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In vitro ACTIVITY OF RO363, A β_1-ADRENOCEPTOR SELECTIVE AGONIST

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1 The β-adrenoceptor stimulant effects of RO363 and (−)-isoprenaline have been compared in a variety of isolated tissue preparations.

2 RO363 is approximately half as potent as (−)-isoprenaline in tissues where actions are due to β_1-receptor activation (guinea-pig atrial and ileal preparations and ventricular strips from the rabbit, rat and guinea-pig).

3 In uterine and lung strip preparations from the guinea-pig, where responses are due to β_2-receptor stimulation, RO363 is 100 to 350 times less active than (−)-isoprenaline and has a low intrinsic activity.

4 In spontaneously contracted tracheal preparations from the guinea-pig, RO363 is a full agonist and is approximately half as potent as (−)-isoprenaline. These effects of RO363 are due to the activation of a population of β_1-receptors in the tissue since RO363 and (−)-isoprenaline have the same relative potencies in trachea, cardiac and ileal preparations. In addition the K_B values for practolol are similar in all these preparations when RO363 is used as the agonist.

5 The results show that RO363 is a potent and highly selective β_1-receptor agonist.

Introduction

In previous work, Dowd, Keh & Raper (1977) have shown that the insertion of an oxymethylene group between the ring and ethanolamine side-chain of the catecholamines adrenaline, isoprenaline and N-*t*-butylnoradrenaline promotes selective agonistic actions at β_1-adrenoceptors. In an attempt to enhance further this selectivity RO363 (Figure 1) was synthesized. In a preliminary report (Raper, McPherson & Iakovidis, 1978), this compound was shown to produce highly selective β_1-receptor stimulant actions in anaesthetized cats; RO363 had a similar potency to (−)-isoprenaline as a positive chronotropic agent but was more than 100 times less potent as a vasodilator. However, in both isolated atrial and tracheal preparations from the guinea-pig, RO363 had a similar order of potency to (−)-isoprenaline. Evidence was presented to suggest that this unexpected result with RO363 in tracheal preparations might be due to actions involving a population of β_1-receptors within the tissue.

In the present experiments the actions of RO363 have been assessed in a number of isolated tissue preparations and investigations into the possibility that its actions in trachea may be initiated by β_1-receptor activation have been extended. In addition, experiments have been performed with RP333, the phenylethanolamine derivative of RO363 (Figure 1).

Methods

Unless otherwise stated tissues were bathed in Krebs solution (NaCl 6.9, KCl 0.4, $MgSO_4.7H_2O$ 0.14, $NaHCO_3$ 2.1, dextrose 2.0, $CaCl_2$ 0.28 and NaH_2PO_4 0.14 g/l) maintained at 37°C and aerated with a mixture of 5% CO_2 in O_2. A resting tension of 1 g was applied to cardiac and 0.5 g to smooth muscle preparations. Records of all responses were obtained with Grass FT03c force-displacement transducers coupled to a Grass 7C polygraph.

Cardiac preparations

Positive chronotropic responses were assessed in spontaneously beating right atrial preparations from the guinea-pig. Positive inotropic activity was assessed in driven guinea-pig left atrial and right ventricular strip preparations from the rat, rabbit and guinea-pig. Atrial preparations were driven at a frequency of 4 Hz with a pulse width of 1 ms and ventricular strips at 1 Hz with pulse widths of 2.5 ms

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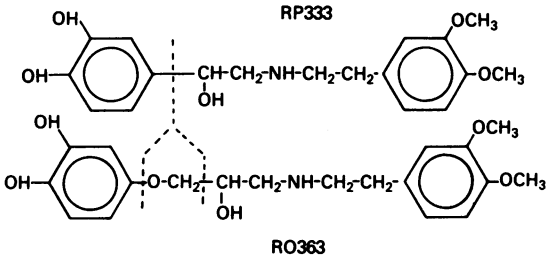

Figure 1 Structures of the compounds RP333 and RO363. Racemic mixtures of the hydrochloride (RP333) and oxalate (RO363) salts were used in the present study.

duration. The voltage used was 2 to 3 times greater than that required to elicit contractions.

Ileal preparations

Segments of ileum from reserpine-pretreated guinea-pig (2.5 mg/kg 24 h previously) were set up in Krebs solution maintained at 32°C. β-Receptor mediated inhibitory activity was monitored as a reduction in responses to exogenous acetylcholine (approx. 80% E_{max}) using a 3 min dose cycle. The catecholamines were added to the bathing solution 1.5 min before a succeeding acetylcholine response. This contact time was sufficient to allow maximal β-receptor-mediated responses to be obtained at each dose level.

