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The costo-uterine muscle of the rat contains a homogeneous population of β-adrenoceptors

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1 The effects of two selective β-adrenoceptor antagonists on the inhibitory responses to some sympathomimetic amines of electrically-stimulated preparations of costo-uterine muscle, taken from virgin rats, have been examined quantitatively.

2 pA_2 values for the antagonist, atenolol (β_1-selective) and ICI 118,551 (β_2-selective) were obtained using as agonists, fenoterol (β_2-selective agonist) and noradrenaline (α- and β-adrenoceptor agonist, β_1-selective); and in addition, with ICI 118,551 only, isoprenaline (β-agonist, non-selective) and adrenaline (α- and β-adrenoceptor agonist, β_2-selective). Catecholamine uptake mechanisms and α-adrenoceptors were not blocked in any of these experiments.

3 Atenolol competitively antagonized the effects of fenoterol and noradrenaline to a similar extent, the pA_2 values being 5.4 and 5.7, respectively.

4 ICI 118,551 competitively antagonized the effects of fenoterol, isoprenaline, adrenaline and noradrenaline to a similar extent; pA_2 values ranged from 8.7 with noradrenaline to 9.1 with isoprenaline.

5 These results extend our previous observations which indicated that the adrenoceptors mediating inhibition of electrically-evoked contractions of costo-uterine muscle of the virgin rat are homogeneous and of the β_2-subtype.

6 The potency of the β_1-selective agonist RO 363 in producing inhibition of electrically-evoked contractions of this tissue was also examined. RO 363 was 200 times less potent than isoprenaline but was a full agonist. This indicates that there is efficient coupling between β_2-adrenoceptor activation and tissue response in this non-innervated preparation.

Introduction

We have previously studied the inhibitory effects of sympathomimetic amines on electrically-evoked contractions of preparations of the isolated costo-uterine muscle taken from virgin rats during the oestrous cycle. Both phenylephrine and isoprenaline reduced the size of contractions. These effects were unaffected by phentolamine but were antagonized though not reversed by propranolol (Hartley & Pennefather, 1981). We, therefore, suggested that the adrenoceptors within the tissue are exclusively of the β-type.

More recently, we found that the relative order of the potencies of six sympathomimetic agonists in producing inhibition of electrically-evoked contractions (fenoterol≥isoprenaline>salbutamol≥adrenaline > noradrenaline > phenylephrine) indicated that the adrenoceptors were of the β_2-subtype (Hartley & Pennefather, 1984a).

To investigate further the homogeneity of the β-adrenoceptor population and to classify the adrenoceptor subtype within the rat costo-uterine muscle, we have examined the antagonism of fenoterol and noradrenaline by the β_1-adrenoceptor selective antagonist, atenolol; and of isoprenaline, fenoterol, adrenaline and noradrenaline by the β_2-adrenoceptor selective antagonist, ICI 118,551 (erythro-DL-1 (7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol).

In addition, we have examined the potency of the newly developed β_1-adrenoceptor selective agonist, RO 363 ((±)-1-(3, 4,-dimethoxyphenethylamino)-3-(3, 4,-dihydroxyphenoxy)-2-propanol; Raper *et al.*, 1978), in causing inhibition of electrically-evoked contractions of preparations of rat costo-uterine muscle.

A preliminary account of these results has been presented to the Australian Physiological and Pharmacological Society (Hartley & Pennefather, 1984b).

Methods

Virgin female Long Evans Hooded rats (180–220 g) were housed at 22°C with a photoperiod of 12 h dark and 12 h light. Vaginal smears were taken from each rat before experimentation and cycle stage determined histologically; we have previously shown that cycle stage does not influence the inhibitory potencies of either isoprenaline or phenylephrine on this preparation (Hartley & Pennefather, 1981).

