Br. J. Pharmac. (1981), **74**, 547–552

DEMONSTRATION OF BOTH β_1- AND β_2-ADRENOCEPTORS MEDIATING RELAXATION OF ISOLATED RING PREPARATIONS OF RAT PULMONARY ARTERY

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1 Cumulative concentration-response (relaxation) curves to three β-adrenoceptor agonists, fenoterol (β_2-selective), isoprenaline (non-selective) and noradrenaline (β_1-selective) were obtained on isolated ring preparations of rat pulmonary artery contracted with 15 mM KCl. α-Adrenoceptors and neuronal and extraneuronal uptakes were blocked with phenoxybenzamine. The agonist concentration-response curves were reproducible.

2 Responses to each of the three agonists could be blocked by the β-adrenoceptor antagonists atenolol (β_1-selective) or ICI 118,551 (β_2-selective) confirming the presence of β-adrenoceptors.

3 The relative potencies of the agonists were isoprenaline : fenoterol : noradrenaline = 100 : 38 : 1.4. This indicated that the predominant β-adrenoceptor type was β_2.

4 Schild plots were obtained for atenolol and ICI 118,551 using the three different agonists. For each antagonist the location of the Schild plot varied depending on which agonist was used. This indicated that the β-adrenoceptor population mediating relaxation responses to β-adrenoceptor agonists was not homogeneous.

5 Atenolol was most potent when noradrenaline was the agonist and ICI 118,551 was most potent when fenoterol was the agonist.

6 It is concluded that isolated pulmonary artery ring preparations of the rat contain a mixed population of β_1- and β_2-adrenoceptors both mediating relaxation.

Introduction

When β-adrenoceptors were subdivided into β_1 and β_2 subtypes, vascular β-adrenoceptors were classified as β_2 (Lands, Arnold, McAuliff, Luduena & Brown, 1967; Lands, Luduena & Buzzo, 1967). Subsequently, some authors proposed that the β-adrenoceptors in blood vessels might represent a third subtype (Wasserman & Levy, 1974; Wardell, Colella, Shetzline & Fowler, 1974) although others found this further subdivision unnecessary (O'Donnell & Wanstall, 1976). Also, the β-adrenoceptor type in some blood vessels has subsequently been classified as β_1, viz. in the coronary blood vessels (Baron, Speden & Bohr, 1972; de la Lande, Harvey & Holt, 1974) and in some cerebral blood vessels (Edvinsson & Owman, 1974). These, and most other studies which have attempted to classify vascular β-adrenoceptors, have used relative potency values of selective agonists or selective antagonists.

Recently, a mixture of both β_1- and β_2-adrenoceptors has been demonstrated in various non-vascular preparations (Carlsson, Ablad, Brandstrom & Carlsson, 1972; Furchgott, 1976; O'Donnell & Wanstall, 1979a,b) and Furchgott (1976) predicted that this might also be found in vascular preparations. Determination of relative potency values would reveal only the predominant β-adrenoceptor type present and a different approach is needed to demonstrate a mixed β-adrenoceptor population. So far the only isolated blood vessels in which a mixed β-adrenoceptor population has been demonstrated are rat jugular and rat portal veins (Cohen & Wiley, 1978). The present study has been carried out on a rat artery preparation, the pulmonary artery, a blood vessel in which the β-adrenoceptor subtype(s) has not previously been classified. The approach used to characterize the adrenoceptor population was that previously used to demonstrate the presence of both β_1- and β_2-adrenoceptors in guinea-pig trachea (Furchgott, 1976; O'Donnell & Wanstall, 1979b) and in the atria from some but not all species (O'Donnell & Wanstall, 1979a; Bryan, Cole, O'Donnell & Wanstall, 1981).

0007-1188/81/110547-06 $01.00

Methods

Rat isolated pulmonary artery preparation

Male Wistar rats, 5 to 8 weeks old (130 g to 210 g) were used throughout the study. Rats of this age range were chosen because Fleisch & Hooker (1976) showed that isoprenaline responses of rat arteries were greatest in young animals. The rats were pretreated with reserpine (1 mg/kg i.p. 18–24 h beforehand), to avoid possible complications from the release of endogenous catecholamines, and were killed by a blow on the head. A small ring segment (1–3 mm in length) of the main pulmonary artery was immediately removed and set up around two horizontal stainless steel pins (one fixed and one linked to a Statham Universal transducer, UC3 + UL5) in an organ bath containing Krebs solution at 37°C gassed with 95% O_2 and 5% CO_2. The resting tension of the preparation was adjusted to 1 g and the preparation was left to equilibrate for a period of 1 h. Preparations were treated with phenoxybenzamine (50 μM for 30 min followed by a 20 min period of thorough washing) to block α-adrenoceptors, neuronal and extraneuronal uptakes. This treatment caused complete α-adrenoceptor blockade for at least 5 h after removal of the phenoxybenzamine. At the conclusion of the equilibration period the preparations were contracted by the addition of 15 mM KCl (i.e. total bath concentration of KCl was 20.9 mM) which produced a submaximal and sustained contraction.