In further experiments ileal segments from reserpine-treated guinea-pigs were stimulated transmurally at a frequency of 1 Hz; β-receptor-mediated inhibitory responses were monitored as a reduction in the height of the elicited contractions (O'Donnell & Wanstall, 1975).

Uterine preparations

β-Receptor-mediated inhibitory responses were obtained in uterine preparations from guinea-pigs pretreated with stilboestrol (0.1 mg/kg, 24 h previously).

In one set of experiments the preparations were bathed in de Jalon solution maintained at 32°C and catecholamine-induced reductions in responses to acetylcholine (3 min dose cycle) were monitored as described for ileal preparations. In further experiments, inhibitory effects were directly assessed in potassium-depolarized preparations bathed in a modified Krebs solution (O'Donnell, Persson & Wanstall, 1978).

Bronchial smooth muscle preparations

Tracheal and lung strip preparations from guinea-pigs were prepared as described by Mylecharane & Raper (1973) and Lulich, Mitchell & Sparrow (1976). Both these preparations exhibited spontaneous tone and thus the direct relaxant effects of the catecholamines could be monitored. In other experiments tone was induced in tracheal preparations with carbachol (0.5 μmol/l) before assessing β-receptor-mediated inhibitory effects (Davey, Malta & Raper, 1974).

β-Receptor-mediated responses were monitored by means of cumulative concentration-effect curves except in experiments in the uterus and ileum where reductions in acetylcholine responses to single doses of the test compounds were assessed.

In all experiments constant concentration-effect curves to (−)-isoprenaline were first obtained and thereafter the effects of either RO363 or RP333 were monitored. Responses were expressed as a percentage of the maximal response to (−)-isoprenaline (Iso) in each experiment. The relative activities (RA) of the compounds were expressed in terms of their individual EC_{50} concentrations (RA = EC_{50} Drug:EC_{50} Iso) and intrinsic activities measured with respect to (−)-isoprenaline (=1).

In experiments where the test compounds had low intrinsic activities, their possible β-receptor antagonistic effects were assessed from superimposed cumulative concentration-effect curves to (−)-isoprenaline using the method of Malta & Raper (1974). In other studies the interactions of (−)-isoprenaline, RO363 and salbutamol with the β-receptor antagonists propranolol, practolol, metoprolol and butoxamine were monitored. In all cases antagonist contact times of 30 min were allowed before agonist curves were re-established. Antagonism was expressed in terms of dissociation constants (K_B values, Furchgott, 1972) or pA_2 values (Arunlakshana & Schild, 1959).

The drugs used were (−)-isoprenaline bitartrate (Wyeth), salbutamol base (Glaxo-Allenbury), RO363 and RP333 (synthesized in the School of Chemistry, Victorian College of Pharmacy), reserpine (Ciba-Geigy), acetylcholine chloride (Sigma), disodium stilboestrol diphosphate (Bristol), carbachol chloride (BDH), butoxamine hydrochloride (Burroughs Wellcome), propranolol and practolol hydrochlorides (ICI) and metoprolol bitartrate (Astra).

Stock solutions of the catecholamines and salbutamol were made up in 0.01 mol/l HCl and dilutions prepared in physiological salt solutions containing 20 μg/ml ascorbic acid.

Results

Cardiac preparations

In right atrial preparations from the guinea-pig, RO363 was approximately half as potent as (−)-isoprenaline as a positive chronotropic agent. The con-

