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The affinity and efficacy of the selective β_1-adrenoceptor stimulant RO363 at β_1- and β_2-adrenoceptor sites

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1 (−)-Isoprenaline and the selective β_1-adrenoceptor agonist RO363 were tested for their inotropic effects in left atrial (β_1) and relaxant effects in K^+-depolarized uterine (β_2) preparations from the guinea-pig. The drugs had similar activities as positive inotropic agents but RO363 was approximately 400 times less active than (−)-isoprenaline as a uterine relaxant. RO363 had intrinsic activities of 0.8 and 0.25 ((−)-isoprenaline = 1) in atrial and uterine preparations, respectively.

2 Apparent dissociation constants (K_D values) determined from the ability of the agonists to displace (−)-[^{125}I]-iodocyanopindolol ([^{125}I]-CYP) bound to membranes prepared from both tissues were used as a measure of affinity. The [^{125}I]-CYP binding sites possessed the characteristics of homogeneous populations of β_1-adrenoceptors in atrial and β_2-adrenoceptors in uterine membrane preparations.

3 The pK_D values for (−)-isoprenaline were similar in the two tissues (left atria 6.4, uterus 6.0) whilst for RO363 the atrial value (7.8) was considerably greater than that for the uterus (6.0). The latter value is very similar to the pK_B value determined from shifts in (−)-isoprenaline curves produced by RO363 in uterine preparations.

4 Graphical plots of the fraction of receptors occupied *vs* response were constructed. The relative efficacy of (−)-isoprenaline with respect to RO363 was calculated to be 25 in atrial and 2633 in uterine preparations.

5 The selective β_1-adrenoceptor stimulant actions of RO363 are a reflection of both its greater affinity and efficacy for β_1- as opposed to β_2-adrenoceptor sites. The potent actions of (−)-isoprenaline in both tissues are largely dependent on efficacy.

Introduction

Previous *in vitro* studies (Iakovidis *et al.*, 1980) have shown that the catecholamine derivative RO363 behaves as a selective β_1-adrenoceptor stimulant. The basis for this classification is that RO363 is approximately equi-active with (−)-isoprenaline as a positive chronotropic and inotropic agent in a number of species and as a relaxant in guinea-pig ileal preparations, but is some 100–300 times less active than (−)-isoprenaline as a relaxant in lung strip and uterine preparations from the guinea-pig.

The response of a tissue to an agonist is dependent on two factors, namely the affinity of the drug for the receptor and the efficacy of the drug-receptor complex (Stephenson, 1956). Thus the selectivity of an agonist for production of effects mediated via different receptor subtypes may be due to selective affinity, efficacy or a combination of both. The aim of the present study was to delineate the roles of affinity and efficacy in the production of the selective β_1-adrenoceptor mediated responses elicited by RO363.

The tissues used were the left atria and uterus from the guinea-pig, since mechanical responses in these tissues have been shown to be initiated via homogeneous populations of β_1- and β_2-adrenoceptors respectively (Broadley, 1982; Krstew *et al.*, 1982). Of crucial importance in such a study is the accurate assessment of the dissociation constant (1/affinity constant) of the agonist, since the computation of efficacy utilizes this value. Dissociation constants of (−)-isoprenaline and RO363 were calculated from radioligand binding experiments using

(−)-[^{125}I]-iodocyanopdinolol ([^{125}I]-CYP) as a molecular probe for the β-adrenoceptors present in the two tissues. Full characterization of [^{125}I]-CYP binding to membranes prepared from left atria and uteri was undertaken, since there were no previous reports utilizing these tissues. The relative efficacies of (−)-isoprenaline and RO363 were calculated using the method described by Furchgott & Bursztyn (1967).

