J. Pharm. Pharmacol. 1983, 35: 752–754
Communicated April 27, 1983

Chronotropic responses of rabbit isolated atria to β-adrenoceptor agonists are mediated by only β_1-adrenoceptors

B. I. COSTIN, STELLA R. O'DONNELL*, JANET C. WANSTALL, *Pharmacology Section, Department of Physiology and Pharmacology, University of Queensland, St Lucia, Brisbane, 4067 Australia*

When Lands et al (1967a, b) suggested that β-adrenoceptors could be subdivided into β_1- and β_2-sub-types, they studied a range of tissues and preparations, one of which was rabbit isolated heart, both chronotropic and inotropic responses being examined. On the basis of their results on rabbit heart, cardiac responses in general were assigned to the group of responses said to be mediated by β_1-adrenoceptors. It is now known from radioligand binding studies that β_1- and β_2-adrenoceptors can co-exist in a single tissue and both β_1- and β_2-adrenoceptor binding sites have been found in rat heart (Minneman et al 1979b) and guinea-pig and cat atria (Hedberg et al 1980). In cat atria, chronotropic responses to sympathomimetic amines are mediated by both of these sub-types (O'Donnell & Wanstall 1979a), but in rat atria only the β_1-adrenoceptors are involved (Bryan et al 1981). Guinea-pig atrial results are still controversial, in that Kaumann et al (1978) and Hayes et al (1982) have suggested that a minor β_2-adrenoceptor population may be involved in the chronotropic response of the atria to salbutamol, whereas other authors (O'Donnell & Wanstall 1979a, 1981a; Zaagsma et al 1979; Broadley & Hawthorn 1983) found no evidence for the involvement of β_2-adrenoceptors in responses to noradrenaline, fenoterol, adrenaline or isoprenaline. In light of the above species differences, this study was designed to determine whether the chronotropic response of rabbit atria was mediated by a homogeneous (β_1 only) or heterogeneous (β_1 plus β_2) β-adrenoceptor population.

Materials and methods

Rabbits of either sex (6–12 weeks old, 1–2 kg) were pretreated with reserpine (1 mg kg^{-1} 18 h before use) to minimize possible complications from the release of endogenous catecholamines. Rabbits were killed by a blow to the back of the neck and the hearts immediately excised. Spontaneously beating preparations were set up for recording atrial rate as described by O'Donnell & Wanstall (1979b) for guinea-pig atria. Preparations were treated with phenoxybenzamine (50 μM for 30 min followed by thorough washing in phenoxybenzamine-free Krebs solution) to block α-adrenoceptors, neuronal uptake and extraneuronal uptake. After an initial exposure of the preparation to a supramaximal concentration of either isoprenaline or noradrenaline, cumulative concentration-response curves to noradrenaline or fenoterol were obtained in the absence and presence of increasing concentrations of a β-adrenoceptor antagonist (atenolol or ICI 118,551), using a contact time of 60 min. EC50 values (concentration of agonist giving 50% of the maximum response in that particular concentration-response curve) were obtained and used to calculate values for concentration ratio (CR, i.e. EC50 antagonist present, divided by EC50 antagonist absent). In a separate series of experiments it was shown that there was no change in sensitivity of the tissues to the agonists with time or pre-exposure to the agonists and therefore no correction of the CR values was necessary. CR values were used to obtain plots of log (CR−1) vs log molar concentration of antagonist [B] as described by O'Donnell & Wanstall (1979b). 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. The slopes of the Schild plots were not significantly different from 1·0 and therefore pA_2 values were calculated from the equation $pA_2 = \log (C\ R - 1) - \log [B]$. A mean pA_2 for each atrial preparation was obtained and these were used to calculate a mean $pA_2 \pm$ s.e. from preparations from a number of animals.

In preparations from 4 more rabbits, cumulative concentration-response curves to procaterol were obtained (concentration range 1 nM to 100 μM, 2-fold increments).

The effect of corticosterone on isoprenaline responses was determined by obtaining concentration-response curves to isoprenaline in the absence and presence of one or two increasing concentrations of corticosterone. Potentiation was expressed, in log units, as the difference between the isoprenaline negative log EC50 values in the presence and absence of corticosterone respectively.

