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pA_2 values of selective β-adrenoceptor antagonists on isolated atria demonstrate a species difference in the β-adrenoceptor populations mediating chronotropic responses in cat and guinea-pig

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pA_2 values for atenolol (β_1-selective) and α-methylpropranolol (β_2-selective) have been determined on isolated atria of cat and guinea-pig using noradrenaline (β_1-selective) and fenoterol (β_2-selective) as agonists. On guinea-pig atria, the pA_2 values did not vary with the agonist used. On cat atria the pA_2 for atenolol was greater with noradrenaline than with fenoterol and the pA_2 for α-methylpropranolol was greater with fenoterol than with noradrenaline. Fenoterol was 20 times more potent on cat than on guinea-pig atria whereas noradrenaline was approximately equipotent in the two species. The results have been interpreted as suggesting that both cat and guinea-pig atria contain one receptor type in common (β_1) but that only cat atria contain β_2-adrenoceptors as well.

When β-adrenoceptors were subdivided into β_1- and β_2-types (Lands et al 1967), responses of tissues to β-adrenoceptor agonists were described as being mediated via either β_1- or β_2-adrenoceptors but not both. More recently it has been suggested that tissues may contain a mixture of β_1- and β_2-adrenoceptors, both of which mediate the same response, but which are present in differing proportions in different tissues (Åblad et al 1975; Carlsson & Åblad 1976; Furchgott 1976). The potencies of β_2-selective agonist drugs, when related to that of the non-selective agonist isoprenaline, are known to differ on cat and guinea-pig heart preparations (Davey et al 1974; Malta & Raper 1975). One of the possible explanations for this species difference, is that the hearts of the two species might have different mixed populations of β_1- and β_2-adrenoceptors. The experiments described in this paper were designed to explore this possibility. When the study commenced no information was available on whether guinea-pig heart contained a mixed population of β-adrenoceptors or not. Åblad et al (1975), on the basis of experiments carried out with selective agonists and antagonists, had suggested that cat heart contained both β_1 and β_2-adrenoceptors. However, their experiments were carried out on whole perfused heart preparations and their experimental conditions were not optimal since neither extraneuronal uptake nor α-adrenoceptors were blocked. Moreover, their method of quantifying the data, i.e. percentage inhibition by the antagonists of standard agonist responses, did not provide any absolute values for the drugs which could be used for comparison of the receptor types in that tissue with those in other tissues or in other species.

Thus, in the present study selective antagonists and agonists have been used but experiments have been carried out on guinea-pig and cat isolated atria, rather than whole heart, and optimal experimental conditions (Furchgott 1972) identical for both species, were employed. In addition pA_2 values for the antagonist drugs have been obtained.

MATERIALS AND METHODS

Female guinea-pigs (350–550 g) and cats of either sex (1·6 to 5 kg) were pretreated with reserpine, intraperitoneally, 16–24 h before the experiments (5 mg kg^{-1} in guinea-pigs; 0·1 mg kg^{-1} in cats). Isolated preparations of atria (right atrium of cat and both atria of guinea-pig) were set up in Krebs solution containing 1·1 mM ascorbic acid, gassed with 5% CO_2 in oxygen and maintained at 37 °C. Atrial rate was recorded on a digital counter activated by the interruption of a light beam. Each tissue was treated with phenoxybenzamine (50μM for 30 min followed by thorough washout) to block neuronal and extra-neuronal uptakes and α-adrenoceptors. Neither cocaine nor pargyline caused any further potentiation of noradrenaline after phenoxybenzamine treatment in either cat or guinea-pig atria. Therefore these inhibitors were not included. Cumulative concentration-response lines to the agonists were obtained in the absence (control) and the presence of increasing concentrations of an antagonist drug. On guinea-pig

* Correspondence.

atria, two control lines were obtained and the results from the first one discarded, since the slope of the first line was frequently lower than that of subsequent lines. On cat atria this procedure was unnecessary. EC50 values (concentration producing 50% of the maximum response in each individual line) were interpolated and used to obtain the potency of the agonists (− ve log EC50 values) and also the agonist concentration ratios for each concentration of antagonist. pA_2 values were determined from plots of log (concentration ratio −1) vs log antagonist concentration (molar) according to the method of Arunlakshana & Schild (1959). These plots have been referred to as "Schild plots". Each "Schild plot" represented the line of best fit for the combined data from a number of experiments, calculated using a linear least squares regression analysis (Snedecor & Cochran 1967). pA_2 values are the negative log of the molar concentration of antagonist at the point where log (concentration ratio −1) equals zero. The contact time for the antagonist drugs was 60 min.