Methods

Organ bath studies

General All guinea-pigs (400–700 g) were pretreated with reserpine (1 mg kg^{-1}, i.p. 18 h). Those animals from which uterine tissues were taken were additionally treated with stilboestrol (0.1 mg kg^{-1}, i.p. 24 h). Left atria and uteri were dissected free and set up under 0.5 g tension in Krebs solution (composition mM; NaCl 118.4, KCl 4.7, $CaCl_2$ 1.9, $NaHCO_3$ 25, $MgSO_4$ 1.2, glucose 11.7, NaH_2PO_4 1.2, EDTA 0.1 and ascorbic acid 0.1), gassed with 5% CO_2 in O_2, and maintained at 37°C. Left atria were driven at a frequency of 2.5 Hz with square wave pulses of 1 ms duration at 1.5 times the threshold voltage using a Grass S6 stimulator. Tone in uterine preparations was induced using a K^+-depolarizing solution in which all Na^+ salts in the Krebs solution were replaced by K^+salts (Krstew *et al.*, 1982). Interference from possible α-adrenoceptor-mediated effects and neuronal and extraneuronal uptake mechanisms was reduced by pretreating tissues with phenoxybenzamine (50 μM, 30 min incubation, followed by 6 washes in 30 min). Changes in isometric tension in the tissues were recorded on a Grass model 7c polygraph using a Grass FT03c transducer coupled to a Grass 7P1 pre-amplifier.

The tissues were washed three times during an initial 30 min stabilization period. Thereafter, cumulative concentration-effect curves for the positive inotropic and uterine relaxant effects of (−)-isoprenaline and RO363 were established at 30 min intervals. Responses to each concentration of agonist were expressed as the change in tension from basal values. Following each curve, the tissues were washed 4 times in 10 min and allowed to stabilize for 20 min before the establishment of the next curve. Curves to (−)-isoprenaline were established before those for RO363. Responses to RO363 were expressed as a percentage of the maximal response to (−)-isoprenaline. From individual experiments, pD_2 values (for half-maximal effects of each drug), and intrinsic activity (α, (−)-isoprenaline = 1) were calculated.

Estimation of apparent K_p values for RO363 The apparent K_p value of RO363 was calculated using the procedure described by Barlow *et al.* (1967) as modified by Kenakin & Black (1978). The slope of the plot of [A] against [A]/[P] for equi-effective concentrations of the full agonist A ((−)-isoprenaline) and the partial agonist P (RO363) is equal to the apparent K_p value. The apparent K_p value approaches the true K_p when the efficacy of agonist A is far greater than that of P. In the present study, apparent K_p values were calculated by linear regression analysis of 5 equi-effective pairs of concentrations. In the text, the negative log of the apparent K_p value (i.e. pK_p) is used.

Estimation of pK_B/pA_2 values for RO363 Due to the low intrinsic activity of RO363 in uterine preparations, it was possible to quantitate its antagonistic effects against the relaxant effects of (−)-isoprenaline using the method described by Malta & Raper (1974). After constant cumulative concentration-effect curves to (−)-isoprenaline had been obtained, curves were re-established in the presence of 50 and 100 μM RO363. An equilibration time of 40 min was allowed at each concentration used. Dose-ratios for half-maximal effects of (−)-isoprenaline were calculated and used to assess the presence of competitive antagonism (Arunlakshana & Schild, 1959). K_B values (Furchgott, 1972) were calculated for both concentrations of RO363 in each experiment and are expressed as $-\log K_B$ (pK_B) in the text.

The high intrinsic activity of RO363 in atrial preparations, precluded the assessment of pK_B values using this method.

Using similar methodology the pK_B values for the β-adrenoceptor antagonists propranolol, metoprolol, betaxolol, ICI 118, 551 and L643, 717–01J10 were determined using (−)-isoprenaline as the agonist in both uterine and atrial preparations.

Relative intrinsic efficacy of RO363 The response (R) of left atrial or uterine preparations was regarded as a function (f) of the stimulus (S) given to the tissue by the agonists, i.e. R = f(S). The stimulus (S) was taken to be the product of the fractional receptor occupancy (y) by the agonist and its efficacy (e), i.e. S = y × e. For the two agonists, (−)-isoprenaline (I) and RO363 (RO), assuming that equi-active responses correspond to identical stimuli,