Drugs and solutions used: Atenolol (ICI); corticosterone (Sigma); (±)-fenoterol hydrobromide (Boehringer Ingelheim); ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol, ICI); (±)-isoprenaline sulphate (Sigma); (−)-noradrenaline acid tartrate (Sigma); phenoxybenzamine hydrochloride (Smith Kline and French); procaterol (Warner-Lambert); reserpine (Serpasil ampoules, Ciba). Stock solutions (10, 50 or 100 mM) of fenoterol, isoprenaline, noradrenaline and ICI 118,551 were made up in 10 mM HCl, of atenolol in deionized water, of

* Correspondence.

corticosterone in absolute ethanol and of phenoxybenzamine in absolute ethanol containing 10 mM HCl. A 10 mM solution of procaterol was made up in Krebs solution immediately before use. 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.

Statistical analyses

The significance of any deviation from unity of the slopes of the Schild plots has been calculated according to the methods described in Snedecor & Cochran (1967). Mean pA_2 values have been expressed together with their standard errors (s.e.). pA_2 values have been compared using Student's *t*-test. The significance of any potentiation of isoprenaline by corticosterone was obtained using a paired *t*-test.

Results and discussion

Schild plots for the β_1-selective antagonist, atenolol, and the β_2-selective antagonist ICI 118,551, using two agonists of opposite selectivity, viz noradrenaline (β_1-selective) and fenoterol (β_2-selective) are shown in Fig. 1. The pA_2 values calculated from the data used to obtain these Schild plots were: atenolol, noradrenaline as agonist, 7·12 ± 0·09 (n = 6), fenoterol as agonist, 7·18 ± 0·08 (n = 7); ICI 118,551, noradrenaline as agonist, 6·82 ± 0·12 (n = 4), fenoterol as agonist 6·89 ± 0·08 (n = 5). For each of the antagonists the Schild plots obtained when using the two different agonists were superimposed. The pA_2 value with noradrenaline as agonist was not significantly different from that with fenoterol as agonist ($P > 0·05$, Student's *t*-test). The lack of separation of the Schild plots provided evidence (O'Donnell & Wanstall 1981a) that only a single population of β-adrenoceptors was involved in the chronotropic response of rabbit atria. In tissues in which responses are mediated by a mixed population of β-adrenoceptors, the Schild plots for either of these two selective antagonists, using noradrenaline and fenoterol as agonists were not superimposed, e.g. cat trachea and cat atria (O'Donnell & Wanstall 1979a, 1983), guinea-pig trachea (O'Donnell & Wanstall 1979b, 1980), rat pulmonary artery (O'Donnell & Wanstall 1981b).

The pA_2 values indicated that the single β-adrenoceptor sub-type involved was β_1, in accordance with the classification of Lands et al (1967b), since these pA_2 values were the same as those obtained for these two antagonists on β_1-adrenoceptors but were different from their pA_2 values on β_2-adrenoceptors (refer O'Donnell & Wanstall 1979b, 1980). This was confirmed by the relative potencies of isoprenaline : noradrenaline : fenoterol which were 100 : 9·3 : 3·5, i.e. noradrenaline was more potent than fenoterol. This is characteristic of tissues containing β_1-adrenoceptors. If the receptors had been β_2-adrenoceptors fenoterol would have been expected to be more potent than noradrenaline.

To confirm that no β_2-adrenoceptors were involved in the chronotropic response of rabbit atria, the effects of the β_2-selective agonist, procaterol, were examined. Using this drug, Hedberg & Mattsson (1981) recently demonstrated the involvement of β_2-adrenoceptors in responses of cat papillary muscle, a tissue in which the β_2-adrenoceptor population had previously been shown to be as little as 2% of the total β-adrenoceptor population (Hedberg et al 1980). No responses were obtained to procaterol in rabbit atria at concentrations considered to activate β_2-adrenoceptors (i.e. 1 nM to 1 μM). Small responses were obtained in concentrations thought to activate β_1-adrenoceptors (i.e. 1 to 100 μM), but the mean maximum response to procaterol was only