The K^+-contracted preparations relaxed on addition of isoprenaline, fenoterol or noradrenaline and cumulative concentration-response curves could be obtained. At the end of each concentration-response curve a supramaximal concentration of isoprenaline was added. Responses to the agonists were calculated as a percentage of this maximum isoprenaline relaxation and plotted against log concentration agonist. The EC_{50}, i.e. concentration producing 50% of the maximum response, was then interpolated.

Effects of β-adrenoceptor antagonists

Concentration-response curves to isoprenaline, fenoterol or noradrenaline were obtained in the absence (control) and presence of increasing concentrations of either atenolol (β_1-selective antagonist) or ICI 118,551 (β_2-selective antagonist). Neither of these antagonists, in the concentrations used, caused any alteration to the K^+-induced contraction. Two (or occasionally three) concentrations of antagonist were used on any one preparation and the antagonist contact time was 60 min. Values of concentration ratio (CR) were obtained by dividing the EC_{50} in the presence of the antagonist by the EC_{50} in the control curve. Separate experiments were carried out in which the agonist concentration-response curves were repeated at 1 h intervals in the absence of antagonist to determine whether there was any change in sensitivity of the preparations due to time and/or pre-exposure to the agonist. Preparations did not change in sensitivity to either isoprenaline or fenoterol but there was a progressive decline in sensitivity to noradrenaline (1.2 fold in the second curve and a further 1.2 fold in the third curve). Therefore, as advocated by Furchgott (1972), the CR values obtained when noradrenaline was the agonist were corrected to allow for this change in sensitivity as described by O'Donnell & Wanstall (1979b). Plots of log molar antagonist concentration, (log [B]), against log (CR − 1) were obtained as proposed by Arunlakshana & Schild (1959). A linear least squares regression analysis (Snedecor & Cochran, 1967) was used to obtain the line of best fit, using the combined data points from a number of animals, and this line is referred to as a Schild plot. If a Schild plot had a slope which was not significantly different from 1.0, a pA_2 value for the antagonist was calculated from the formula $pA_2 = \log (CR - 1) - \log [B]$ as described by O'Donnell & Wanstall (1979b).

Drugs and solutions

Drugs used were:- atenolol (I.C.I.), fenoterol hydrobromide (Boehringer Ingelheim), ICI 118,551 hydrochloride (erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol, I.C.I.), (±)-isoprenaline sulphate (Sigma), (−)-noradrenaline acid tartrate (Sigma), phenoxybenzamine hydrochloride (Smith, Kline and French), phentolamine methanesulphonate (Regitine, Ciba) and reserpine (Serpasil, Ciba). Phentolamine and reserpine were obtained as solutions in ampoules. All other drugs were obtained as pure powders. Stock solutions (10 or 100 mM) of atenolol, fenoterol, ICI 118,551, isoprenaline and noradrenaline were made up in 10 mM HCl. Stock solutions (100 mM) of phenoxybenzamine were prepared in absolute ethanol containing 10 mM HCl. Dilutions of all drugs were made in Krebs solution and kept on ice during the course of the experiment.

The composition of the Krebs solution was (mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.7, ascorbic acid 1.1.

Statistical analyses

All mean values are quoted together with standard errors (s.e.). Comparisons have been made using paired *t* test.

Table 1 Mean negative log EC_{50} values and relative potency values for three β-adrenoceptor agonists on rat pulmonary artery contracted with KCl (15 mM)

Agonist	*Mean neg. log EC_{50} value*	*Relative potency*[a]
(±)-Isoprenaline (Iso)	7.58 ± 0.05 (19)[b]	100
(±)-Fenoterol	7.16 ± 0.07 (18)	38
(−)-Noradrenaline	6.03 ± 0.07 (20)	1.4[c]

[a]Relative potency = antilog (mean neg. log EC_{50} agonist − mean neg. log EC_{50} Iso) × 100.
[b]Number of preparations.
[c]This value is one half of the experimental value and so allows for the fact that (−)-noradrenaline was used whereas (±)-isoprenaline and (±)-fenoterol were used.

Results

Relative potencies of β-adrenoceptor agonists

The first addition of 15 mM KCl to rat pulmonary artery preparations caused an increase in tension ranging from 104 to 580 mg (314.9 ± 15 mg, $n = 57$). A cumulative concentration-response (relaxation) curve could then be obtained to a β-adrenoceptor agonist. The mean maximum relaxation to isoprenaline was 96.9% (s.e.mean 1.7, $n = 57$) of the magnitude of the KCl contraction. Papaverine (1 mM) produced a slightly greater relaxation response. Maximum relaxations to noradrenaline and fenoterol were between 90 and 100% of that to isoprenaline. The potencies (mean negative log EC_{50} values) and relative potencies of isoprenaline, fenoterol and noradrenaline, were calculated from the control concentration-response curves and are summarized in Table 1.