$$e_I \times y_I = e_{RO} \times y_{RO}$$

and

$$\frac{e_I}{e_{RO}} = \frac{y_{RO}}{y_I}$$

thus $\log(e_I) - \log(e_{RO}) = \log(y_{RO}) - \log(y_I)$

Thus, for equi-active responses, the antilog of the differences in fractional receptor occupancies for two agonists (right hand side of equation) equals the relative efficacies of the drugs (left hand side of equation). The relative efficacy of the drugs represents their relative intrinsic efficacy ($\varepsilon_I/\varepsilon_{RO}$), since $e = \varepsilon R_t$ where R_t is the total number of receptors, and is assumed to be constant in any experiment in which the same piece of tissue is used to construct curves to both agonists (Furchgott & Bursztyn, 1967). From the law of mass action, y (or RA/Rt) can be shown to be equal to $[A]/([A] + K_A)$, where A is the concentration of agonist which elicits the response and K_A is the dissociation constant of the agonist-receptor complex.

In each experiment, the efficacy of RO363 relative to (−)-isoprenaline was calculated from the antilog of the distance between the (−)-isoprenaline and RO363 fractional receptor occupancy-response curves (Furchgott, 1966; Furchgott & Bursztyn, 1967) using 5 pairs of equi-active responses corresponding to 0.20, 0.35, 0.50, 0.65 and 0.80 of the maximum response to RO363. Dissociation constants were calculated using radioligand binding techniques.

Radioligand binding studies Left atria from non-pretreated, and uteri from stilboestrol (0.1 mg kg^{-1}; 24 h) pretreated animals were removed and homogenized (Polytron, setting 10 for 15 s) in ice-cold Krebs-phosphate buffer (composition mM: NaCl 119, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 1.9, glucose 11.7, NaH_2PO_4 1.3, Na_2HPO_4 8.7, pH 7.4) An initial low speed centrifugation (30 *g* for 5 min) was used to remove connective tissue from uterine homogenates. Both tissue homogenates were then centrifuged twice at 39,000 *g* for 10 min and the resulting pellets resuspended in Krebs-phosphate buffer. Atrial and uterine membrane pellets were finally resuspended in 300 vol. and 100 vol. (original wet weight), respectively, to obtain the final stocks of membrane suspensions.

The radioligand used was [^{125}I]-CYP (approximately 2, 000 Ci $mmol^{-1}$, Amersham). Binding assays were performed in disposable polystyrene tubes. In both saturation and drug-displacement studies, 150 μl of homogenate was combined with Krebs-phosphate buffer in a final volume of 250 μl. Also present were 0.1 mM GTP, 1 mM ascorbic acid and 0.1 mM EDTA. The tubes were incubated for 70 min at 37°C and the assay terminated by the addition of 8 ml ice-cold buffer followed by rapid filtration through Whatman GF/B filters. Each filter was then washed with additional 2 × 4 ml aliquots of ice-cold buffer. Radioactivity retained on the filters was measured using a Packard γ Counter (Model 5320) at an efficiency of approximately 46%. Specific binding of the radioligand was defined as the difference in the amount of [^{125}I]-CYP bound in the absence and in the presence of propranolol (1 μM).

Specific binding in left atria and uteri ranged from approximately 87% to 55% (at 10 pM) and 68% to 42% (at 200 pM), respectively. The concentrations of radioligand used for saturation experiments were 10, 20, 50, 80, 100 and 200 pM, whereas for kinetic and displacement studies, 50–80 pM [^{125}I]-CYP was used.

For each drug displacement experiment, 12–14 concentrations of each drug covering a 10,000–100,000 fold range were employed.

Satuaration and drug displacement data were analyzed using two computer programs, EBDA (McPherson, 1983a, b) which performed preliminary Scatchard, Hill and Hofstee analyses and created a file for the second program, LIGAND (Munson & Rodbard, 1980), which was used to obtain final parameter estimates.

In experiments in which the association rate constant was determined, the membrane suspensions and the radioligand were incubated separately at 37°C for 20 min before being combined. The rate of association of [^{125}I]-CYP to atrial and uterine membranes was determined at 0, 6, 10, 20, 50 and 120 min. The association rate constant (k_I) was calculated as described by Hoyer *et al.*, (1982).

