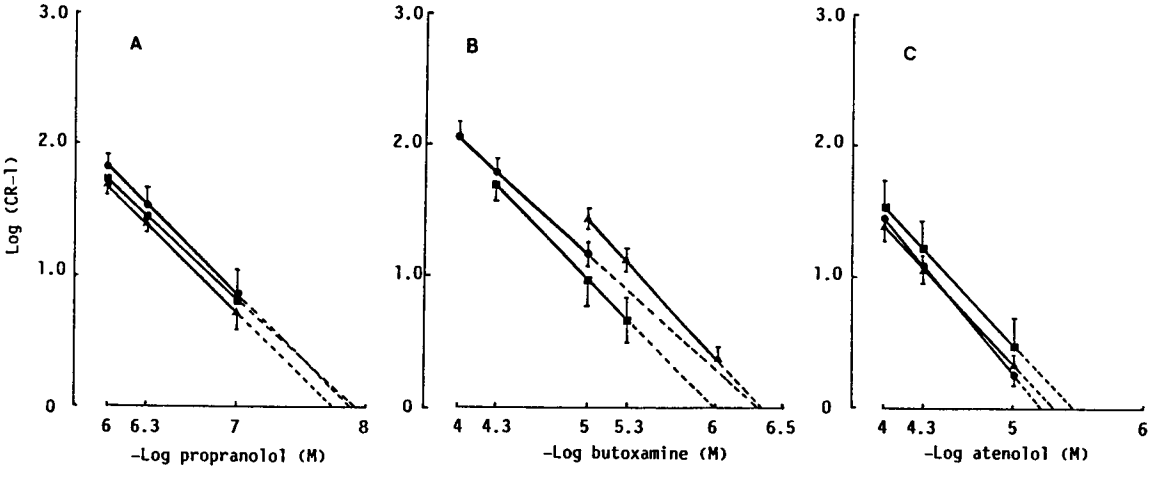

FIG. 1. Schild plots for the antagonism of isoprenaline (●), salbutamol (▲) and noradrenaline (■) by A, propranolol; B, butoxamine; and C, atenolol on rat lung parenchymal strip. The plots represent the mean of the calculated lines of best fit through the data obtained from each animal used. The vertical lines represent the s.e. of the mean values of log (CR − 1) at points corresponding to the antagonists concentration used.

Table 2. pA_2 and pK_B values obtained on rat lung strip for β-adrenoceptor antagonists using the agonists isoprenaline, salbutamol and noradrenaline.

β-adrenoceptor antagonist	Isoprenaline as agonist		Salbutamol as agonist		Noradrenaline as agonist	
	pA_2	pK_B	pA_2	pK_B	pA_2	pK_B
Propranolol	7·86±0·22 (6)	7·79±0·07 (17)	7·72±0·15 (6)	7·75±0·03 (18)	7·89±0·23 (7)	7·78±0·07 (20)
Atenolol	5·19±0·05 (52)	5·39±0·11 (14)	5·33±0·07 (5)	5·34±0·06 (15)	5·47±0·22 (5)	5·49±0·12 (14)
Butoxamine	6·31±0·11 (5)	6·10±0·07 (15)	6·34±0·03 (6)	6·38±0·04 (16)	5·99±0·23 (6)	6·00±0·07 (18)

Results show mean values ± s.e.
Number of data points are given in parentheses.

Discussion

In a previous study, we found that noradrenaline was 150 times less potent than isoprenaline and about 29 times less potent than salbutamol on loaded rat lung strip, which indicates that β_2-adrenoceptors are the predominant receptor subtype mediating β responses in this tissue (Candenas et al 1986). If one adheres to the method described by O'Donnell & Wanstall (1979, 1981), to establish whether the β-adrenoceptor population in a tissue is homogeneous or heterogeneous, Schild plots for selective antagonists with agonists of different selectivity must be obtained to assess whether these plots for a determined antagonist are superimposed (homogeneous population) or separated (heterogeneous population).

In our study, the Schild plots obtained for propranolol are superimposed and the pA_2 values do not vary with the agonist employed, as could be expected for a non-selective antagonist regardless of the β-adrenoceptor agonist used and the β-adrenoceptor population in a tissue. However, the pA_2 values for propranolol (Table 2), were lower than usual, comparing with previous estimates of the pA_2 values for this antagonist in other respiratory tissues (Siegl et al 1979; O'Donnell & Wanstall 1979; Goldie et al 1983).

When the β_1-selective antagonist atenolol was used, the Schild plots obtained with the agonists isoprenaline (non-selective), salbutamol (β_2-selective) and noradrenaline (plus phentolamine) (β_1-selective) were in a similar location and none of the slopes of these plots are significantly different from unity, even when isoprenaline was used as agonist. Furthermore, the pA_2 values calculated for atenolol (5·19, 5·33 and 5·47) were very similar to those found for this antagonist on β_2-adrenoceptor in other tissues (O'Donnell & Wanstall 1983; Goldie 1984; Hartley & Pennefather 1985). In contrast, pA_2 values determined for atenolol on β_1-adrenoceptors ranged from 6·78 to 7·37 (O'Donnell & Wanstall 1983).

The Schild plots obtained with butoxamine, a β_2-selective antagonist, were also similar in location and the pA_2 values (6·31, 6·34 and 5·99) are consistent with those reported for this antagonist when acting on β_2-adrenoceptor (Kenakin 1982).

In conclusion, the results obtained in the present study are consistent with the presence, in rat lung strip, of an apparently homogeneous population of adrenoceptors of the β_2-subtype. However, the recent use of the highly β_1-selective agonist procaterol has demonstrated the presence of a minor population of β_2-adrenoceptors in tissues previously described as β_1-homogeneous (Johansson & Persson 1983; O'Donnell & Wanstall 1985). Thus, our conclusions may need to be modified, when highly β_1-selective adrenoceptor agonists are available to us as an alternative to noradrenaline.

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