Isolated organ bath studies

The methods for setting up isolated preparations of costo-uterine muscle, for electrical stimulation and for recording of contractions, have been described by us previously (Hartley & Pennefather, 1981; 1984a). In brief, the tissues were attached to holders incorporating platinum electrodes. They were then placed in 30 ml organ baths containing (mmol l^{-1}): NaCl 120, KCl 5, $CaCl_2$ 2.5, $NaHCO_3$ 25, NaH_2PO_4 1, $MgSO_4$ 1, glucose 11, sucrose 10, disodium edetate 0.03, sodium ascorbate 0.09. The bathing medium was maintained at 37°C and aerated with 5% CO_2 in O_2. Tissues were subjected to 200 mg resting tension. Pulses of 2 ms and 30–50 V were delivered from Grass S88, S48 or S44 stimulators for 5 s every 100 s. Contractions were recorded isometrically using FT.03 transducers and Grass 7B or 79C polygraphs. All preparations were allowed to equilibrate for 1 h before the addition of agonist drugs. Only one sympathomimetic amine was added to any one tissue; increasing concentrations of the amines were added non-cumulatively as described by us previously. For determination of pA_2 values, antagonists were used in three increasing concentrations: atenolol at 1, 10 and 100 μmol l^{-1}; ICI 118,551 at 5, 50 and 500 nmol l^{-1}. Each concentration was allowed to equilibrate with the tissue for at least 30 min before repeating full concentration-response curves to the agonist. All experiments were carried out in the absence of inhibitors of catecholamine uptake mechanisms and α-adrenoceptors.

Drugs

(−)-Noradrenaline-D-bitartrate (Sigma), (−)-adrenaline-D-bitartrate (Sigma), (−)-isoprenaline-D-bitartrate (Sigma), and fenoterol hydrobromide (Boehringer Ingelheim) were made up daily as stock solutions of 10 μmol l^{-1} in a diluent containing (mmol l^{-1}) NaCl 154, NaH_2PO_4 1, ascorbic acid 0.2. Atenolol and ICI 118,551 (ICI, Australia) were made up in distilled water. A stock solution of RO 363 [(±)-1-(3, 4,-dimethyphenethylamino)-3-(3, 4,-dihydroxyphenoxy) -2-propanol] oxylate (synthesized in the School of Chemistry, Victorian College of Pharmacy) was made up in 0.01 mol l^{-1} HCl and diluted in the bathing medium.

Statistical analysis

The potencies of agonists were expressed as mean EC_{50} values which are the mean negative log molar concentrations producing 50% of the maximal effect. Dose ratios (DR) were determined from the concentration causing 50% of maximal inhibition of electrically-evoked contractions at each concentration of the antagonist. The pooled log (dose ratio − 1) values thus obtained in each experiment were plotted against log antagonist concentration in the form of a Schild plot (Arunlakshana & Schild, 1959). From these plots of pooled data a mean slope and the pA_2 value together with their 95% confidence limits were calculated (Documenta Geigy, 1970).

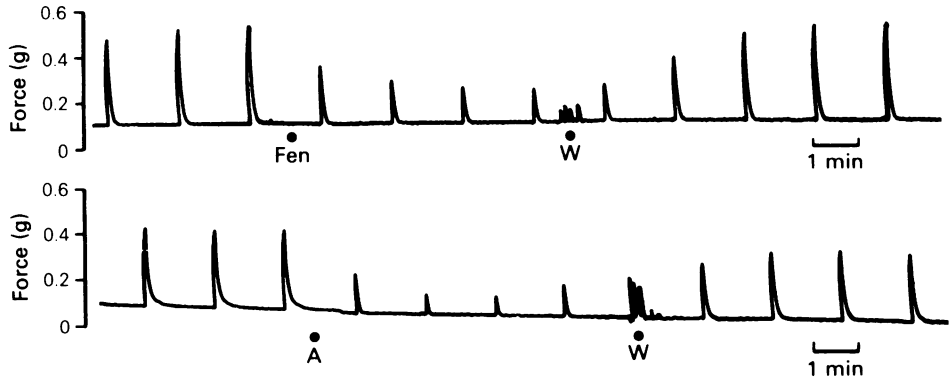

Figure 1 The effect of fenoterol (Fen), 5 nmol l^{-1}, and adrenaline (A), 0.1 μmol l^{-1} on electrically-stimulated preparations of costo-uterine muscle taken from a rat in oestrus. W indicates drug washout. Stimulation parameters are: 30 Hz, 2 ms, 40 V for 5 s every 100 s.

Results

Isolated organ bath studies

As we have shown previously (Hartley & Pennefather, 1981; 1984a), preparations responded regularly to electrical field stimulation for periods of 5–6 h. All amines used produced only inhibition of electrically-evoked contractions. Figure 1 shows the inhibitory effects of fenoterol and adrenaline upon evoked-contractions of costo-uterine muscle preparations.