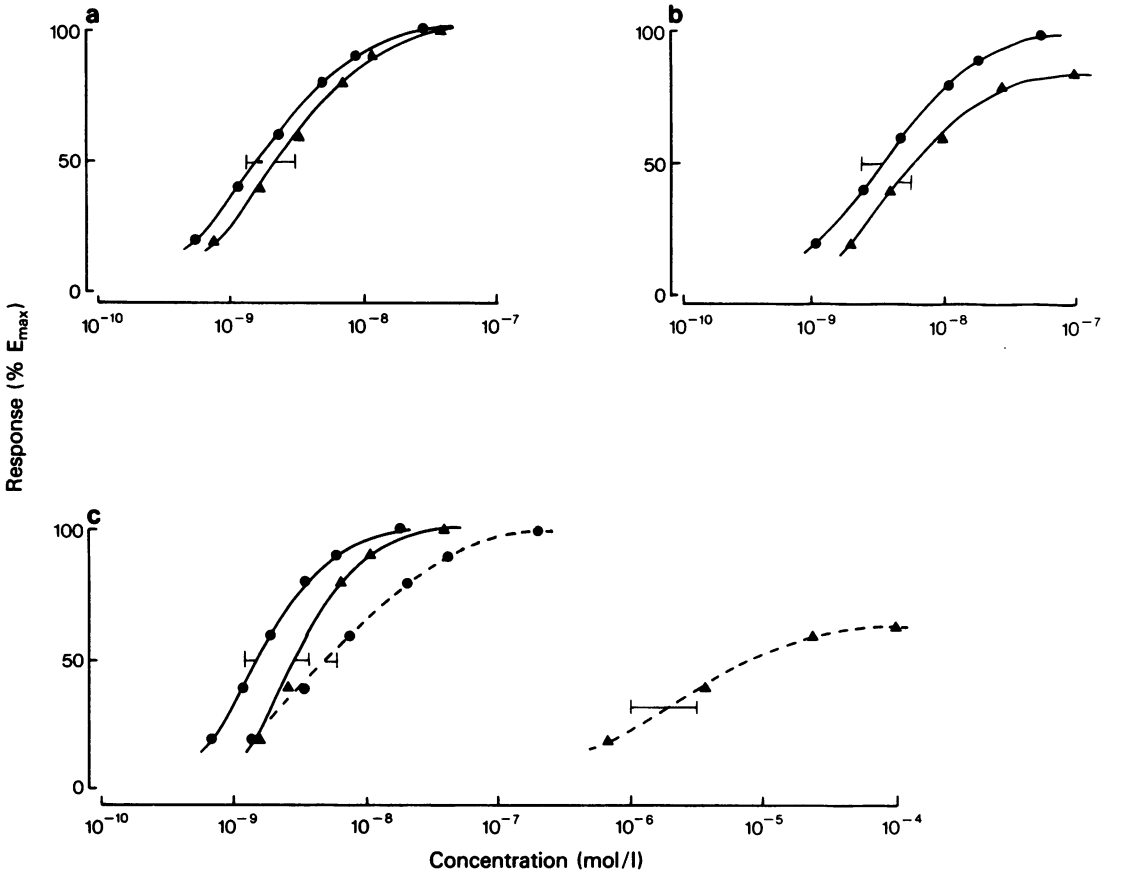

Figure 2 Mean concentration-effect curves for the effects of (−)-isoprenaline (●) and RO363 (▲) in guinea-pig isolated tissue preparations (a) right atria (chronotropic) (b) left atria (inotropic) and (c) spontaneously contracted tracheal (solid lines) and potassium-depolarized uterine (broken line) preparations. Responses are expressed as mean concentrations required to produce a given percentage response (E_{max} (−)-isoprenaline = 100%). Horizontal bars at 50% E_{max} for each individual curve show s.e. mean.

centration-effect curves to the two agonists were parallel and both produced the same maximal response (Figure 2). Similar results were obtained for the inotropic actions of RO363 in guinea-pig left atrial preparations (Figure 2) and in right ventricular strips from the guinea-pig, rat and rabbit; however, in these preparations the intrinsic activity of RO363 was marginally lower than that of (−)-isoprenaline (Table 1).

In all cardiac and smooth muscle preparations used, the rate of development of β-receptor mediated activity with RO363 was generally slower than with (−)-isoprenaline.

Similar results were obtained in right atrial preparations from reserpine-treated (2.5 mg/kg, 24 h previously) and non-reserpine-treated guinea-pigs. The mean relative activity of RO363 with respect to (−)-isoprenaline in the former preparations was 1.64 (s.e. mean = 0.11, $n = 4$). This value is not significantly different from that found in non-reserpinized preparations (t test, $P < 0.05$).

The effects of both (−)-isoprenaline and RO363 in cardiac preparations from all species studied were antagonized by practolol. The K_B values obtained for practolol were independent of the agonist used in each preparation, however, there were small interspecies variations in the K_B values obtained (Table 2).

The phenylethanolamine derivative, RP333, was without inotropic and chronotropic effects in left and right atrial preparations from the guinea-pig when used in concentrations up to 0.1 mmol/l. At concentrations of 5 μmol/l, RP333 shifted (−)-isoprenaline curves to the right without affecting the maximal inotropic effects obtained (mean dose-ratio = 3.42,

$n = 3$). Higher concentrations produced little further shift in the concentration-effect curves.