The rates of dissociation (k_{-1}) of [^{125}I]-CYP from atrial and uterine binding sites were determined by the addition of 1 μM propranolol after equilibration of membranes with [^{125}I]-CYP for 70 min at 37°C. Dissociation was determined at 0, 2, 4, 6, 8, 10, 30, 50, 70, 90, 120, 150, 180, 210 and 240 min after the addition of 1 μM propranolol. The dissociation rate constant was evaluated by the polyexponential curve fitting programme ESTRIP (Brown & Manno, 1978). Non-specific binding was also determined at each time point.

With the exception of the dissociation rate studies which were done in duplicate, all assays were performed in triplicate. Protein was determined according to the method of Lowry *et al.*, (1951) using bovine serum albumin as standard.

Drugs

The drugs used were: (−)-isoprenaline bitartrate (Wyeth); RO363 ((±)-1-(3,4-dimethoxy-phenethylamino)-3-(3,4-dihydroxyphenoxy)-2-propanol) oxalate) (synthesized at the Victorian College of Pharmacy); (+)-isoprenaline bitartrate (Sterling Winthrop); propranolol hydrochloride and ICI 118,551 hydrochloride (erythro-DL-1 (7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) (Imperial Chemical Industries); metoprolol tartrate (Astra); betaxolol (L.E.R.S. Synthelabo);

L643,717-01J10 ((S)-2(P-[3-(3, 4-dimethoxyphenethylamino)-2-hydroxypropoxy] phenyl) -4-(2-thienyl) imidazole dihydrochloride) (Merck, Sharp & Dohme); guanosine triphosphate (Sigma); phenoxybenzamine hydrochloride (Smith, Kline & French); reserpine (Serpasil, Ciba-Geigy) and disodium stilboestrol diphosphate (Honvan, Bristol).

Stock solutions (10 mM) of (−)- and (+)-isoprenaline, RO363, betaxolol, ICI 118, 551 and metoprolol were prepared in 0.01 M HCl; L643, 717-01J10 in equal parts of 95% ethanol and 0.01 M HCl; phenoxybenzamine (0.1 M) in 95% ethanol containing 1 μl 10M HCl ml^{-1} and the remaining drugs in distilled water. Dilutions were made using Krebs-bicarbonate solution (Na^+ or K^+ as appropriate) or Krebs-phosphate solution (both containing 1 mM ascorbic acid).

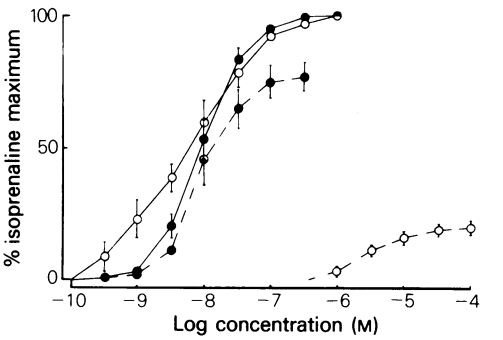

Figure 1 Mean cumulative concentration-effect curves for (−)-isoprenaline (solid lines) and RO363 (broken lines) in driven left atrial (●) and K^+-depolarized uterine preparations (○) from the guinea-pig. Responses are expressed as a percentage of the maximum response to (−)-isoprenaline. Points show mean values together with s.e.means (vertical lines if greater than the size of the symbol) from 4 experiments in each tissue.

Results

Organ bath studies

Agonist activity of (−)-isoprenaline and RO363 Both compounds produced positive inotropic responses in electrically driven left atrial and relaxant effects in K^+-depolarized uterine preparations. Whereas the two agonists were almost equi-active in atria, there was a marked difference in the activity of the drugs in the uterus. Table 1 shows the mean pD_2 and intrinsic activity values for (−)-isoprenaline and RO363 in the two tissues and Figure 1 shows mean concentration-effect curves to both agonists.

Estimates of the apparent pK_p value for RO363 were obtained from analysis of equi-effective concentrations of both agonists. The mean pK_p values for RO363 in atrial and uterine preparations were 7.43 (s.e.mean = 0.12, n = 4) and 5.80 (s.e.mean = 0.11, n = 4), respectively.