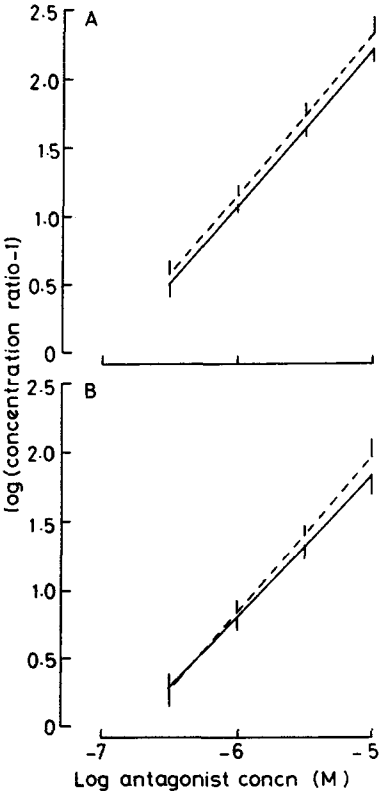

FIG. 1. Schild plots for atenolol (A) and ICI 118,551 (B) on rabbit isolated atria (chronotropic responses) using fenoterol (– – –) or noradrenaline (——) as agonist. The plots represent the calculated lines of best fit through the combined data from a number of animals. The vertical bars represent the s.e. of the estimated values of log (CR − 1) at points corresponding to the antagonist concentrations used. The slopes of the Schild plots were: atenolol, noradrenaline as agonist 1·15 ± 0·10 (12 data points, 6 animals) fenoterol as agonist 1·18 ± 0·12 (12, 7); ICI 118,551, noradrenaline as agonist 1·05 ± 0·17 (8, 4), fenoterol as agonist 1·14 ± 0·13 (9, 5).

19·5 ± 2·2% (n = 4) of the maximum response to isoprenaline. These data supported the conclusion that there are no pharmacologically detectable β_2-adrenoceptors contributing to the chronotropic response of rabbit atria, and also suggested that the overall β_1-adrenoceptor population is small, when compared with that of cat papillary muscle, in which the maximum response to procaterol was 92% of the isoprenaline maximum (Hedberg & Mattsson 1981).

Thus with respect to the nature of the β-adrenoceptor population mediating the chronotropic response, rabbit resembled rat (β_1-only) but differed from cat (β_1 plus β_2). We therefore determined whether chronotropic responses to isoprenaline in rabbit atria were modulated by extraneuronal uptake, since this is another way in which rat has been shown to differ from cat (Bryan et al 1981). In rat and guinea-pig, chronotropic responses to isoprenaline were not potentiated by inhibitors of extraneuronal uptake (Wöppel & Trendelenburg 1973; Goldie 1976; Bryan et al 1981), as they were in the cat (Kaumann 1972; Goldie 1976). Corticosterone 10 and 50 μM, concentrations which have been shown to potentiate isoprenaline responses in cat trachea (O'Donnell & Wanstall 1983), caused no significant change in sensitivity of rabbit atria to isoprenaline. The mean differences between negative log EC50 values in the presence and absence of corticosterone were 10 μM, 0·01 ± 0·04 (n = 4) log units and 50 μM, −0·12 ± 0·15 (n = 4) log units ($P > 0·05$, paired t-test). Thus it appears that responses of rabbit atria, as those of guinea-pig and rat atria, are not affected by extraneuronal uptake, and this is in keeping with the apparent inability of rabbit myocardial cells to accumulate catecholamines (de la Lande et al 1974; Bryan & O'Donnell, unpublished observations).

The data obtained on rabbit atria support the view that, although responses of many tissues are now known to be mediated by a mixed population of both β_1- and β_2-adrenoceptors, there are some tissues in which only one β-adrenoceptor sub-type seems to be involved (O'Donnell & Wanstall 1979a). This view is in agreement with observations from radioligand binding studies (Minneman et al 1979a). The reason why some tissues have a mixed β-adrenoceptor population while others do not is still not resolved. The hypothesis that β_1-adrenoceptors may be innervated receptors, and that β_2-adrenoceptors may be hormonal receptors, possibly more closely associated with extraneuronal uptake (refer Bryan et al 1981) is an attractive hypothesis, and the data obtained on rabbit atria (an adrenergically innervated tissue in which responses are mediated only by β_1-adrenoceptors and in which responses to isoprenaline are not potentiated by the extraneuronal uptake inhibitor, corticosterone) are compatible with this hypothesis. In contrast, there are data on other tissues which do not appear to support the hypothesis. For example, rat pulmonary artery (Wanstall & O'Donnell 1982) and rat jugular vein (Cohen & Wiley 1978; Cohen et al 1979), both contain β_1-adrenoceptors but little or no adrenergic innervation.

