Br. J. Pharmac. (1985), **84**, 227–235

Responses to the β_2-selective agonist procaterol of vascular and atrial preparations with different functional β-adrenoceptor populations

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1 Relaxant responses to the β-adrenoceptor agonist, procaterol, have been examined on preparations of guinea-pig pulmonary artery (β_2-adrenoceptors only), rat and rabbit pulmonary artery and rat aorta ($\beta_2 > \beta_1$), and these responses have been compared with responses of dog left circumflex coronary artery (β_1 only).

2 Low concentrations of procaterol (3 nM to 100 nM) relaxed KCl-contracted preparations of rat aorta and pulmonary artery from rat, rabbit and guinea-pig whereas high concentrations ($> 1\ \mu$M) were required to relax preparations of the dog left circumflex coronary artery.

3 The dissociation constant (K_P value) for procaterol on β_1-adrenoceptors was 4.9 μM (determined on dog coronary artery) and on β_2-adrenoceptors was 0.008 μM (rabbit pulmonary artery). Procaterol therefore had a $\beta_2 : \beta_1$ selectivity value of 612.

4 K_P values obtained on guinea-pig atria for procaterol, on which the concentration-response curve was biphasic, confirmed that both β_2- and β_1-adrenoceptors mediate responses of this tissue. The K_P values were 0.009 μM (data from the first phase of the control concentration-response curve) and 3.5 μM (data from the concentration-response curve in the presence of the β_2-selective antagonist, ICI 118,551, 10 nM).

5 Data obtained on rat atria indicated that chronotropic responses of preparations from some rats, but not others, involved a minor population of β_2-adrenoceptors, but the β_2-adrenoceptors, when present, were less important than in guinea-pig atria.

6 Procaterol appears to be a particularly useful drug for detecting a functional population of β_2-adrenoceptors in tissues, whether they are the minor or the predominant receptor sub-type present.

Introduction

The β_2-selective adrenoceptor agonists commonly used in pharmacological β-adrenoceptor classification studies (e.g. fenoterol, terbutaline, salbutamol) have only a moderate degree of selectivity, e.g. the $\beta_2 : \beta_1$ selectivity of fenoterol, as assessed in functional studies on isolated tissue preparations, is only about 20 fold (O'Donnell & Wanstall, 1981a). Another β_2-selective agonist, procaterol (previously known as OPC 2009; Yabuuchi, 1977), has not been widely employed in functional studies designed to characterize β-adrenoceptor populations, although it has been used in a number of radioligand binding studies (Minneman *et al.*, 1979; Hedberg *et al.*, 1980; Dickinson & Nahorski, 1981). However this drug was recently examined on guinea-pig right and left atrial preparations (predominantly β_1-adrenoceptors), and the data revealed a minor population of β_2-adrenoceptors mediating responses of these preparations (Johansson & Persson, 1983). The β_2-adrenoceptors mediating chronotropic and inotropic responses of guinea-pig atria had not been observed in earlier studies (O'Donnell & Wanstall, 1979; 1981a; Zaagsma *et al.*, 1979), and Johansson & Persson (1983) suggested that agonists (e.g. procaterol) which were more selective than those used in the earlier studies were needed to demonstrate the minor population of β_2-adrenoceptors in this tissue. Experiments with procaterol have subsequently shown that, in contrast to guinea-pigs, there was no minor population of β_2-adrenoceptors in rabbit atria (chronotropic responses; Costin *et al.*, 1983). In the present study, the effects of procaterol have been

examined on rat atria, another tissue in which chronotropic responses have previously been described as involving only β_1-adrenoceptors (Bryan *et al.*, 1981).

This paper also describes the responses to procaterol of some isolated vascular preparations, namely the pulmonary artery from guinea-pig, rat and rabbit, and rat aorta. Procaterol has not previously been examined on these vessels, although it was used on isolated preparations of dog left circumflex coronary artery to confirm that the relaxation of this vessel did not involve any β_2-adrenoceptors (O'Donnell & Wanstall, 1984a). In contrast to dog coronary artery (β_1-adrenoceptors only), the rat pulmonary artery and aorta were already known to contain predominantly β_2-adrenoceptors (O'Donnell & Wanstall, 1981b; 1984b), but it was necessary to characterize the β-adrenoceptor population of the guinea-pig and rabbit pulmonary arteries in the present study. Data obtained on these blood vessel preparations and on guinea-pig atrial preparations have allowed us to obtain an estimate of the $\beta_2 : \beta_1$ selectivity of procaterol, from functional studies, and to compare this estimate with values reported in the literature from radioligand binding studies.