Smooth muscle preparations

Bronchial In spontaneously contracted guinea-pig tracheal preparations both (−)-isoprenaline and RO363 (0.1 to 10 nmol/l) produced relaxant effects (Figure 2) while RP333 was inactive when used in concentrations up to 0.1 mmol/l. In these preparations (−)-isoprenaline and RO363 produced similar maximal relaxant effects. RO363 had a lower intrinsic activity in carbachol-contracted trachea (Table 3), however, with both types of preparation, comparison of EC_{50} concentrations showed that RO363 was approximately half as active as (−)-isoprenaline as a relaxant.

In spontaneously contracted tracheal preparations, initial qualitative experiments suggested that the relaxant effects of RO363 might be due to the activation of a population of β_1-receptors within the tissue.

Figure 3 shows traces from experiments in which the relaxant effects produced with submaximal concentrations of RO363, (−)-isoprenaline and salbutamol were differentially antagonized by butoxamine and practolol. The β_2-receptor selective antagonist, butoxamine, antagonized responses to salbutamol to the greatest extent, while RO363 was preferentially antagonized by the β_1-receptor selective compound practolol. The non-selective antagonist, propranolol, reduced the effects of all three agonists to a similar extent.

In further, more detailed studies, cumulative concentration-effect curves to (−)-isoprenaline and RO363 were first obtained and thereafter their interactions with the β-receptor antagonists propranolol

Table 1 Cardiac activities of (−)-isoprenaline and RO363

Tissue	*Iso* EC_{50}	*RO363* EC_{50}	*Relative activity*	α
Guinea-pig atria	1.70	2.78	1.87	1.01
(chronotropic)	(0.23)	(0.36)	(0.22)	(0.01)
Guinea-pig atria	2.82	6.30	2.20	0.85
(inotropic)	(0.45)	(1.92)	(0.48)	(0.05)
Guinea-pig ventricle	121	286	1.90	0.91
(inotropic)	(71)	(207)	(0.78)	(0.09)
Rabbit ventricle	287	432	2.02	0.87
(inotropic)	(50)	(57)	(0.56)	(0.08)
Rat ventricle	498	543	1.67	0.82
(inotropic)	(178)	(141)	(0.87)	(0.03)

Mean EC_{50} concentrations (nmol/l) are shown for (−)-isoprenaline (Iso) and RO363 together with the mean relative activity of the compounds (EC_{50} RO363: EC_{50} Iso) and the intrinsic activity (α) of RO363 with respect to (−)-isoprenaline (= 1). Figures in parentheses represent s.e. mean from four to nine experiments.

Table 2 Mean dissociation constants K_B values ($\times 10^{-6}$) for practolol in cardiac preparations using either (−)-isoprenaline or RO363 as agonists

Tissue	*Agonists* (−)-*Isoprenaline*	*RO363*
Guinea-pig atria	0.47	0.35
(chronotropic)	(0.09)	(0.07)
Guinea-pig ventricle	1.61	1.64
(inotropic)	(0.15)	(0.07)
Rat ventricle	3.36	4.09
(inotropic)	(1.76)	(1.43)
Rabbit ventricle	1.02	1.34
(inotropic)	(0.10)	(0.61)

Figures in parentheses show s.e. mean from three experiments with each agonist/antagonist combination.

Table 3 Agonistic activities of (−)-isoprenaline (Iso) and RO363 in smooth muscle preparations from the guinea-pig

Tissue	*Iso* EC_{50}	*RO363* EC_{50}	*Relative activity*	α
Lung strip	125	20,300	172	0.24
$n = 4$	(12)	(6400)	(64)	(0.08)
Uterus (ACh)	7.88	833	102	0.56
$n = 4$	(1.61)	(272)	(22)	(0.14)
Uterus (K^+)	5.25	2030	351	0.65
$n = 4$	(0.48)	(1070)	(172)	(0.01)
Trachea (spon)	1.62	2.55	1.62	0.99
$n = 14$	(0.23)	(0.95)	(0.31)	(0.02)
Trachea (CCh)	45.5	87.5	1.94	0.20
$n = 4$	(2.2)	(8.5)	(0.21)	(0.01)
Ileum (ACh)	21.2	36.7	2.12	0.63
$n = 6$	(3.4)	(6.1)	(0.57)	(0.06)
Ileum (stim)	10.8	25.4	1.82	0.97
$n = 6$	(2.4)	(11.3)	(0.46)	(0.02)

Agonistic activities of (−)-isoprenaline and RO363 in lung strips, acetylcholine-(ACh) and potassium-depolarized (K^+) uteri, spontaneously (spon) and carbachol (CCh)-contracted trachea, and acetylcholine (ACh) and transmurally stimulated (stim) ileal preparations. Mean EC_{50} values are expressed as nmol/l. The relative activity of RO363 with respect to (−)-isoprenaline (EC_{50} RO363: EC_{50} Iso) and its intrinsic activity (α, Iso = 1) are also shown. Figures in parentheses represent s.e. mean from n experiments.