Schild plots for β-adrenoceptor antagonists

The Schild plots for ICI 118,551 and atenolol were in different locations depending upon the agonist used (Figure 1). ICI 118,551 was most potent against fenoterol and least potent against noradrenaline. Atenolol was most potent against noradrenaline and least potent against fenoterol. For each antagonist the Schild plot with isoprenaline as agonist was located between the plots with fenoterol and noradrenaline. The slopes and pA_2 values relating to these Schild plots are summarized in Table 2.

Table 2 Slopes of Schild plots and pA_2 values for ICI 118,551 and atenolol on rat pulmonary artery

Antagonist	*Agonist*	*Slope of Schild plot* ± s.e.	*Mean pA_2* ± s.e.
ICI 118,551	Fenoterol	0.83 ± 0.12 (12)[a]	9.16 ± 0.09 (6)[b]
	Isoprenaline	0.65 ± 0.06*** (17)	c
	Noradrenaline	1.23 ± 0.14 (16)	7.31 ± 0.10 (7)
Atenolol	Fenoterol	1.08 ± 0.22 (12)	5.03 ± 0.11 (7)
	Isoprenaline	0.99 ± 0.13 (11)	5.54 ± 0.11 (5)
	Noradrenaline	0.64 ± 0.05*** (15)	c

[a]Number of data points
[b]Number of animals
[c]pA_2 values not calculated because the slope of the Schild plot was significantly less than 1.0. Extrapolation of these Schild plots to log (CR − 1) = 0 would give pA_2 values of 8.99 for ICI 118,551 and 6.59 for atenolol.
***Slope of Schild plot significantly less than 1.0; $P < 0.001$.

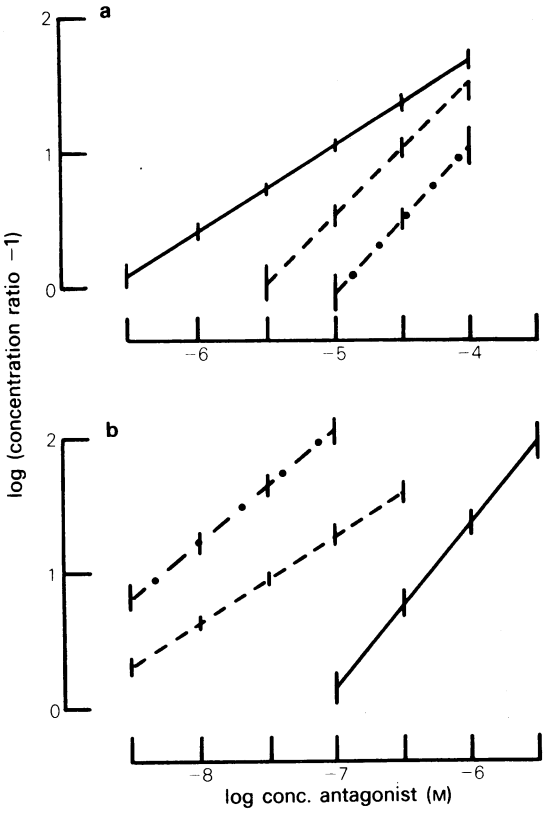

Figure 1 Schild plots for (a) atenolol and (b) ICI 118,551 on rat isolated pulmonary artery ring preparations using fenoterol (-·-·-·), isoprenaline (----) or noradrenaline (——) as agonist. The plots are calculated regression lines of best fit through data points from a number of animals. The vertical bars represent the s.e. of the estimated values of log (CR − 1) at those points corresponding to the antagonist concentrations used.

Discussion

The experiments described in this paper have demonstrated that relaxation responses to β-adrenoceptor agonists can be obtained on isolated ring preparations of rat pulmonary artery contracted with KCl. The responses were shown to be blocked by two β-adrenoceptor antagonists, ICI 118,551 and atenolol, confirming that the relaxation responses were mediated by β-adrenoceptor stimulation. Fleisch & Hooker (1976) had observed relaxation responses to isoprenaline on rat pulmonary artery preparations. They assumed that the responses were the result of β-adrenoceptor stimulation and, since the present study commenced, Chand & Altura (1980) have confirmed this. However, neither group of workers investigated the β-adrenoceptor subtype(s) involved.