A preliminary account of some of these data was presented to the 5th meeting on Adrenergic Mechanisms, Porto, October 1983 (O'Donnell & Wanstall, 1984c).

Methods

Female guinea-pigs (270 to 480 g), male rats (90 to 260 g) and rabbits of either sex (1.3 to 1.6 kg) were used. All animals were pretreated with reserpine (1 mg kg^{-1} i.p. 18–24 h before the experiment) to minimize possible complications due to the release of endogenous catecholamines.

Blood vessel preparations

Isolated ring preparations of main pulmonary artery from the rabbit, rat or guinea-pig and aorta from the rat were set up as described by O'Donnell & Wanstall (1981b), under a resting tension of 1.0 g (rat pulmonary artery and aorta) or 1.5 g (rabbit and guinea-pig pulmonary arteries). Preparations were pre-exposed to phenoxybenzamine (50 μM for 30 min followed by washout) and contracted with KCl. The concentration of KCl added to the organ bath was 15 mM, giving a final concentration of 20.9 mM, with the exception of the Schild plot experiments (*vide supra*) on guinea-pig pulmonary artery, when 20 mM KCl was added to the organ bath. Cumulative concentration-response curves to isoprenaline and procaterol were obtained. The procaterol concentration range was 3 nM to 2 μM (4 fold increments). Responses were expressed as a percentage of the maximum response to isoprenaline, which was determined at the completion of each procaterol concentration-response curve. The maximum response to procaterol, expressed as a fraction of the maximum response to isoprenaline, is termed intrinsic activity. The maximum relaxation to isoprenaline expressed as a percentage of the KCl-induced contraction is termed responsiveness.

Atrial preparations

Spontaneously beating atrial preparations from guinea-pig and rat were set up in Krebs solution at 37°C aerated with 95% O_2 and 5% CO_2. Atrial rate was recorded as described by O'Donnell & Wanstall (1979). Preparations were pre-exposed to phenoxybenzamine (50 μM for 30 min followed by thorough washing in phenoxybenzamine-free Krebs solution) to block α-adrenoceptors, neuronal uptake and extraneuronal uptake. A cumulative concentration-response curve to isoprenaline using 10 fold increments in concentration, was obtained first. After washing the tissue, this was followed by a cumulative concentration-response curve to procaterol (concentration range 1 nM to 100 μM, using 2 fold increments in concentration). At the end of the procaterol concentration-response curve a maximum response to isoprenaline was obtained and all responses were expressed as a percentage of this maximum response. Intrinsic activity of procaterol is as defined above for blood vessel preparations.

Schild plots for atenolol on pulmonary artery preparations

Concentration-response curves to noradrenaline (β_1-selective agonist) or fenoterol (β_2-selective agonist) were obtained in the absence and presence of increasing concentrations of atenolol (60 min contact time). Maximum responses to noradrenaline and fenoterol were the same as the maximum response to isoprenaline, and the concentration giving 50% of the maximum response to isoprenaline (EC_{50}) was determined from each concentration-response curve. EC_{50} values were used to calculate values of concentration ratio (CR) i.e. EC_{50} in the presence of antagonist divided by EC_{50} in the absence of antagonist. In the absence of the antagonist, repeated agonist concentration-response curves were shown to be reproducible. Plots of log $(CR - 1)$ versus log molar concentration of antagonist [B] were obtained. A linear least squares regression analysis (Snedecor & Cochran, 1967) was used to obtain the line of best fit through the combined data points from a number of animals. These plots are a modification of the plot

proposed by Arunlakshana & Schild (1959) for the determination of pA_x values, and, for convenience, are referred to as Schild plots. pA_2 values were obtained by extrapolation of the Schild plots to log $(CR-1)=0$. The slopes of the Schild plots were not significantly different from 1.0. Therefore, assuming a slope of unity, pK_B values were calculated, for each concentration of atenolol used, from the equation $pK_B = \log (CR-1) - \log [B]$ (Mackay, 1978). A mean pK_B value for each preparation was obtained and these were used to calculate a mean $pK_B \pm$ s.e. from preparations from a number of animals.