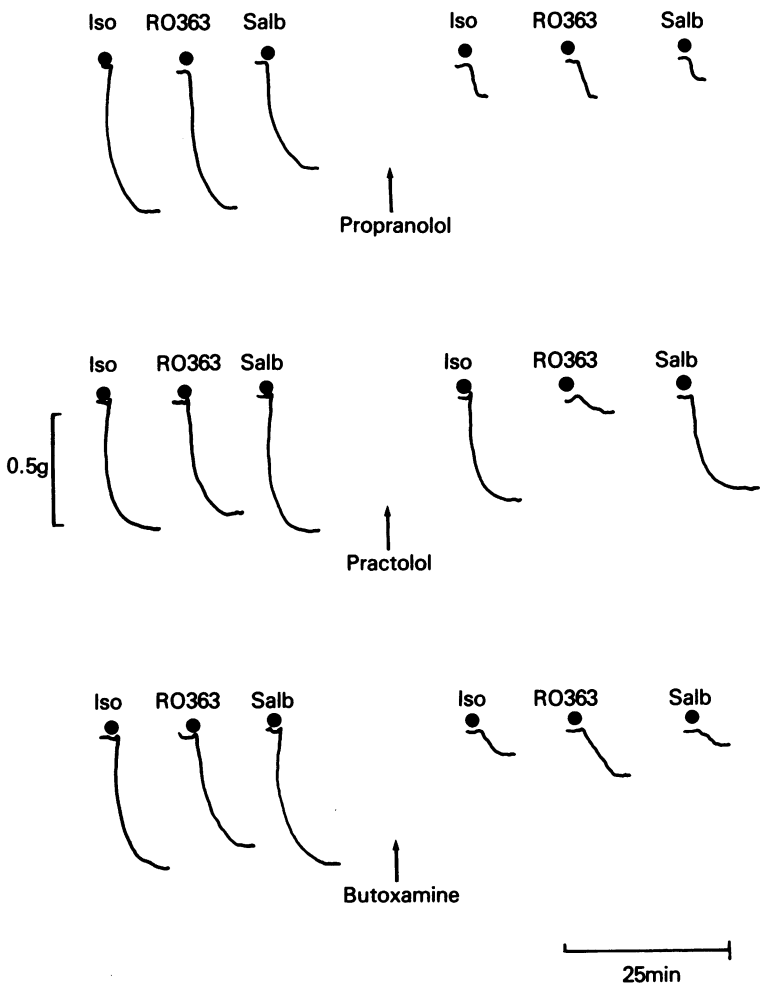

Figure 3 Submaximal relaxant responses (*circa* 60 to 80% E_{max}) to (−)-isoprenaline (Iso), salbutamol (Salb) and RO363 in spontaneously contracted tracheal preparations from the guinea-pig. Responses to a given concentration of each agonist are shown in the absence (left) and in the presence (right) of propranolol (5 nmol/l), practolol (10 μmol/l) and butoxamine (10 μmol/l).

(0.1 μmol/l), metoprolol (10 μmol/l), practolol (10 μmol/l) or butoxamine (50 μmol/l) examined.

Propranolol produced a similar shift in the concentration-effect curves to both agonists, while practolol, at a concentration which had little effect on responses to (−)-isoprenaline, caused a marked shift to the right of the curve to RO363. Butoxamine, which is not highly selective as a β_2-receptor antagonist (Siegel, Rossi & Orzechowski, 1979), shifted (−)-isoprenaline curves to the greatest extent (Figure 4). The mean K_B values obtained in these experiments are shown in Table 4. Metoprolol, which is less selective than practolol as a β_1-receptor antagonist (Harms, 1976), produced a similar but quantitatively smaller differential blocking action (Table 4).

In the carbachol-contracted preparations, where RO363 has an intrinsic activity of 0.2, it was shown that the compound produced a shift to the right of superimposed concentration-effect curves to (−)-isoprenaline. The mean pA_2 value obtained by the method of Arunlakshana & Schild (1959) was 5.74 (s.e. mean = 0.06, n = 4). The mean slope of the relationship between log (dose-ratio − 1) and log (RO363 concentration) was close to unity (0.89, s.e. mean = 0.06) which suggests that RO363 is acting as a classical dualist in this preparation.