Drugs and solutions used. Atenolol (ICI); butoxamine hydrochloride (Burroughs Wellcome); fenoterol hydrobromide (Boehringer Ingelheim); α-methylpropranolol (ICI); noradrenaline acid tartrate (Sigma); phenoxybenzamine hydrochloride (Smith, Kline & French) and reserpine (Serpasil ampoules, Ciba). Stock solutions (10 or 100 mM) of atenolol, butoxamine, fenoterol and noradrenaline were made up in 0·01 M HCl and stock solutions of α-methylpropranolol in distilled water. A stock solution of phenoxybenzamine (100 mM) was made up in absolute ethanol containing 0·001 ml 10 M HCl ml^{-1}. Dilutions of all drugs were made in Krebs solution and kept ice-cold during the course of each experiment.

The composition of the Krebs solution (mM) was: 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

Mean control concentration-response lines to noradrenaline and fenoterol on atria of guinea-pig and cat are shown in Fig. 1. For noradrenaline there was only a 2 fold difference in potency between the two species (− ve log EC50 values; cat, 7·97 ± 0·09, n = 10, and guinea-pig 7·66 ± 0·05, n = 13). For fenoterol a 20 fold difference in potency was seen (cat, 7·81 ± 0·10, n = 11; guinea-pig, 6·51 ± 0·05, n = 14).

The pA_2 values for α-methylpropranolol and atenolol, shown in Table 1, were obtained from the

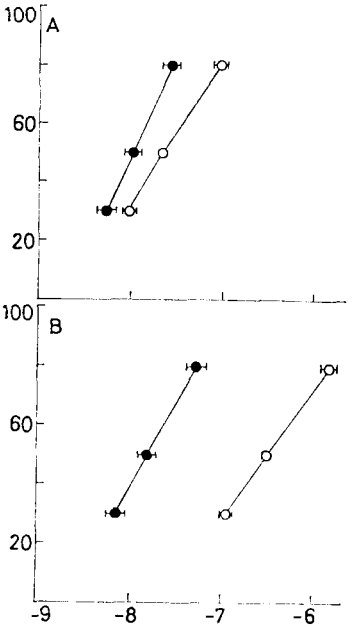

FIG. 1. Mean log concentration-response lines to (A) noradrenaline and (B) fenoterol obtained on cat (●) and guinea-pig (○) atria. The mean lines were plotted by the method of Langer & Trendelenburg (1969). Horizontal bars represent standard errors of the mean EC30, EC50 and EC80 values respectively. Ordinate: % maximum response. Abscissa: log agonist concentration (M).

Table 1. pA_2 values and slopes (±s.e.) of the 'Schild plots' for α-methylpropranolol and atenolol on atria of guinea-pig and cat using noradrenaline and fenoterol as agonists.

	Guinea-pig				Cat			
	Noradrenaline		Fenoterol		Noradrenaline		Fenoterol	
	pA_2	slope ± s.e.	pA_2	slope ± s.e.	pA_2	slope ± s.e.	pA_2	slope ± s.e.
α-Methylpropranolol	7·64	1·01 ± 0·09 (8, 16)*	7·69	0·95 ± 0·09 (7, 14)	7·44	0·96 ± 0·14 (5, 8)	8·00	0·99 ± 0·13 (5, 10)
Atenolol	7·02	1·08 ± 0·20 (5, 10)	7·18	0·90 ± 0·15 (7, 12)	7·03	0·92 ± 0·14 (5, 9)	5·81	0·90 ± 0·22 (6, 10)

* Numbers of animals and experimental points respectively are shown in parentheses.

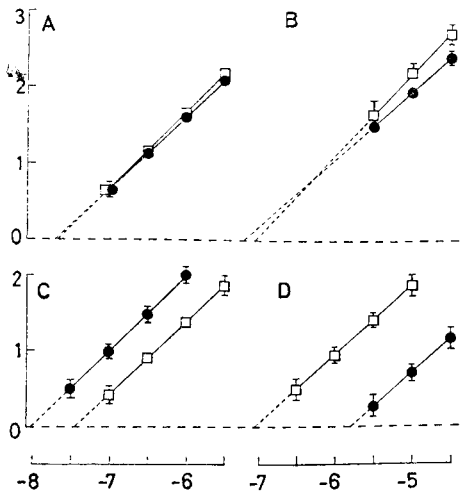

FIG. 2. "Schild plots" for the antagonism of noradrenaline (□) and fenoterol (●) by α-methylpropranolol (A & C) and atenolol (B & D) which were used to obtain pA_2 values. Results in A & B are from guinea-pigs and in C and D from cats. The lines represent lines of best fit for combined data from 5–8 animals and a minimum of 8 observations. These were calculated using a linear least squares regression analysis of y on x and the estimates of y for the antagonist concentrations (x) used in each group of experiments are shown. The vertical bars indicate the standard errors of these estimated y values. Ordinate: log (concentration ratio − 1). Abscissa: log antagonist concentration (M).