Determination of K_P values for procaterol

The dissociation constant (K_P) for the partial agonist, procaterol, on β-adrenoceptors in the various tissues was determined by obtaining equiactive concentrations of procaterol [P] and the full agonist, isoprenaline, [A]. The method used was that advocated by Kenakin & Beek (1980) and is based on the following equation derived from occupation theory:

$$[A] = \frac{e_P K_A}{e_A - e_P} - \frac{[A]}{[P]} \times \frac{e_A}{e_A - e_P} \times K_P$$

K_A and e_A are the dissociation constant and efficacy, respectively, of the full agonist and K_P and e_P are the corresponding values for the partial agonist. Plots of [A] versus [A]/[P] were obtained. The slope of this line yields an estimate of K_P, provided $e_A \gg e_P$. In determining the K_P value in a tissue in which isoprenaline acts on both β_1- and β_2-adrenoceptors (e.g. rabbit pulmonary artery), it has been assumed that the dissociation constant and intrinsic efficacy of isoprenaline are not different on β_1- and β_2-adrenoceptors, respectively. The available evidence suggests that this is an acceptable assumption (see Kenakin, 1982).

Drugs and solutions

The following drugs were used: atenolol (I.C.I.); fenoterol hydrobromide (Boehringer–Ingelheim); ICI 118,551 (erythro-DL-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol; I.C.I.); (±)-isoprenaline sulphate (Sigma); (−)-noradrenaline acid tartrate (Sigma); phenoxybenzamine hydrochloride (Smith, Kline and French); procaterol hydrochloride (5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril; Warner–Lambert); reserpine (Serpasil ampoules, Ciba). Stock solutions (10 or 100 mM) of fenoterol, ICI 118,551 isoprenaline and noradrenaline were made up in 10 mM HCl, and of atenolol in de-ionized water. Procaterol (10 mM) was made up daily in Krebs solution and phenoxybenzamine was made up in absolute ethanol containing 10 mM HCl. Dilutions of all drugs were made in Krebs solution and kept ice-cold throughout 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.

Results

(i) Characterization of the β-adrenoceptor population mediating relaxation of guinea-pig and rabbit pulmonary arteries

On guinea-pig pulmonary artery, fenoterol (β_2-selective) was 129 times more potent than noradrenaline (β_1-selective). This indicated that the responses were mediated predominantly by β_2-adrenoceptors. There was no evidence for a minor population of β_1-adrenoceptors in that (a) the Schild plots for atenolol, using either fenoterol or noradrenaline as the agonist, were superimposed (Figure 1) and the pA_2 values for atenolol (Table 1) were within the range of previous estimates of the pA_2 values for atenolol on β_2- but not β_1-adrenoceptors (O'Donnell & Wanstall, 1983); and (b) isoprenaline was 438 times more potent than noradrenaline, a difference in potency which approaches the value of 600 predicted by Furchgott (1981) for a tissue containing only β_2-adrenoceptors.

On rabbit pulmonary artery fenoterol was also more potent than noradrenaline (4 fold), indicating that responses were mediated predominantly by β_2-

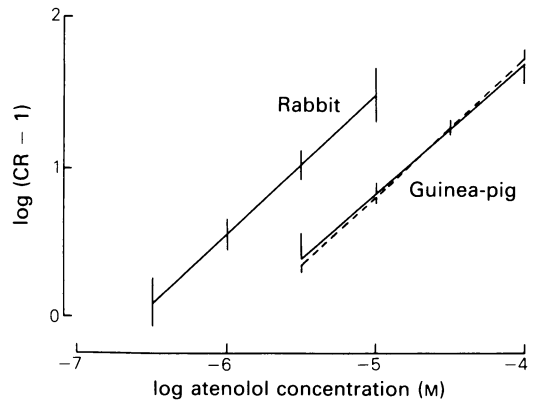

Figure 1 Schild plots for atenolol on pulmonary artery preparations from rabbit and guinea-pig using noradrenaline (——) or fenoterol (----) as agonist. The plots represent the calculated lines of best fit through the combined data from a number of animals. The vertical lines represent the s.e. of the estimated values of log (concentration ratio (CR) − 1) at points corresponding to the antagonist concentrations used.