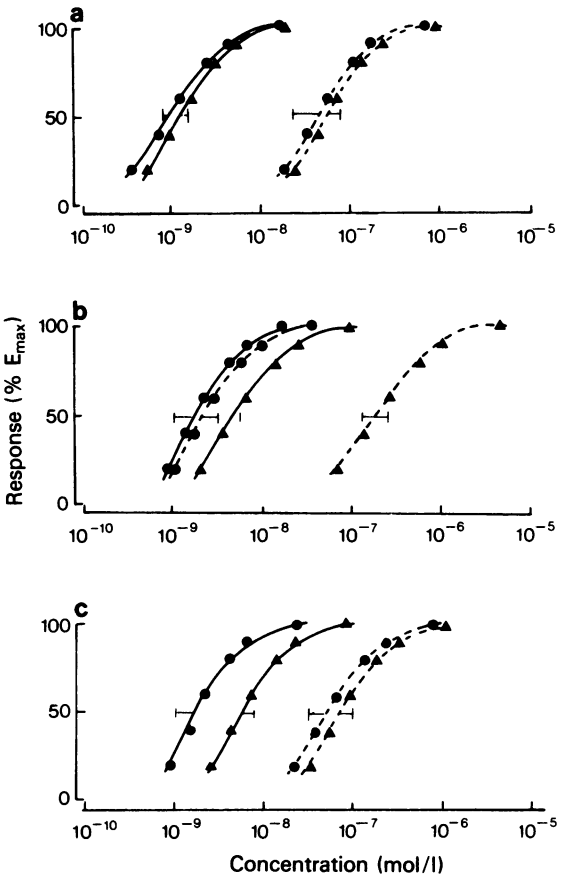

Figure 4 Mean cumulative concentration-effect curves for the relaxant effects of (−)-isoprenaline (●) and RO363 (▲) in spontaneously contracted tracheal preparations from the guinea-pig. Curves are shown in the absence (solid lines) and in the presence (broken lines) of (a) propranolol (0.1 μmol/l), (b) practolol (10 μmol/l) and (c) butoxamine (10 μmol/l). Responses are expressed as mean concentrations required to produce a given percentage of the maximal response to (−)-isoprenaline and horizontal bars at 50% E_{max} show s.e. mean from four experiments.

Both (−)-isoprenaline and RO363 produced concentration-related relaxant effects in guinea-pig lung strips (Table 3). However, unlike the effects obtained in tracheal preparations, RO363 was much less active than (−)-isoprenaline and had a very low intrinsic activity (range 0.09 to 0.36).

This precluded investigations into differential antagonism of the responses using shifts in concentration-effect curves to the agonists. However, in two experiments it was found that propranolol (1 μmol/l) abolished responses to single concentrations of RO363 and (−)-isoprenaline, and in two further experiments practolol (10 μmol/l) produced no differential blockade.

Uterine In uterine preparations, as in lung strips, RO363 was much less active and had a lower intrinsic activity than (−)-isoprenaline (Figure 2). There was a marked variation in the relative activities of RO363 with respect to (−)-isoprenaline in acetycholine-contracted (range 41 to 136) and potassium-depolarized uteri (range 100 to 833). The relaxant effects to single concentrations of the two agonists were abolished by propranolol (1 μmol/l) and no differential blockade of the responses was obtained with practolol (10 μmol/l).

In potassium-depolarized uteri, the possible antagonistic actions of RO363 were assessed from shifts in superimposed concentration-effect curves to (−)-isoprenaline. The (−)-isoprenaline curves were shifted to the right without affecting the maximal relaxation. The mean K_B value for RO363 (4.61×10^{-6}, s.e. mean = 2.08, $n = 4$) is similar to that obtained with the compound in carbachol-contracted tracheal preparations.

Ileal In ileal preparations (−)-isoprenaline and RO363 produced a concentration-related inhibition of responses to exogenous and neurally released acetylcholine. Maximal β-receptor mediated effects of (−)-isoprenaline resulted in a 40 to 50% inhibition of responses to exogenous acetylcholine and a 70 to 80% inhibition of contractions elicited by transmural stimulation. In the latter preparations RO363 and (−)-isoprenaline produced similar maximal responses,

Table 4 Dissociation constants (K_B values) for propanolol, butoxamine, metoprolol and practolol in guinea-pig spontaneously contracted tracheal preparations using either (−)-isoprenaline or RO363 as agonists