"Schild plots" shown in Fig. 2. Since the slopes of these lines (Table 1) did not differ significantly from 1·0 it was considered valid to compare these pA_2 values between species and between agonists. For both α-methylpropr-anolol and atenolol the pA_2 values on guinea-pig atria were independent of the agonist used whereas on cat atria the pA_2 values varied with the agonist used (Table 1, Fig. 2). For both antagonists, the pA_2 values on cat atria with noradrenaline as agonist were the same as the pA_2 values on guinea-pig, with either agonist (Table 1). However, on cat atria with fenoterol as the agonist, the pA_2 value for α-methylpropranol was high and that for atenolol low compared with the other three values obtained for each of these antagonists (Table 1).

Insufficient data were obtained for a "Schild plot" for butoxamine on cat atria. This was because butoxamine at the concentrations required frequently caused the atrial preparations to stop beating. In two experiments with each agonist in which some data for butoxamine were obtained, the pA_2 values from individual experimental points were calculated according to the formula $pA_2 = \log$ (concentration ratio −1)-log antagonist concentration (molar). The mean pA_2 values of butoxamine were 6·10 ± 0·15, n = 4 with fenoterol as agonist and 5·07 ± 0·13, n = 3 with noradrenaline as agonist. Thus, like the other two antagonists studied, the pA_2 value for butoxamine on cat atria varied with the agonist used. In contrast, on guinea-pig atria the pA_2 value for butoxamine did not vary with the agonist used (pA_2 values from "Schild plots"; noradrenaline as agonist, 5·31, slope 1·09 ± 0·14, number of points 23; fenoterol as agonist, 5·37, slope 1·08 ± 0·23, number of points, 13).

DISCUSSION

The results in this paper showed that, in guinea-pig atria, the pA_2 values of the selective β-adrenoceptor blocking drugs atenolol, α-methylpropranolol and butoxamine were not dependent on whether the agonist used was noradrenaline or fenoterol. In contrast, for each of these three antagonists on cat atria a different pA_2 value was obtained with noradrenaline than with fenoterol as agonist. These observations have been interpreted as evidence that guinea-pig atria, unlike cat atria, possesses a single β-adrenoceptor type and not a β-adrenoceptor mixture.

Interpretation of the data in this way depends on three principles which underly the definition of the pA_2 value of a competitive antagonist i.e., provided that the experimental conditions are optimal, that a single receptor type is involved and that the antagonism is purely competitive (1) the pA_2 value for an antagonist should be a measure of the affinity of the antagonist for that receptor, (2) the pA_2 value for an antagonist should be the same whatever agonist drug is used, and (3) identical receptors in different tissues should give rise to the same pA_2 value for a given antagonist (see Arunlakshana & Schild 1959; Schild 1973). It thus follows that (1) if in any given tissue the same pA_2 value is not obtained for a competitive antagonist using different agonists, then more than one receptor type is involved in the response of that tissue to the agonists and (2) if, in different tissues the pA_2 values for a competitive antagonist are not the same, then the receptor populations (whether single or multiple) in the two tissues are not identical.

Thus the constancy with different agonists of the pA_2 values for atenolol, α-methylpropranolol or butoxamine obtained on guinea-pig atria in the present study, indicated that noradenaline and fenoterol and also the antagonist drugs, were all acting on the same receptor type in this tissue, i.e.

there was no evidence for more than one β-adrenoceptor type in guinea-pig atria. This receptor type can be called β_1 according to the terminology of Lands et al (1967). Moreover, if one accepts the conclusion that guinea-pig atria have only β_1-adrenoceptors, then the pA_2 values obtained for the β-adrenoceptor antagonists on this tissue can be taken as a measure of their affinity for β_1-adrenoceptors. Likewise the potencies of the two agonists reflect their action only on β_1-adrenoceptors.