Table 1 pA_2 and pK_B values and slopes of Schild plots for atenolol on preparations of pulmonary artery from guinea-pig and rabbit, using noradrenaline or fenoterol as agonists

Tissue	*Agonist*	*Slope of Schild plot*	pA_2[a]	pK_B[b]
Guinea-pig pulmonary artery	Fenoterol	0.92 ± 0.07 (8)[c]	5.88	5.81 ± 0.06 (4)[d]
	Noradrenaline	0.87 ± 0.15 (12)	5.95	5.82 ± 0.10 (8)
Rabbit pulmonary artery	Noradrenaline	0.94 ± 0.18 (9)	6.59	6.55 ± 0.11 (5)

[a] Obtained by extrapolation of Schild plot to log (CR − 1) = 0.
[b] pK_B = log (CR − 1) − log [B].
[c] Number of data points.
[d] Number of animals.
Results show mean values ± s.e.

adrenoceptors but, in contrast to guinea-pig, there was some evidence from Schild plot data that β_1- as well as β_2-adrenoceptors were involved in the response. Although it was not possible to obtain a Schild plot for atenolol using fenoterol as agonist (because of difficulties in obtaining more than one concentration-response curve to fenoterol on any one preparation), the Schild plot for atenolol, with noradrenaline as the agonist, was not superimposed on the Schild plots for atenolol on β_2-adrenoceptors,

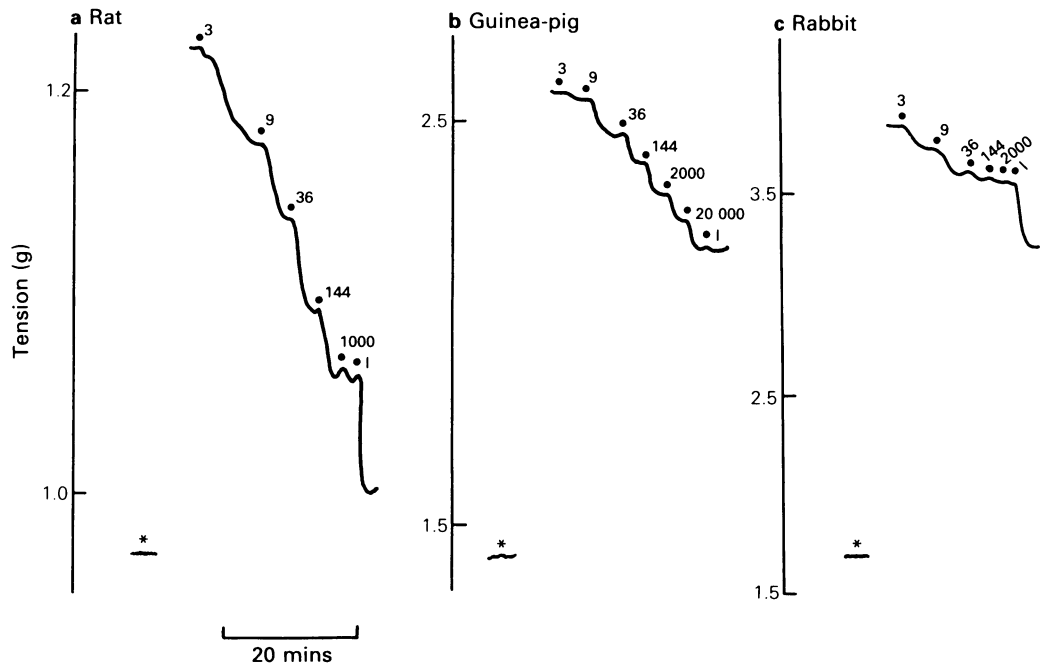

Figure 2 Responses to procaterol of pulmonary artery preparations from (a) rat, (b) guinea-pig and (c) rabbit. Preparations were pre-exposed to phenoxybenzamine (50 μM for 30 min followed by washout) and contracted with KCl (15mM). Procaterol concentrations are in nM. At I a supramaximal concentration of isoprenaline (10 μM) was added. * Shows the resting tension before addition of KCl. The mean responsiveness to isoprenaline (expressed as % of the KCl-induced contraction) on these pulmonary artery preparations was rat 92 ± 12.2% (n = 4); guinea-pig 38 ± 3.4% (n = 4); rabbit 38 ± 6.5% (n = 6). The value for rat aorta was 66 ± 19.0% (n = 4).

i.e. those obtained on guinea-pig pulmonary artery (*vide supra*). In fact the higher pA_2 value obtained for atenolol on the rabbit pulmonary artery (Table 1) approached previous estimates for the pA_2 of atenolol on β_1-adrenoceptors (O'Donnell & Wanstall, 1983).