The financial support of the National Health and Medical Research Council of Australia and the excellent technical assistance of Miss Jane Baxter are gratefully acknowledged. J.C.W. is an NH & MRC Senior Research Officer.

REFERENCES

Arunlakshana, O., Schild, H. O. (1959) Br. J. Pharmacol. 14: 48–58

Broadley, K. J., Hawthorn, M. H. (1983) Ibid. 78: 136P

Bryan, L. J., Cole, J. J., O'Donnell, S. R., Wanstall, J. C. (1981) J. Pharmacol. Exp. Ther. 216: 395–400

Cohen, M. L., Landry, A. S., Hemrick, S. K., Fuller, R. W. (1979) Can. J. Physiol. Pharmacol. 57: 1246–1250

Cohen, M. L. Wiley, K. S. (1978) Life Sci. 23: 1997–2006

de la Lande, I. S., Harvey, J. A., Holt, S. (1974) Blood Vessels 11: 319–337

Goldie, R. G. (1976) Clin. Exp. Pharmacol. Physiol. 3: 225–233

Hayes, R., Howard, J. C., Nasmyth, P. A. (1982) Br. J. Pharmacol. 76: 195–200

Hedberg, A., Mattsson, H. (1981) J. Pharmacol. Exp. Ther. 219: 798–808

Hedberg, A., Minneman, K. P., Molinoff, P. B. (1980) Ibid. 212: 503–508

Kaumann, A. J. (1972) Naunyn-Schmiedeberg's Arch. Pharmacol. 273: 134–153

Kaumann, A. J., Birnbaumer, L., Wittmann, R. (1978) in: Birnbaumer, L., O'Malley, B. W. (eds) Receptors and Hormone Action, Academic Press, New York 3 pp 133–177

Lands, A. M., Arnold, A., McAuliff, J. P. Luduena, F. P., Brown, T. G. (1967a) Nature (London) 214: 597–598

Lands, A. M., Luduena, F. P., Buzzo, H. J. (1967b) Life Sci. 6: 2241–2249

Minneman, K. P., Hedberg, A., Molinoff, P. B. (1979a) J. Pharmacol. Exp. Ther. 211: 502–508

Minneman, K. P., Hegstrand, L. R., Molinoff, P. B. (1979b) Mol. Pharmacol. 16: 34–46

O'Donnell, S. R., Wanstall, J. C. (1979a) J. Pharm. Pharmacol. 31: 686–690

O'Donnell, S. R., Wanstall, J. C. (1979b) Naunyn-Schmiedeberg's Arch. Pharmacol. 308: 183–190

O'Donnell, S. R., Wanstall, J. C. (1980) Life Sci. 27: 671–677

O'Donnell, S. R., Wanstall, J. C. (1981a) J. Auton. Pharmacol. 1: 305–312

O'Donnell, S. R., Wanstall, J. C. (1981b) Br. J. Pharmacol. 74: 547–552

O'Donnell, S. R., Wanstall, J. C. (1983) Ibid. 78: 417–424

Snedecor, G. W., Cochran, W. G. (1967) Statistical methods 6th ed. The Iowa State University Press. Iowa, pp 137–171

Wanstall, J. C., O'Donnell, S. R. (1982) Proc. Aust. Physiol. Pharmacol. Soc. 13: 9P

Wöppel, W., Trendelenburg, U. (1973) Eur. J. Pharmacol. 23: 302–305

Zaagsma, J., Oudhof, R., van der Heijden, P. J. C. M., Plantje, J. F. (1979) in: Usdin, E., Kopin, I. J., Barchas, J. (eds) Catecholamines: Basic and Clinical Frontiers, Pergamon New York, 1: pp 435–437