Short communications

Characterization of the coronary vascular β-adrenoceptor in the pig

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The β-adrenoceptor in pig coronary vascular smooth muscle has been characterized by the use of a selective agonist, salbutamol and a selective antagonist, practolol. In coronary artery strips contracted with KCl, salbutamol had only marginal relaxant activity and was a partial agonist compared with isoprenaline. Practolol had relatively high antagonist potency against isoprenaline (pA_2 6·59). It is concluded that the pig coronary vascular β-adrenoceptor is of the β_1-type.

Introduction.—There is conflicting evidence about the nature of the β-adrenoceptor in coronary vascular smooth muscle. Thus it has been variously suggested that this receptor:

1. resembles the peripheral vascular β-adrenoceptor (β_2-type) (Parratt & Wadsworth, 1970; Broadley, 1970; Mark, Abboud, Schmid, Heistad & Mayer, 1972);
2. differs from the peripheral vascular β-adrenoceptor (Bohr, 1967);
3. resembles the myocardial β-adrenoceptor (β_1-type) (Somani, Laddu & Hardman, 1970; Lucchesi & Hodgeman, 1971);
4. differs from the myocardial β-adrenoceptor (Adam, Boyles & Scholfield, 1970; Bussmann, Rauh & Krayenbuehl, 1970);
5. differs from both the vascular and myocardial β-adrenoceptors (Ross & Jorgensen, 1970).

Several species were used in these studies, but since each category contains at least one report based on results obtained in the dog, the different conclusions cannot be attributed to species difference.

Furchgott (1972) has stressed the need for proper control of experimental conditions if data are to be used to differentiate receptors within a single class. Most of the work cited above was carried out in intact animals, in which satisfactory control of experimental variables is difficult to achieve. Analysis of direct responses to adrenoceptor agonists and antagonists in the coronary vascular bed is especially complicated because of the pronounced influence of accompanying changes in the metabolic activity of the myocardium (Parratt, 1969).

These difficulties can be avoided by removing the coronary vessels from the myocardium and observing the direct effects of adrenoceptor agonists and antagonists on the vascular smooth muscle *in vitro* (Zuberbuhler & Bohr, 1965; Bohr, 1967). In the present work, the β-adrenoceptor in pig isolated coronary artery strips has been characterized by the use of salbutamol, an agonist with selectivity for peripheral vascular β-adrenoceptors (β_2 type) and practolol, an antagonist with selectivity for myocardial β-adrenoceptors (β_1-type).

Methods.—Hearts were obtained from pigs immediately after slaughter. Helical strips, approximately 3 mm wide and 3 cm long were cut from the right coronary artery. On a few occasions, strips were also obtained from the left circumflex coronary artery. The preparations were mounted in 30 ml tissue baths containing Krebs solution plus ascorbic acid, 100 μg/ml, maintained at 37° C and bubbled with 5% carbon dioxide in oxygen. Each preparation was stretched to a tension of 0·5 g and allowed to equilibrate for 1–2 h until it gave constant contractor responses to KCl, 3–60 mM. A concentration of KCl producing approximately 30% maximum contraction was used thereafter—usually 20–30 mM. KCl producing 0·6–0·8 g increase in tension. In a previous study it was shown that the relaxant effect of catecholamines on coronary artery strips is unaffected by the type of spasmogen used (Zuberbuhler & Bohr, 1965).

Cumulative concentration-effect curves were obtained for (−)-isoprenaline and (±)-salbutamol, each curve taking 15–20 min to complete. An interval of 20 min elapsed between completion of one curve and the start of the next. During this time the bathing solution was changed 5 or 6 times. Similar results were obtained, irrespective of whether isoprenaline or salbutamol was tested first.

Antagonist potencies of propranolol and practolol were determined with isoprenaline as the agonist. Two or three

control cumulative concentration-effect curves for isoprenaline were obtained and the antagonist added to the bathing solution for 45 min before responses to isoprenaline were re-examined. Only one concentration of antagonist was tested in each preparation. The results for each antagonist were plotted as log (isoprenaline concentration-ratio − 1) against −log (antagonist concentration) (Arunlakshana & Schild, 1959). The pA_2 value and slope were obtained from the regression line calculated by the method of least squares.

Values in the text and table are means with 95% confidence limits in parentheses.