(ii) Effects of procaterol on isolated blood vessel preparations

Responses of KCl-contracted pulmonary artery preparations from rat, guinea-pig and rabbit are shown in Figure 2. These blood vessels relaxed to procaterol concentrations in the range 3 nM to 1 μM (which was a maximal or supramaximal concentration). These data are in contrast to those obtained previously on dog left circumflex coronary artery preparations (O'Donnell & Wanstall, 1984a) on which 1 μM was the threshold concentration of procaterol (Figure 3). This difference between the procaterol concentrations which relaxed the vessels examined in the present study and those required to relax dog coronary artery, reflects the marked selectivity of procaterol between tissues containing predominantly β_2- and β_1-adrenoceptors, respectively. Procaterol was approximately equipotent on the different pulmonary arteries and on rat aorta. It was of comparable potency to isoprenaline on each of these vessels (see negative log EC_{50} values, Table 2), but the intrinsic activity ranged from 0.48 on rabbit pulmonary artery to >0.79 on the other three vessels studied (Figure 3).

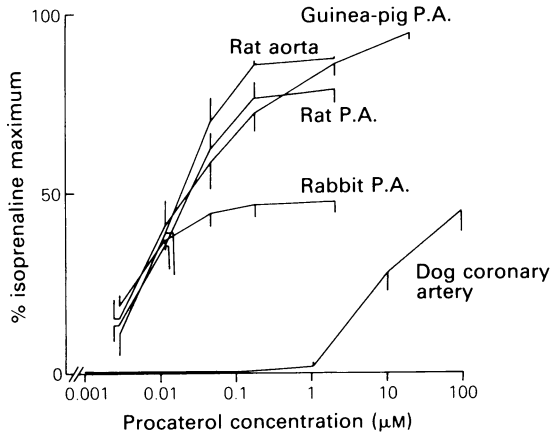

Figure 3 Mean concentration-response (relaxation) curves to procaterol on isolated ring preparations of pulmonary artery (P.A.) from rat ($n=4$), rabbit ($n=6$) and guinea-pig ($n=4$), aorta from rat ($n=4$) and left circumflex coronary artery from dog ($n=6$). Preparations were pre-exposed to phenoxybenzamine (50 μM for 30 min followed by washout) and contracted with KCl 15 mM or 20 mM (dog left circumflex coronary artery). Responses were expressed as a percentage of the maximum response to isoprenaline. The vertical lines represent the s.e. of the mean responses at each of the procaterol concentrations tested. The concentration-response curve on dog left circumflex coronary artery is based on data previously described by O'Donnell & Wanstall (1984a).

Table 2 Mean negative log EC_{50} values for isoprenaline and procaterol and maximum responses (intrinsic activity) to procaterol on isolated blood vessel preparations

Tissue	*β-Adrenoceptor population*	*− log EC_{50}[a]* *Isoprenaline*	*Procaterol*	*Intrinsic activity of procaterol*
Guinea-pig pulmonary artery	β_2	7.37 ± 0.05 (7)	7.65 ± 0.19 (4)	0.95 ± 0.02[d] (4)
Rabbit pulmonary artery	$\beta_2 > \beta_1$	7.74 ± 0.06 (7)	8.38 ± 0.06 (6)	0.48 ± 0.03[e] (6)
Rat pulmonary artery	$\beta_2 > \beta_1$	7.58 ± 0.05[b] (19)	7.84 ± 0.10 (4)	0.79 ± 0.04[e] (4)
Rat aorta	$\beta_2 > \beta_1$	7.32 ± 0.07 (7)	7.87 ± 0.17 (4)	0.88 ± 0.01[e] (4)
Dog left circumflex coronary artery	β_1	8.20 ± 0.13[c] (6)	< 5.2	> 0.45 ± 0.06[f] (6)