Antagonist activity of RO363 In uteri, RO363 shifted concentration-effect curves to (−)-isoprenaline to the right in a parallel fashion without a depression of the maximal relaxant effect. The dose-ratios calculated from half-maximal effects of (−)-isoprenaline ranged from 41–50 at 50 μM and 82–90 at 100 μM RO363. Analysis of the dose-ratios using the method of Arunlakshana & Schild (1959) indicated the presence of competitive antagonism (mean slope 0.94; s.e.mean = 0.06, n = 4). The mean pK_B value for RO363 was 5.74 (s.e.mean = 0.12, n = 8).

Radioligand binding experiments and characterization of binding sites

Saturation experiments The specific binding of [^{125}I]-CYP to atrial and uterine membrane preparations increased with increasing concentrations of the radioligand (5–200 pM). Figure 2 shows saturation curves and Scatchard transformations of the binding data from single experiments using homogenates of atrial and uterine membranes. In both membrane preparations, the binding displayed the characteristics of a bimolecular interaction that was saturable and of high and similar affinity in the two tissues (K_D atria 20.3 pM, uterus 31.6 pM). Table 2 shows the mean dissociation constant (K_D) values, the mean

Table 1 pD_2 values (negative log EC_{50} for half-maximal effects) and intrinsic activities (α, (−)-Iso = 1) for the β-adrenoceptor agonistic activity of (−)-isoprenaline ((−)-Iso) and RO363 in guinea-pig left atrial and uterine preparations

	Atria		*Uterus*	
	pD_2	α	pD_2	α
(−)-Iso	8.04 ± 0.09	1.00	8.24 ± 0.15	1.00
RO363	8.05 ± 0.09	0.77 ± 0.06	5.58 ± 0.04	0.21 ± 0.03

The values shown are mean ± s.e.mean, n = 4 preparations.

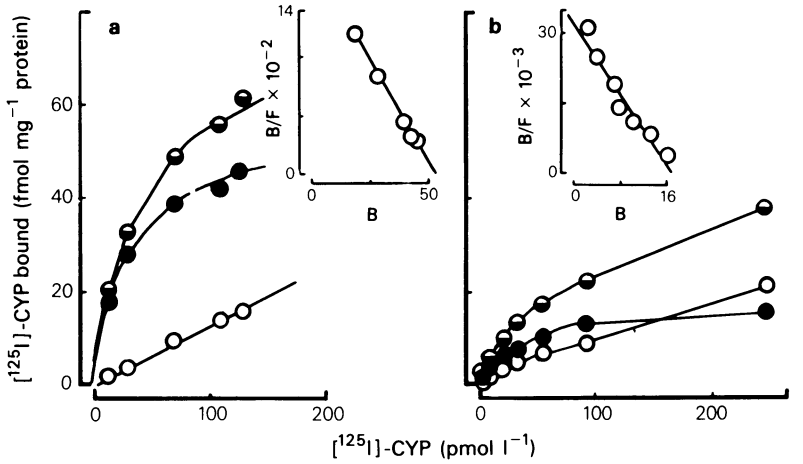

Figure 2 Saturation studies on the binding of various concentrations (10–200 pM) of [^{125}I]-CYP to guinea-pig left atrial (a) and uterine(b) membranes. Specific binding (●) was determined as the difference between total binding (○) and that observed in the presence of 1 μM propranolol (○). Also shown (inset) are the Scatchard transformations (bound/free (B/F) *vs* bound (B)) of data. Computer analysis yielded K_D values of 22.2 pM (atria) and 30.4 pM (uterus) and B_{max} values of 45.8 fmol mg^{-1} protein (atria) and 17.1 fmol mg^{-1} protein (uterus) in these two experiments.

maximal density of binding sites (B_{max}) and the Hill coefficient in the two tissues. The most notable difference between the two tissues was the considerably lower number of binding sites (B_{max}) in uterine (12.5 fmol mg^{-1} protein) as opposed to atrial (51.9 fmol mg^{-1} protein) membrane homogenates. In both instances Hill coefficients were not significantly different from unity indicating the absence of co-operativeness in binding.