	Agonist	
Antagonist	*(−)-Isoprenaline*	*RO363*
Propranolol (0.1 μmol/l)	2.97×10^{-9} (0.74)	3.09×10^{-9} (0.87)
Butoxamine (50 μmol/l)	2.91×10^{-6} (0.92)	6.00×10^{-6} (1.56)
Metoprolol (10 μmol/l)	4.2×10^{-7} (1.5)	0.41×10^{-7} (0.01)
Practolol (10 μmol/l)	8.9×10^{-6} (1.32)	0.28×10^{-6} (0.03)

Mean K_B values were calculated from shifts in agonist concentration-effect curves produced with the stated concentration of the antagonists. Figures in parentheses represent s.e. mean from four experiments with each agonist/antagonist combination.

while in the former, RO363 had a lower intrinsic activity (Table 3). In both preparations RO363 was approximately half as active as (−)-isoprenaline, and the relaxant effects obtained were abolished by propranolol (1 μmol/l).

In transmurally stimulated preparations practolol shifted the (−)-isoprenaline and RO363 concentration-effect curves to the right without affecting maximal responses. The K_B values obtained were similar with both agonists ((−)-isoprenaline/practolol, mean $K_B = 2.52 \times 10^{-6}$, s.e. mean = 0.80, $n = 6$; RO363/practolol, mean $K_B = 1.45 \times 10^{-6}$, s.e. mean = 0.61, $n = 6$). These K_B values are similar to those obtained in cardiac preparations where the same agonist/antagonist interactions were monitored (Table 2).

Discussion

The results of the present experiments show that RO363 is a potent and highly selective β_1-receptor stimulant. Results obtained with reserpinized and non-reserpinized preparations suggest that its actions are due to direct rather than indirect sympathomimetic effects. These results confirm and extend those outlined in a brief report by Raper *et al.* (1978).

In tissues where pharmacological actions are thought to be mediated through β_1-adrenoceptor stimulation (atria, ventricles and ileum), RO363 has a similar order of potency to (−)-isoprenaline, while in tissues where the activation of β_2-receptors is involved (uterus and lung strips) it is some 100 to 350 times less active and has a low intrinsic activity.

The results obtained with RO363 in guinea-pig tracheal preparations are anomolous in this regard. Although Furchgott, Wakade, Sorace & Stollak (1975) have presented evidence to suggest that a population of β_1-receptors might exist in the tissue, it is commonly accepted that sympathomimetic actions in the trachea are due to the activation of β_2-receptors.

The similar relative activities of RO363 and (−)-isoprenaline in cardiac, ileal and tracheal preparations suggest that the relaxant effects of RO363 in the trachea may be due to the activation of β_1-receptors. This idea is further supported by the finding that K_B values for practolol in all the above preparations are similar when RO363 is used as the agonist. Furthermore, these K_B values fall within the same range as those reported by other workers who have studied the antagonistic actions of practolol at β_1-receptor sites (Drew & Levy, 1972; Cornish & Miller, 1975, Harms, 1976). The differential agonist/antagonist interactions obtained in tracheal preparations also support the above view, since the β_1-receptor selective antagonists practolol and metoprolol preferentially shift curves to RO363, the β_2-receptor selective antagonist butoxamine produces a preferential blockade of responses to (−)-isoprenaline and salbutamol, and the non-selective antagonist propranolol produces a similar blockade of responses to all three agonists.

The weak activity of RO363 in uterine and lung strip preparations, together with the lack of differential β-receptor blockade with practolol when (−)-isoprenaline and RO363 are used as agonists, suggests that the effects obtained with RO363 in these tissues are due to the activation of β_2-receptors rather than stimulation of a subpopulation of β_1-receptors.

The above results suggest that assessment of the β-receptor selectivity of agonists by comparing their relative activities with respect to (−)-isoprenaline in atrial ('β_1') and tracheal ('β_2') preparations may be wrongly interpreted.

In a general sense, the possibility that the actions of a compound are due either to activation of the dominant type of β-receptor in a tissue or to stimulation of a subpopulation of different β-receptors, cannot be differentiated without the use of selective β-receptor antagonists. The present lack of potent and highly selective β_2-receptor antagonists is a practical difficulty in this regard. In the absence of supporting experiments with antagonists, a false impression of the selective effects of an agonist are only likely to occur when a compound has highly potent and selective actions on the subpopulation of β-receptors in a tissue. This is shown in the present experiments where RO363 activates β_1- rather than β_2-receptors in the guinea-pig trachea. Previous workers have presented evidence to suggest that a subpopulation of β_2-receptors occurs in cat and human cardiac pacemaker tissue (Åblad, Carlsson, Carlsson, Dahlöf, Ek & Hultberg, 1974), while in cat trachea the dominant β-receptor appears to be of the β_1-type (Lulich *et al.*, 1976).