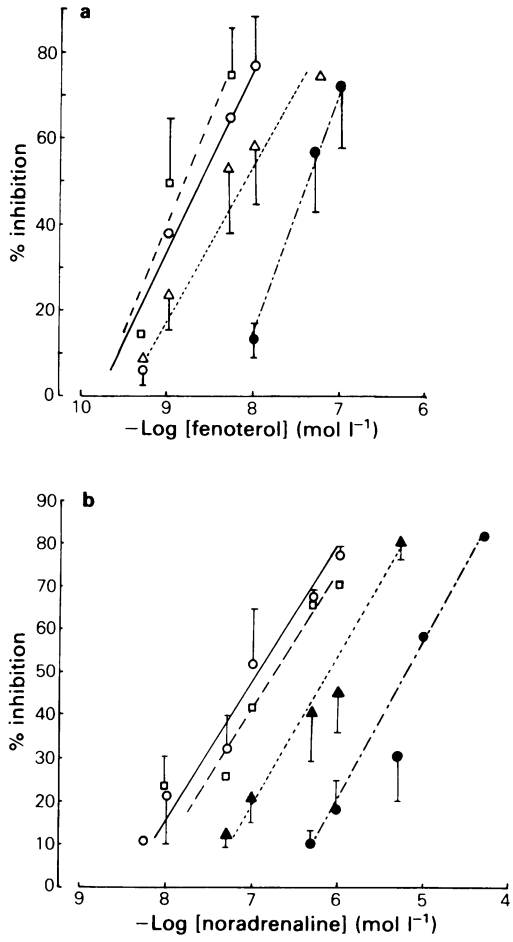

Figure 2 The mean negative log concentration-response curves for fenoterol-(a; $n = 5$) and noradrenaline-(b; $n = 4$) induced inhibition of electrically-evoked contractions, in the absence (open circles) and in the presence of atenolol at concentrations of 1 μmol l^{-1} (open squares), 10 μmol l^{-1} (triangles) and 100 μmol l^{-1} (filled circles). Vertical lines represent s.e.mean.

Effects of antagonists

The mean negative log concentration-response curves for fenoterol and noradrenaline, in the absence and presence of increasing concentrations of atenolol (1, 10 and 100 μmol l^{-1}) are shown in Figure 2. These concentrations of the antagonist did not affect the magnitude of electrically-evoked contractions. Atenolol, at a concentration of 1 μmol l^{-1}, had negligible effects on the control concentration-response curves for either agonist while concentrations of 10 μmol l^{-1} or greater effected rightward shifts in the concentration-response curves for both agonists.

From individual experiments, the log (dose ratio − 1) values were determined and Schild plots of log (dose ratio − 1) versus log concentration of atenolol were constructed (Figure 3). The pA_2 values for atenolol and the slopes of the Schild plots, together with their 95% confidence limits, are shown in Table 1. The slopes were not significantly different from unity, indicating competitive antagonism by atenolol of both fenoterol and noradrenaline. pA_2 values did not differ significantly from one another.

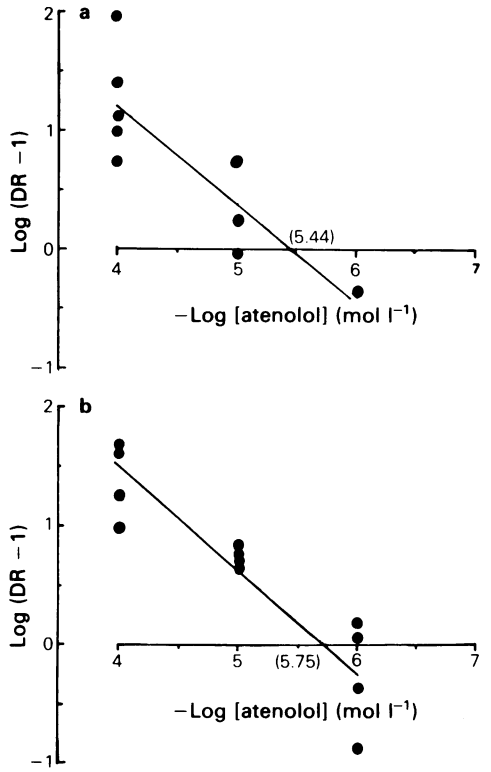

Figure 3 Schild plots for the antagonism of fenoterol (a; $n = 5$) and noradrenaline (b; $n = 4$) by the β_1-antagonist, atenolol.