Kinetics of [^{125}I]-CYP binding to membranes The association of [^{125}I]-CYP to atrial and uterine membrane preparations was time-dependent and equilibrium was reached after 50–70 min incubation of the radioligand with the membranes. The initial association rate constants for binding to atrial and uterine homogenates were 34.5 nM min^{-1} (s.e.mean = 8.0, $n = 3$) and 47.1 nM min^{-1} (s.e. mean = 4.8, $n = 3$) respectively. Analysis of the dissociation of [^{125}I]-CYP from the binding site indicated a monophasic dissociation curve in each tissue homogenate (left atria, $6.35 \pm 0.54 \times 10^{-3}$ min^{-1}; uterus, $1.67 \pm 0.30 \times 10^{-3}$ min^{-1}). The dissociation constants (k_{-1}/k_1) for [^{125}I]-CYP determined from kinetic data were 18.4 pM and 3.5 pM for the atria and uterus, respectively.

Drug displacement studies A number of β-adrenoceptor antagonists which display varying affinities for β_1- and β_2- receptor sites, were assessed for their ability to displace specifically bound [^{125}I]-CYP from membrane preparations of both tissues. Table 3 shows the mean pK_D values and values of slope factors obtained for the antagonists, propranolol (non-selective), betaxolol, metoprolol and L643, 717-01J10 (β_1-selective) and ICI 118, 551 (β_2-selective) against [^{125}I]-CYP binding to atrial and uterine membranes. For all antagonists, in both tissues, the slope factors were not significantly different from unity indicating that the displacements were from an homogeneous population of binding sites. The existence of a single homogeneous binding site in each tissue was also verified by analysing the displacement data with a one and a two site model, using

Table 2 Dissociation constants (K_D), maximal density of binding sites (B_{max}) and Hill coefficients (nH) for (−)-[^{125}I]-iodocyanopindolol binding to guinea-pig left atrial and uterine membranes.

	n	K_D (pM)	B_{max} (fmol mg^{-1} protein)	nH
Atria	3	20.3 ± 3.5	51.9 ± 3.5	0.97 ± 0.02
Uterus	6	31.6 ± 5.4	12.5 ± 3.2	1.02 ± 0.02

Values shown are mean ± s.e.mean from n experiments.

Table 3 Dissociation constants for drugs acting at β-adrenoceptor sites in left atrial and uterine preparations

Drug	Atria pK_B	Atria pK_D	Atria SF	Uterus pK_B	Uterus pK_D	Uterus SF
Antagonists						
Propranolol	8.53±0.09	8.50±0.10	0.95±0.03	9.11±0.05	8.83±0.10	0.90±0.14
ICI 118, 551	6.86±0.04	6.79±0.06	0.91±0.07	8.67±0.04	8.48±0.02	1.05±0.13
Betaxolol	8.50±0.02	8.24±0.10	0.94±0.13	6.46±0.09	5.90±0.20	1.06±0.02
L643, 717-01J10	8.05±0.07	8.76±0.14	0.87±0.09	5.05±0.08	5.60±0.05	1.15±0.19
Metoprolol	7.40±0.08	6.96±0.03	0.94±0.01	5.81±0.04	5.30±0.08	0.91±0.06
Agonists						
(+)-Isoprenaline		4.76±0.11	0.95±0.10		4.84±0.08	0.96±0.17
(−)-Isoprenaline		6.41±0.04	1.30±0.28		5.98±0.06	1.01±0.09
(±)-RO363		7.81±0.10	0.91±0.20		6.01±0.08	0.94±0.07

Shown are mean pK_B ($-\log K_B$) values from organ bath studies, and mean values for pK_D ($-\log K_D$) and slope factors (SF) from displacement studies with [^{125}I]-CYP. Values shown are means ± s.e.mean from 3–6 experiments with each drug.

the LIGAND programme. Statistical tests performed by LIGAND indicated a significant preference ($P<0.05$) for a one site as opposed to a two site model in all cases.