[a] EC_{50} is the concentration producing 50% of the maximum response to the particular agonist.
[b] O'Donnell & Wanstall (1981b).
[c] O'Donnell & Wanstall (1984a).
[d] Procaterol concentration used to produce maximum response = 20 μM.
[e] Procaterol concentration used to produce maximum response = 2 μM.
[f] Procaterol concentration used to produce maximum response = 100 μM.
Results shown are mean ± s.e. with numbers of animals in parentheses. The preparations were pre-exposed to phenoxybenzamine (50 μM for 30 min followed by washout) and contracted with KCl (15 mM or 20 mM).

On rat pulmonary artery, a concentration of 10 nM ICI 118,551 was shown to antagonize procaterol, and from these data a pK_B for ICI 118,551 of 9.56 was estimated. This value is characteristic of ICI 118,551 acting on β_2-adrenoceptors (O'Donnell & Wanstall, 1984b) and confirmed that the concentrations of procaterol which relaxed rat pulmonary artery were activating β_2-adrenoceptors.

(iii) Effects of procaterol on guinea-pig and rat atrial preparations (chronotropic responses)

Mean concentration-response curves to procaterol on spontaneously beating atrial preparations from guinea-pig and rat are illustrated in Figure 4. On guinea-pig atria the concentration-response curve was biphasic i.e. the data resembled those previously found by Johansson & Persson (1983). The initial phase of the curve occurred at low concentration (1 nM to 100 nM) of procaterol, and the second phase at high concentrations (1 μM to 100 μM). ICI 118,551 10 nM antagonized the responses to low concentration of procaterol, causing a shift in the first phase of the concentration-response curve of about 100 fold. This shift corresponds to a pK_B of about 10.00 which is characteristic of ICI 118,551 acting on β_2-adrenoceptors and indicates that the responses to low concentrations of procaterol are mediated by β_2-adrenoceptors. In the presence of 100 nM ICI 118,551 there was no further shift in the concentration-response curve to procaterol, indicating that the concentration-response curve to procaterol in the presence of 10 nM ICI 118,551 represented activation of β_1-adrenoceptors.

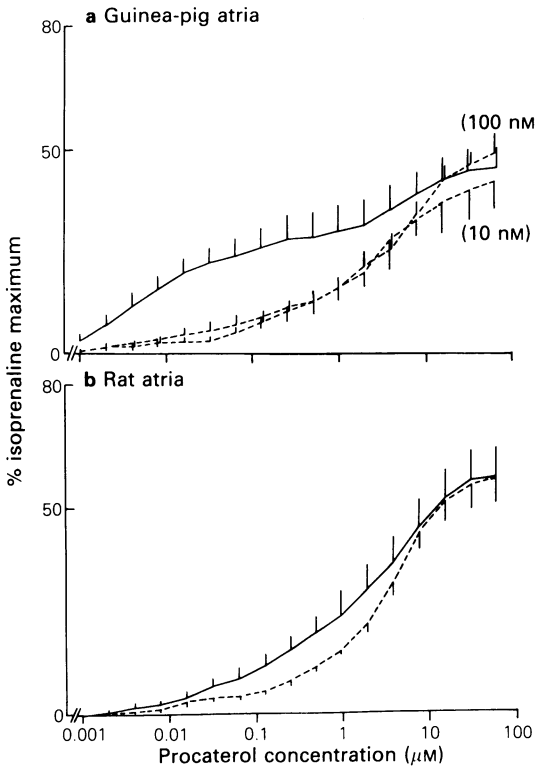

Figure 4 Mean concentration-response (rate) curves to procaterol on spontaneously beating atrial preparations from guinea-pig (a) and rat (b) in the absence (——, control) and presence (----) of ICI 118,551 10 nM (a and b) or 100 nM (a). Responses are expressed as a percentage of the maximum response to isoprenaline. The vertical lines represent the s.e. of the mean responses at each of the procaterol concentrations tested. The mean resting rates and maximum rates were (beats min^{-1}): guinea-pig ($n = 15$) 158 ± 4.7, 288 ± 3.3; rat ($n = 12$) 199 ± 8.2, 404 ± 11.8.