The same conclusion cannot be drawn for cat atria since, for all three antagonists, the pA_2 values were different for noradrenaline and fenoterol. Thus the two agonists cannot be stimulating identical receptors i.e. more than one β-adrenoceptor type is present in this tissue. If one considers the evidence that the potency of noradrenaline and also the pA_2 values of the antagonist drugs with noradrenaline as agonist, were the same in cat and guinea-pig atria, and, if one accepts that noradrenaline is acting only on β_1-adrenoceptors in guinea-pig atria (vide supra) then the conclusion can be drawn that one of the receptors in cat is β_1, the same as in guinea-pig. The data with fenoterol provided evidence on the nature of the second receptor in cat atria in that the potency of fenoterol was greater in cat than in guinea-pig. Although this difference in potency could be due, at least in part, to a difference in β-adrenoceptor reserves in the two tissues, it does suggest that the second receptor type is probably β_2, since fenoterol is known to be a potent and selective β_2-adrenoceptor agonist (O'Donnell 1970). The observation that, when fenoterol was the agonist the β_2-selective antagonist α-methylpropranolol had a greater pA_2 in cat than in guinea-pig whereas the β_1-selective antagonist atenolol had a lower pA_2 in cat than in guinea-pig, provides additional support for the proposal that the second receptor type is β_2.

This conclusion does depend on the β-adrenoceptor agonists and antagonists being selective at the receptor level. The compounds used were selected on the basis of evidence in the literature for their tissue selectivity i.e. atenolol (Barrett 1977) and noradrenaline (Furchgott 1976) are considered to be β_1-selective whilst fenoterol (O'Donnell 1970), α-methylpropranolol (Levy 1973) and butoxamine (Imbs et al 1977) are considered to be β_2-selective. Tissue selectivity is not necessarily a reliable indication of receptor selectivity especially if there should be differences in the β-adrenoceptor reserves and/or β-adrenoceptor mixtures in the tissues but, since commencing this study, other data from our laboratory has confirmed that α-methylpropranolol, butoxamine and fenoterol are β_2-selective and atenolol and noradrenaline β_1-selective at the receptor level (O'Donnell & Wanstall, O'Donnell et al, to be published). Although α-methylpropranolol is not highly selective it was used in the present study because of the difficulties encountered in obtaining quantitative data in cats with the more β_2-selective drug, butoxamine.

In summary, it would appear that the simplest explanation for the experimental findings is that in guinea-pig the chronotropic response to β-adrenoceptor agonists is mediated solely by β_1-adrenoceptors whereas that in cat is mediated by two different β-adrenoceptors one of which is the same as the β-adrenoceptor in guinea-pig atria (β_1) and the other probably β_2. The conclusions on cat atria support the view of Carlsson & Åblad (1976) that cat heart contains a mixture of β_1 and β_2-adrenoceptors. The demonstration that guinea-pig atria contain only β_1-adrenoceptors emphasises that (a) not all tissues necessarily possess a mixture of β-adrenoceptor subtypes and (b) the proportion of β_1 to β_2-adrenoceptors in any particular tissue or organ can vary between species.

The fact that cat atria contain β_2-adrenoceptors and guinea-pig atria do not may be sufficient to account for the higher relative potency of β_2-selective agonists on the heart of cat than of guinea-pig (see introduction). The only data obtained in human heart tissue indicated that man, like cat, may also have β_2 receptors (Åblad et al 1974). If this is so, then the cat may well be a better model for predicting the potential incidence of cardiac side effects of the selective bronchodilator β-adrenoceptor agonists in man, as has been suggested by e.g. Davey et al (1974). Nevertheless, guinea-pig atria would be better tissue for examining the properties of β_1-adrenoceptors and for assessing the potency of agonists and antagonists on β_1-adrenoceptors at the receptor level since the tissue does not contain β_2-adrenoceptors.

A number of pharmacological differences have now been demonstrated between the atria from cat and guinea-pig. Apart from the differences in receptor populations shown in the present study, atrial responses to isoprenaline have been shown to be potentiated by inhibitors of extraneuronal uptake and catechol-*O*-methyl transferase in cat but not in guinea-pig (Kaumann 1972; Woppel & Trendelenburg 1973) which is not incompatible with fluorescence histochemical findings that the extraneuronal uptake of isoprenaline into atrial myocardial cells was very poor in guinea-pig compared with that seen in cat (Anning et al 1979). It is tempting to speculate

that there may be a link between the differences in the receptor populations and the differences in extraneuronal uptake of catecholamines in the atria of the two species. It has been suggested that β_2-adrenoceptors might be "hormonal receptors" primarily for circulating adrenaline (Carlsson & Hedberg 1976; Ariëns & Simonis 1976) and it has been proposed that extraneuronal *O*-methylating systems might be involved in regulating the access of circulating adrenal medullary catecholamines to receptors (Trendelenburg 1978). The cat atria, with β_2-adrenoceptors present, have a good extraneuronal metabolizing system whereas the guinea-pig atria, with no β_2-adrenoceptors, do not.

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