Comparison of the pK_D values for the antagonists determined from binding studies with pK_B values determined from the rightward shift of (−)-isoprenaline curves in left atrial and uterine preparations in organ bath experiments are shown in Table 3. The pK_D and pK_B values obtained with the compounds are similar in magnitude and a high and significant linear correlation is evident between pK_D and pK_B estimates in the atria ($r=0.88$, $P=0.048$), and the uterus ($r=0.97$, $P=0.007$).

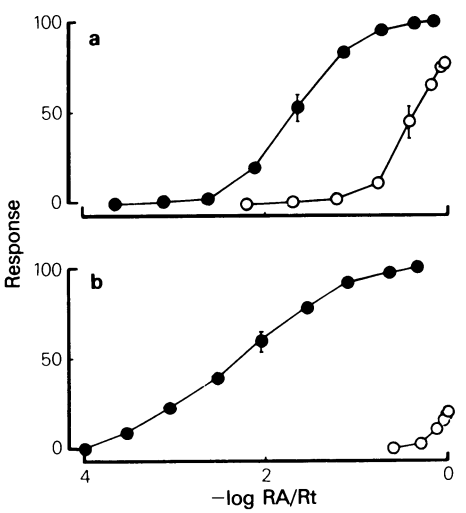

Figure 3 Mean ($n=4$) negative log fractional occupancy (RA/Rt)-response curves for (−)-isoprenaline (●) and RO363 (○) in (a) driven left atrial and (b) K^+-depolarized uterine preparations from the guinea-pig. Responses are expressed as a percentage of the maximum response to (−)-isoprenaline. Points show mean values together with the s.e.means (vertical lines if greater than size of symbol) at the mid range of responses in each curve.

RO363 and the (−)- and (+)-isomers of isoprenaline all displaced specifically bound [^{125}I]-CYP to a similar extent in membrane preparations from both tissues. Table 3 shows the mean pK_D values and values of the slope factors for RO363 and the two optical isomers of isoprenaline. RO363 was considerably more active in its ability to displace [^{125}I]-CYP binding from atrial as opposed to uterine membrane preparations, whereas (−)-isoprenaline was approximately equi-active as a displacing agent in the two tissues. In both tissues, [^{125}I]-CYP binding displayed stereo-selectivity since (+)-isoprenaline was considerably less active than its (−)-isomer.

Relative efficacy of RO363

From the previously described organ bath experiments, fractional receptor occupancies (RA/Rt) were calculated for atrial and uterine responses to RO363 and (−)-isoprenaline. Figure 3 shows mean log (fractional receptor occupancy) *vs* response curves for the atrial and uterine experiments. In atria, the mean intrinsic efficacy of (−)-isoprenaline relative to RO363 ($\varepsilon_I/\varepsilon_{RO}$) was 25.0 (s.e. mean = 0.8, $n=4$) whereas in the uterus it was 2, 633 (s.e.mean = 1,000, $n=4$).

Discussion

A number of compounds (e.g. fenoterol, salbutamol, soterenol and terbutaline) are, at a functional level, selective β_2-adrenoceptor stimulants; however, they appear to display approximately equal affinities for the two subtypes of β-adrenoceptors (Minneman *et al.*, 1979b; Nahorski, 1981). It would thus appear that these agents are selective by virtue of their greater efficacy (Stephenson, 1956) for β_2- as opposed to β_1-adrenoceptor mediated effects.

In the present studies, the contribution of affinity and efficacy to the β_1-adrenoceptor selective actions of RO363 were studied using left atrial and uterine tissues from the guinea-pig. These tissues were used since (a) at a functional level, responses are due to the activation of homogeneous populations of β_1- and β_2-adrenoceptors respectively, and (b) these tissues were found to yield sufficient quantities of membranes for radioligand binding experiments using the high affinity, high specific activity ligand (−)-[^{125}I-iodocyanopindolol ([^{125}I]-CYP) (Engel *et al.*, 1981). Thus both organ bath responses and radioligand binding data can be generated in the same tissues under comparable conditions.