Results.—Isoprenaline (1–100 ng/ml) produced a concentration-dependent relaxation of coronary artery strips contracted with KCl, the maximum effect corresponding to complete abolition of tone. The sensitivity of individual preparations to isoprenaline varied by less than 2 fold over a period of 4 hours.

In contrast to isoprenaline, salbutamol had only marginal relaxant activity in coronary artery strips. In five preparations salbutamol had no effect in concentrations up to 100 μg/ml. In a further five preparations salbutamol (0·1–30 μg/ml) did produce relaxation, but maximal effects were only 20–66% of those produced by isoprenaline. Relative potency was estimated by comparing the concentrations of isoprenaline and salbutamol required to produce 50% of their respective maximal effects (van Rossum, 1963). Salbutamol was estimated to be 632 (589–677) times less potent than isoprenaline. Daly, Farmer & Levy (1971) reported it to be only 3·17 times less potent in the hind limb vessels of the dogs.

Graded concentrations of propranolol (5, 25 and 125 ng/ml) or of practolol (0·1, 0·5 and 2·5 μg/ml) produced parallel shifts to the right in the isoprenaline concentration-effect curve. The results summarized in Table 1 provide evidence of simple competitive antagonism for both drugs (Arunlakshana & Schild, 1959).

Discussion.—The profile of action of salbutamol in pig coronary vascular smooth muscle closely resembles that obtained previously on β_1-adrenoceptors in isolated cardiac muscle preparations (Farmer, Levy & Marshall, 1970; Nayler, 1971). In both tissues, salbutamol had only marginal potency and was a partial agonist compared with isoprenaline. This is in marked contrast to the high potency of salbutamol on peripheral vascular β-adrenoceptors (Farmer, *et al.*, 1970; Daly, *et al.*, 1971).

Both propranolol and practolol were found to be competitive antagonists of isoprenaline on pig coronary vascular β-adrenoceptors. The pA_2 for practolol is almost identical to those obtained in tissues containing β_1-adrenoceptors (see Table 1) and is clearly greater than on β_2-adrenoceptors (Bristow, Sherrod & Green, 1970). These complementary results with salbutamol and practolol enable the coronary vascular β-adrenoceptor of the pig to be characterized as a β_1-type.

The present results were obtained in large coronary arteries but should apply also to small coronary arteries, since Bohr

TABLE 1. *Comparison of β-adrenoceptor antagonist potencies of propranolol and practolol in pig coronary vascular smooth muscle and in some tissues containing β_1-adrenoceptors*

Species	Tissue	Receptor classification*	Propranolol			Practolol		
			pA_2 (45 min)	Slope	*n*	pA_2 (45 min)	Slope	*n*
Pig	Coronary artery	—	8·41 (8·09–8·92)	1·07 (0·81–1·34)	4–6	6·59 (6·41–6·86)	0·99 (0·75–1·22)	6–11
Guinea-pig	Left atria‡	β_1	8·54 (8·32–8·76)	1·25 (0·95–1·34)	12	6·76 (6·44–7·28)	1·08 (0·80–1·35)	3
Guinea-pig	Oesophagus‡	β_1	8·25 (7·91–8·87)	0·99 (0·67–1·32)	6–10	6·68 (6·41–7·08)	0·83 (0·64–1·01)	5–7
Rabbit	Left atria†	β_1	8·36	±0·07 (S.E.M.)	6	6·58	±0·04 (S.E.M.)	6

* Lands, Arnold, McAuliff, Luduena & Brown (1967); Lands, Luduena & Buzzo (1967). † Wale (1970). ‡ Unpublished results from this laboratory. *n*, Number of determinations of potency at each concentration of antagonist tested.

(1967) has shown that β-adrenoceptors at these two sites are similar.

Do these findings extend to coronary vascular β-adrenoceptors in species other than the pig? The only other recent studies *in vitro,* undertaken in coronary vascular strips from dogs, humans, rabbits and monkeys, established an order of β-adrenoceptor agonist potency of isoprenaline> noradrenaline>adrenaline (Bohr, 1967), which is characteristic of the β_1-adrenoceptor (Arnold, 1972). Therefore, it is likely that coronary vascular β-adrenoceptors in these species, as well as in the pig, are of the β_1-type, but further experiments with selective agonists and antagonists in coronary artery strips from these species are needed to confirm this indication.

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