The relative potencies of isoprenaline, fenoterol and noradrenaline on rat pulmonary artery (100: 38: 1.4) were very similar to those previously obtained on guinea-pig trachea (100: 37: 2.5, calculated from the potency data of O'Donnell & Wanstall, 1979b). Thus, on both guinea-pig trachea, which is considered to contain predominantly but not exclusively β_2-adrenoceptors (Furchgott, 1976; O'Donnell & Wanstall, 1979b) and on rat pulmonary artery (this study) fenoterol was much more potent than noradrenaline. In contrast, on a tissue known to contain predominantly, but not exclusively, β_1-adrenoceptors (cat atria), fenoterol and noradrenaline were equipotent (O'Donnell & Wanstall, 1979a) and in two tissues believed to contain only β_1-adrenoceptors (rat and guinea-pig atria, rate responses) fenoterol was less potent than noradrenaline (O'Donnell & Wanstall, 1979a,b; Bryan *et al.*, 1981). Thus the relative potency values of the β-adrenoceptor agonists demonstrated that the predominant subtype of β-adrenoceptor in rat pulmonary artery was β_2 but it was not possible from that data to conclude whether only β_2-adrenoceptors were present.

The difference in potency between isoprenaline and noradrenaline (viz. 71 fold) observed on rat pulmonary artery was not as great as the 300 fold difference which Furchgott (1976) postulated was indicative of a purely β_2-adrenoceptor population in a tissue, suggesting that β_1-adrenoceptors might also be involved. Definitive evidence for this was obtained from further experiments in which Schild plots were obtained for two selective antagonists, using fenoterol (β_2-selective) and noradrenaline (β_1-selective) as agonists. This approach has been used in other tissues (O'Donnell & Wanstall, 1979a,b; Bryan *et al.*, 1981) and has recently been substantiated (Wanstall & O'Donnell, 1980). For each of the antagonists, ICI 118,551 (β_2-selective, O'Donnell & Wanstall, 1980) and atenolol (β_1-selective, O'Donnell & Wanstall, 1979b), the Schild plots on rat pulmonary artery were in different locations depending on the agonist used. If rat pulmonary artery had contained only β_2-adrenoceptors, i.e. a homogenous β-adrenoceptor population, then, for either of these selective antagonists, the Schild plots would have been superimposed whatever the agonist used. The separation of the Schild plots when using β-adrenoceptor agonists of differing selectivity was evidence for a mixture of β_1- and β_2-adrenoceptors mediating the relaxation response (O'Donnell & Wanstall, 1979a,b, 1980; Bryan *et al.*, 1981).

Some of the Schild plots obtained on rat pulmonary artery had slopes which were less than the theoretical value of 1.0. These low slopes were not

peculiar to one particular antagonist or to one particular agonist. They only occurred when the antagonist selective for β_2-adrenoceptors (ICI 118, 551) was used in conjunction with the agonists acting predominantly through β_2-adrenoceptors (isoprenaline or fenoterol) or when the antagonist selective for β_1-adrenoceptors (atenolol) was used in conjunction with the agonist acting predominantly through β_1-adrenoceptors (noradrenaline). These observations were compatible with the prediction made by Furchgott (1976) for tissues in which the response was mediated by a mixed receptor population. In tissues with a homogeneous receptor population the slope of the antagonist Schild plots should be 1.0, irrespective of the agonist, provided that the antagonism is purely competitive (Arunlakshana & Schild, 1959) and the experimental conditions are properly controlled (Furchgott, 1972). Thus, it could be argued that the low slopes obtained for some of the Schild plots in the present study provided additional, albeit indirect, support for the conclusion that the β-adrenoceptor population being studied in the rat pulmonary artery preparation was not homogeneous.

In conclusion, the present study has not only confirmed the presence of β-adrenoceptors in rat pulmonary artery but has demonstrated for the first time the presence of both β_1 and β_2-adrenoceptor subtypes in an isolated artery preparation or in a pulmonary blood vessel. Although there have been previous reports on the presence of both β_1 and β_2-adrenoceptors in e.g. the renal vascular bed (Tairi, Yabuuchi & Yamashita, 1977) and in the vasculature supplying adipose tissue (Belfrage, 1978), the different β-adrenoceptor subtypes could have been in different blood vessel types. The demonstration of β_1 as well as β_2-adrenoceptors in isolated preparations of rat pulmonary artery (present study), rat jugular vein and rat portal vein (Cohen & Wiley, 1978) appears to substantiate the view of Furchgott (1976) that appropriate experimentation may reveal a subpopulation of β_1-adrenoceptors in blood vessels hitherto thought to contain only β_2-adrenoceptors.

This work was supported by a grant from the National Health and Medical Research Council of Australia. One of us (J.C.W.) is a NH & MRC Senior Research Officer. The excellent technical assistance of Miss Jane Baxter is gratefully acknowledged. We are grateful to Dr J.D. Fitzgerald, I.C.I. Ltd, Pharmaceutical Division, Macclesfield, U.K. for generously providing the compound ICI 118,551.

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(*Received February 18, 1981.*
Revised June 17, 1981.)