The determination of the dissociation constants for the two agonists was the most critical aspect of the present study. Using radioligand binding techniques, particular care was taken to ensure that the binding site possessed the same characteristics as the receptor site upon which the agonists act to produce responses in the organ bath. The specific binding of the radioligand [^{125}I]-CYP was shown to exhibit stereoselectivity and to occur in a saturable reversible manner, without co-operativeness, to a single site with equal and high affinity in each tissue.

The results of the kinetic studies indicate a similar association rate to that previously found for the ligand (Hoyer *et al.*, 1982) but indicate a monophasic dissociation rate in the two membrane preparations. Previous studies with [^{125}I]-CYP have shown a biphasic dissociation rate (Brodde *et al.*, 1981; Engel *et al.*, 1981 Hoyer *et al.*, 1982) which presumably arises from two different affinity states of the receptor (Hoyer *et al.*, 1982). One possible explanation for the different number of phases in dissociation studies is that a complete ionic buffer was used in the present study, whereas previous workers used isotonic saline buffered with 10 mM Tris-HCl.

For both atria and uterus, the dissociation constants for five β-adrenoceptor antagonists determined from their ability to displace [^{125}I]-CYP, were similar in magnitude and exhibited a significant linear correlation to the dissociation constant values observed in organ bath studies. In addition, analysis of the displacement of [^{125}I]-CYP by wide ranges of concentrations of the above antagonists which display selectivity for β_1- or β_2-adrenoceptors indicated that the binding sites represented homogeneous populations of β_1- and β_2-receptors in left atria and uterus, respectively.

When the ability of (−)-isoprenaline to displace [^{125}I]-CYP was examined, the pK_D for the catecholamine was marginally greater in atrial than in uterine preparations and was considerably lower than the pD_2 values obtained in the organ bath studies. Similar pK_D values for (−)-isoprenaline have been observed in other tissues in which homogeneous β_1-adrenoceptor populations e.g. rat glomeruli (McPherson & Summers, 1983), cat and guinea-pig left ventricle (Hedberg *et al.*, 1979; Minneman *et al.*, 1979a) and homogeneous β_2-adrenoceptor populations exist e.g. rat liver, cat soleus muscle (Minneman *et al.*, 1979a) and human lymphocytes (Brodde *et al.*, 1981). The concurrence of the available evidence strongly suggests that the dissociation constants obtained in the present study are an accurate estimation of the true values.

The dissociation constant for RO363 determined from radioligand experiments was considerably higher in atrial than in uterine membrane preparations (Table 3). The accuracy of these estimates of the dissociation constants can be verified by alternative methods using organ bath studies in which apparent pK_p and pK_B values are calculated from agonist concentration-effect curves. For RO363 there is a close similarity between the pK_D and apparent pK_p values in the two tissues, and between the pK_D and pK_B value in the uteri.

Comparison of the dissociation constants of (−)-isoprenaline and RO363 in the two tissues indicate that RO363 possesses a 25 times greater affinity for β_1-adrenoceptors than (−)-isoprenaline, but both agonists have approximately equal affinity at β_2-adrenoceptor sites. Thus, selective affinity contributes to the selective β_1-receptor stimulant actions of RO363.

Utilizing the dissociation constants, it is apparent that (−)-isoprenaline possesses a high efficacy, or alternatively, possesses a large receptor reserve in both tissues. At 50% maximal response, (−)-isoprenaline has to occupy only 2.1% and 0.5% of receptors in atrial and uterine preparatations, respectively (see Figure 3). In contrast, RO363 has a lower efficacy and has no receptor reserve. For half maximal effects of RO363 in atria and uteri, approximately 32% and 70% of receptors must be occupied, respectively. Calculation of the relative intrinsic efficacy of (−)-isoprenaline with respect to RO363 indicates that RO363 possesses considerably weaker ef-

ficacy at β_2-than at β_1-adrenoceptors.

In conclusion, the results of the present study indicate that a combination of selective affinity and efficacy, are responsible for the selective β_1-adrenoceptor mediated stimulant actions of RO363. For (−)-isoprenaline, a high degree of efficacy is responsible for the potent effects of this catecholamine observed in organ bath experiments.

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