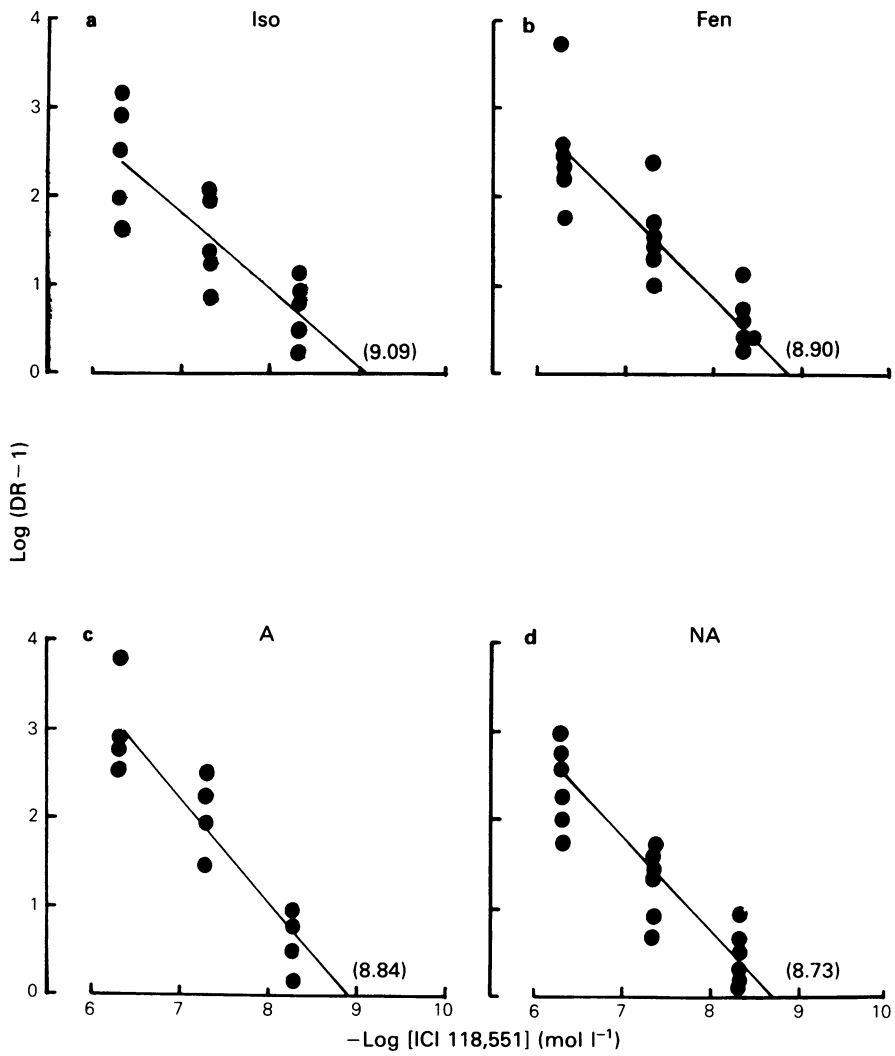

Figure 4 Schild plots for the antagonism of (a) isoprenaline (Iso; $n = 5$), (b) fenoterol (Fen; $n = 6$), (c) adrenaline (A; $n = 4$) and (d) noradrenaline (NA; $n = 6$) by the β_2-antagonist, ICI 118,551.

ICI 118,551 (5, 50 and 500 nmol l^{-1}) caused concentration-dependent rightward shifts in the negative log concentration-response curves to isoprenaline, fenoterol, adrenaline and noradrenaline. From individual experiments, the log (dose ratio − 1) values were determined and Schild plots constructed (Figure 4). Table 1 shows the estimated pA_2 values for ICI 118,551 and the slopes of the Schild plots together with their 95% confidence limits. As with atenolol, the slopes of the Schild plots did not differ significantly from unity and the pA_2 values were similar for each of the agonists used.

Action of RO 363

In six experiments the effects of the β_1-adrenoceptor selective agonist, RO 363, were investigated. The potency of RO 363 was determined from EC_{50} values obtained from concentration-response curve data. RO 363 was a full agonist and approximately 200 times less potent than isoprenaline in producing inhibition of electrically-evoked contractions; the negative log EC_{50} values ± s.e.mean were 9.99 ± 0.05 and 7.85 ± 0.08 for isoprenaline and RO 363, respectively. Thus the potency of RO 363, relative to isoprenaline, was

Table 1 The mean pA_2 values for the antagonists ICI 118,551 and atenolol, and the slopes obtained from Schild plot analysis, together with their 95% confidence limits

	ICI 118,551			*ATENOLOL*		
Agonist	pA_2 (95% confidence limits)	*Slope*	n	pA_2 (95% confidence limits)	*Slope*	n
Isoprenaline	9.09 (8.52, 10.28)	− 0.87 (− 1.20, − 0.53)	5			
Fenoterol	8.90 (8.46, 9.68)	− 0.98 (− 1.29, − 0.68)	6	5.44 (4.98, 6.70)	− 0.85 (− 1.33, − 0.37)	5
Adrenaline	8.84 (8.42, 9.56)	− 1.22 (− 1.58, − 0.85)	4			
Noradrenaline	8.73 (8.38, 9.27)	− 0.97 (− 1.22, − 0.73)	6	5.75 (5.44, 6.23)	− 0.82 (− 1.09, − 0.55)	4

n = number of experiments

0.0047. In four additional experiments, the antagonism by ICI 118,551 of RO 363 was determined. The pA_2 value (8.83) was not different from those values obtained with the other amines (see Table 1).