Three out of six individual preparations of rat atria gave negligible responses to low concentrations of procaterol (1 nM to 100 nM). The other 3 preparations responded to these concentrations, and biphasic concentration-response curves were obtained, but the responses to these concentrations were less than were seen on guinea-pig atria at the same concentrations. Because of the difference between individual preparations of rat atria, the mean concentration-response curve to procaterol was not obviously biphasic (Figure 4) and 10 nM ICI 118,551 produced only a small shift in the curve (Figure 4). The intrinsic activity of procaterol on rat atria was 0.57 ± 0.07 ($n = 6$) compared with 0.45 ± 0.05 ($n = 6$) on guinea-pig atria. These values represent the intrinsic activity of procaterol on β_1-adrenoceptors, since they correspond to maximum responses obtained with 60 μM procaterol (i.e. a concentration which activates β_1-adrenoceptors).

(iv) Estimation of the dissociation constants (K_P) for procaterol on β_1- and β_2-adrenoceptors

Estimates of K_P values for procaterol on β_1- and β_2-adrenoceptors are summarized in Table 3. The K_P value for β_1-adrenoceptors was obtained from data on dog coronary artery, and the K_P value for β_2-adrenoceptors was obtained from data on rabbit pulmonary artery. Meaningful values could not be calculated from data on guinea-pig or rat pulmonary artery or rat aorta because procaterol was almost a full agonist on those tissues. K_P values were also obtained on guinea-pig atria using (a) data from the first phase of the control concentration-response curve and (b) data from the concentration-response curve in the presence of 10 nM ICI 118,551 (Table 3). Table 3 indicates, also, the range of K_D values for procaterol which have been reported in the literature

Table 3 Estimated K_P values for procaterol on β_1- and β_2-adrenoceptors

Receptor sub-type	*Tissue*	K_P (μM)	K_D[a] (*range*; μM)
β_1	Dog coronary artery	4.9	2.1[d] to 9.8[e]
	Guinea-pig atria[b]	3.5	
β_2	Rabbit pulmonary artery	0.008	0.026[f] to 0.26[d]
	Guinea-pig atria[c]	0.009	

[a] Values reported in the literature.
[b] Obtained from concentration-response curve in presence of 10 nM ICI 118,551.
[c] Obtained from first phase of biphasic concentration-response curve.
[d] Minneman *et al.* (1979).
[e] Hedberg *et al.* (1980).
[f] Dickinson & Nahorski (1981).

from radioligand binding studies. From the K_P values on rabbit pulmonary artery and dog coronary artery, the $\beta_2:\beta_1$ selectivity of procaterol is 612.

Discussion

Data obtained with procaterol on blood vessels in the present study and in a previous study (O'Donnell & Wanstall, 1984a) have illustrated the high degree of $\beta_2:\beta_1$ selectivity of this drug. Relaxant responses of four blood vessel preparations in which β_2-adrenoceptors predominate (rat, guinea-pig and rabbit pulmonary artery and rat aorta) were obtained with concentrations of procaterol as low as 3 nM. In contrast, relaxant responses were seen on dog coronary artery only at concentrations above 1 μM (O'Donnell & Wanstall, 1984a) a concentration which caused a maximum response on the other blood vessels. The difference between the negative log EC_{50} values (see Table 2) on dog coronary artery (β_1-adrenoceptors) and on the other vessels (predominantly β_2-adrenoceptors) suggest a $\beta_2:\beta_1$ selectivity value for procaterol of between 100 and 1000. A selectivity of this order of magnitude was confirmed by using some of the data on blood vessels to obtain dissociation constants (K_P values) for procaterol on β_1- (dog coronary artery data) and β_2- adrenoceptors (rabbit pulmonary artery data). The K_P values obtained differed from one another by a factor of 612 and were in reasonable agreement with K_D values for procaterol cited in the literature from radioligand binding studies.