The latter point highlights the dangers of interspecies extrapolation regarding the types of β-receptor found in any given tissue. In addition, is suggests that more work is required in defining the nature of possible mixed receptor populations in commonly used tissues.

As with the oxymethylene derivatives of noradrenaline, adrenaline, isoprenaline and N-*t*-butylnoradrenaline (Keh, Raper & Dowd, 1978), RO363 produced weak β-receptor antagonistic effects in tracheal preparations in which the influence of spare receptors was minimized by the use of carbachol (Van den Brink, 1973). Similar antagonistic actions were also obtained with RO363 in experiments with uterine preparations. These results suggest that the inclusion of an oxymethylene link in phenylethanolamines possessing a 3,4-dihydroxy (catechol) substitution may lead to the unmasking of potential antagonistic actions.

The influence of the oxymethylene link on antagonistic activity is highlighed in β-receptor agonists possessing other than a 3,4-dihydroxy phenyl substituent. Thus although orciprenaline (3, 5-dihydroxy) is a full agonist in guinea-pig atrial and spontaneously-contracted tracheal preparations, its oxymethylene derivative (H59/36) is a partial agonist (mean α atria = 0.09, s.e. mean = 0.02, $n = 5$; mean α trachea = 0.16, s.e. mean = 0.03, $n = 4$), and displays antagonistic actions against (−)-isoprenaline (pA_2 atria = 7.66, s.e. mean = 0.02, $n = 4$; pA_2 trachea = 7.16, s.e. mean = 0.08, $n = 4$) (McPherson, unpublished observations). The low intrinsic activity of H59/36 compared with orciprenaline has also been noted by Åblad, Brogärd & Corrodi, (1970) who studied the positive chronotropic effects of the compounds in anaesthetized guinea-pigs. Likewise, H133/22 (N-isopropylphenoxypropanolamine with a 4-OH ring substituent) appears to be a β_1-receptor selective agonist in anaesthetized cats (Carlsson, Dahlöf, Hedbery, Persson & Tängstand, 1977) but has only weak stimulant effects in guinea-pig right atrial ($\alpha = 0.47$, s.e. mean = 0.11, $n = 4$) and spontaneously contracted tracheal preparations ($\alpha = 0.55$, s.e. mean = 0.01, $n = 4$). In both preparations H133/22 antagonizes responses to (−)-isoprenaline (mean pA_2 right atria = 7.08, s.e. mean = 0.08, $n = 4$; mean pA_2 trachea = 6.27, s.e. mean = 0.17, $n = 4$) (McPherson, unpublished observations). Its corresponding phenylethanolamine derivative (1-(4-hydroxyphenyl)-2-isopropylaminoethanol) has also been shown to possess β-receptor agonistic actions, and though details are lacking, it appears to be a full β-receptor agonist in variety of pharmacological preparations (Lands, Rickards, Nash & Hooper, 1947; Lands & Brown, 1967; Pratesi, Grana & Villa, 1968).

In view of the above, it would appear that the high agonistic potency of RO363 is probably due to its catechol substitution, while its β_1-receptor selective actions are due to a combination of the inclusion of an oxymethylene link and a 3,4-dimethoxyphenethyl (homoveratryl) amine substitution. In previously studied catecholamines (Dowd *et al.*, 1977), the inclusion of an oxymethylene group produced a small degree of β_1-receptor selectivity; the oxymethylene derivative of isoprenaline was the most active compound in this respect, being some five times more active as a β_1- than as a β_2-receptor agonist. In β-receptor antagonists based on a phenoxypropanolamine nucleus, homoveratryl as opposed to isopropyl or N-*t*-butyl amine substitution, produces little change in antagonistic potency at β_1-receptors while causing a marked diminution of activity at β_2-receptor sites: hence the compounds show β_1-receptor selective antagonistic actions (Hoefle, Hastings, Meyer, Corey, Holmes & Stratton, 1975; Leclerc, Mann, Wermuth, Bieth & Schwartz, 1977; Shtacher, Rubenstein & Somani, 1978).

In conclusion, the results of the present experiments show that the phenylethanolamine RP333 is devoid of β-receptor agonistic actions, while its phenoxypropanolamine derivative, RO363, is a potent and highly selective β_1-receptor agonist. The β_1-receptor selectivity of RO363 found in the isolated tissue preparations is in accord with that obtained in anaesthetized cats (Raper *et al.*, 1978). The results obtained in tracheal preparations suggest that RO363 is a useful tool for unmasking the presence of subpopulations of β_1-receptors in tissues where the dominant receptor population is of the β_2-type.

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