Discussion

The present findings confirm that the adrenoceptors mediating inhibitory effects upon the costo-uterine muscle of the virgin rat are homogeneous and of the β_2-subtype. Despite the fact that an α-adrenoceptor antagonist was not present in the bathing medium in this investigation, adrenaline and noradrenaline, both agonists at α- as well as at β-adrenoceptors, produced exclusively inhibitory effects. Moreover, the β-adrenoceptor antagonists, atenolol and ICI 118,551, antagonized but never reversed the inhibitory responses to catecholamines. These findings are consistent with those of our earlier study in which propranolol was used (Hartley & Pennefather, 1984a).

The slopes and locations of Schild plots obtained for the β_1-adrenoceptor selective antagonist, atenolol, using the agonists fenoterol (β_2-selective) and noradrenaline (β_1-selective) were similar, indicating that these agonists activate identical receptors. The pA_2 values obtained (5.4, 5.7) are consistent with those found for this antagonist at β_2-adrenoceptors in other tissues. For example, using fenoterol as the agonist, O'Donnell & Wanstall (1980; 1981) have demonstrated pA_2 values of 5.6 and 5.0 in preparations of guinea-pig trachea, and rat pulmonary artery, respectively. In contrast, the pA_2 value for atenolol, using noradrenaline as the agonist, on the β_1-adrenoceptors in rat atrium is 7.4 (Bryan *et al.*, 1981).

Despite the varying selectivities for adrenoceptors, of the amines we have used, the Schild plots obtained with ICI 118,551 were similar in slope and location. The pA_2 values (range: 9.09 to 8.73) are consistent with those recently found by Henry *et al.* (1984) using carbachol-contracted preparations of this tissue, and with those reported in the literature for this antagonist at β_2-adrenoceptors in other tissues. For example O'Donnell & Wanstall (1980; 1983) have found pA_2 values of 8.69 and 9.16 with fenoterol as the agonist on guinea-pig trachea and rat pulmonary artery, and of 8.59 with adrenaline as the agonist on guinea-pig trachea.

In 1976, Ariëns & Simonis noted that β_2-adrenoceptors, which are particularly sensitive to adrenaline, are usually located extra-junctionally (i.e. not innervated). For example, in tissues such as the rat uterus which receive very little sympathetic innervation, the β-adrenoceptors are predominantly of the β_2-type. The present results with rat costo-uterine muscle add further weight to this concept, since this tissue is not sympathetically innervated (Musgrove *et al.*, 1978; Lawrence & Burden, 1980; Hartley & Pennefather, 1984a).

In 1970, Melton & Saldivar recorded electrical and mechanical activity of the rat costo-uterine muscle *in vivo*. To our knowledge this is the only study of the contractility of the costo-uterine muscle *in vivo*. We would predict, on the basis of our studies, that circulating adrenaline might play an important role in modulating contractile activity in the rat costo-uterine muscle *in vivo*.

We also compared the potency of the newly developed β_1-adrenoceptor agonist, RO 363, with that of isoprenaline. The relative potency of this phenoxypropanolamine varies depending upon the subtype of β-adrenoceptor activated (Iakovidis *et al.*, 1980). Thus it is approximately equipotent with isoprenaline in tissues in which β_2-adrenoceptors predominate. In contrast, in tissues where β_1-adrenoceptors are the predominant subtype, it is 100 to 350 times less active than isoprenaline. The finding that RO 363 was some 200 times less potent than isoprenaline, coupled with a

pA_2 value of 8.83 obtained for the antagonism of RO 363 by ICI 118,551, indicates that this compound is acting at β_2-adrenoceptors in this preparation.

Although RO 363 was 200 times less potent than isoprenaline, it nevertheless acted as a full agonist. This indicates that in the rat costo-uterine muscle, as in the longitudinal myometrium of this species (Kenakin, 1982), the coupling of β_2-adrenoceptor stimulation to the response may be very efficient.

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