It was also possible to estimate K_P values for procaterol from the data obtained on guinea-pig atria in the present study. In the control biphasic concentration-response curves to procaterol, the lower concentration phase (shifted by 10 nM ICI 118,551) was assumed to represent β_2-adrenoceptor activation and the higher concentration phase (not shifted by 10 nM ICI 118,551) to represent β_1-adrenoceptor activation. The complete concentration-response curve in the presence of 10 nM ICI 118,551 can also be assumed to represent β_1-adrenoceptor activation. The latter assumption can be made on the basis of the known pA_2 values for ICI 118,551 on β_1- and β_2-adrenoceptors (O'Donnell & Wanstall, 1980; 1981b; 1984b), and the observations that curves in the presence of 10 and 100 nM ICI 118,551 were superimposed (i.e. 100 nM, which would block β_2- but not β_1-adrenoceptors, did not shift the curve further). The K_P values substantiated the above assumptions as the K_P value obtained from the data in the presence of 10 nM ICI 118,551 was in very good agreement with that obtained on dog coronary artery (β_1), and the K_P value obtained from the first phase of the biphasic control curve was in excellent agreement with that obtained in rabbit pulmonary artery (β_2). Thus the K_P values support the conclusion of Johansson & Persson (1983) that the biphasic concentration-response curve on guinea-pig atria does indeed reflect the involvement of both sub-types of β-adrenoceptor in the chronotropic response.

The data obtained with procaterol on rat atria in the present study were inconsistent, and therefore did not conclusively resolve whether β_2-adrenoceptors might contribute to chronotropic responses in this tissue, which have previously been described as involving only β_1-adrenoceptors (Bryan *et al.*, 1980). The data obtained on rat atria could best be interpreted if one concludes that there is variation between rats i.e. some atria, but not all, have a small population of β_2-adrenoceptors which can contribute to the tissue response. Nevertheless, even in rat atrial preparations which did respond to low concentrations (β_2) of procaterol the responses were small compared with those seen on guinea-pig atria,

whereas the maximum response to procaterol (intrinsic activity on β_1-adrenoceptors) was higher than in guinea-pig. Thus, the relative importance of β_2-adrenoceptor to β_1-adrenoceptor activation is less in rat than in guinea-pig. Since no β_2-adrenoceptors were detected, using procaterol, in rabbit atria (Costin *et al.*, 1983) and since the β_2-adrenoceptor population was large enough in cat atria to be detected with fenoterol, a drug much less selective than procaterol (O'Donnell & Wanstall, 1979), one can conclude that the comparative importance of β_2-adrenoceptors in chronotropic responses in atria appears to be cat > guinea-pig > rat > rabbit.

The experiments with the highly β_2-selective agonist procaterol require us to amend our previous view that only β_1-adrenoceptors are involved in the chronotropic responses of guinea-pig atria and they also emphasize that we can only tentatively conclude from the data in the present study that relaxation of guinea-pig pulmonary artery involves only β_2-adrenoceptors. The possibility remains that this conclusion may also need modification when we have highly β_1-selective adrenoceptor agonists available, as an alternative to noradrenaline. Nevertheless, at the present time, guinea-pig pulmonary artery (relaxant responses) can be added to the short list of tissues so far described in which responses appear to involve only β_2-adrenoceptors (i.e. no β_1-adrenoceptors). Other tissues in this category include the guinea-pig uterus and rat vas deferens (Krstew *et al.*, 1982).

In conclusion, the functional studies described in this paper have demonstrated that procaterol is a highly β_2-selective agonist, and have thus confirmed the findings of radioligand binding studies with this drug (Minneman *et al.*, 1979; Hedberg *et al.*, 1980). The study has shown that procaterol is so selective that there is virtually no overlap in the concentrations required to produce pharmacological responses via β_1- or β_2-adrenoceptors. This makes it a particularly useful drug for the rapid and reliable detection of β_2-adrenoceptors mediating a response. The results have suggested that any new tissue which responds to concentrations of procaterol between 1 and 100 nM probably contains some β_2-adrenoceptors. Determination of the dissociation constants for procaterol on guinea-pig atria have confirmed the findings of Johansson & Persson (1983) that atrial rate in this species can be mediated by β_2- as well as β_1-adrenoceptors, and data obtained in rat suggest that this may be so in some, but not all, atrial preparations from this species.

This work was supported by a grant from the National Health and Medical Research Council of Australia. J.C.W. is a NH and MRC Senior Research Officer. We would like to thank Miss Tina Greco for her excellent technical assistance. We are grateful to Dr B. Maguire of Parke-Davis, Australia, for providing procaterol.

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(Received July 31, 1984.
Revised September 7, 1984.
Accepted September 25, 1984.)