Br. J. Pharmac. (1974), 52, 407-417

POTENCY AND SELECTIVITY *in vitro* OF COMPOUNDS RELATED TO ISOPRENALINE AND ORCIPRENALINE ON β-ADRENOCEPTORS IN THE GUINEA-PIG

STELLA R. O'DONNELL & JANET C. WANSTALL

Department of Physiology, University of Queensland, St Lucia, Brisbane, Queensland, 4067, Australia

1 Amines related in structure to either isoprenaline (catechol series) or orciprenaline (resorcinol series) were examined for activity on isolated trachea (relaxation), atria (chronotropic action) and perfused hind-limb (vasodilatation) of the guinea-pig.

2 Compounds with a resorcinol nucleus were less potent on all three preparations but more selective for trachea than were compounds with a catechol nucleus.

3 In both catechol and resorcinol compounds potency on trachea was enhanced by and selectivity for trachea was favoured by substitution of a *p*-OH phenyl group in the *N-iso*propyl, or by replacement of the *N-iso*propyl with an *N-t*-butyl, with or without a *p*-OH phenyl group.

4 Most of the compounds, particularly the resorcinols, had much lower potencies, relative to isoprenaline, on hind-limb than on trachea.

5 Some of the problems associated with the quantitative measurement of selectivity and with sub-classification of β-adrenoceptors are discussed.

Introduction

The classification of β-adrenoceptors into two sub-groups, β_1 and β_2 (Lands, Arnold, McAuliff, Luduena & Brown, 1967a; Lands, Luduena & Buzzo, 1967b), accelerated a search for agonist and antagonist compounds with selectivity of action on different tissues containing β-adrenoceptors. Compounds have subsequently been described which cause bronchodilatation (β_2) in doses which give rise to minimal cardiac stimulation (β_1). Salbutamol (Cullum, Farmer, Jack & Levy, 1969), terbutaline (Persson & Olsson, 1970) and Th 1165a (O'Donnell, 1970) are examples.

O'Donnell (1972) examined some compounds related to Th 1165a for their selectivity of action as tracheal relaxants in the guinea-pig. Further compounds have now been examined on trachea and atria so that conclusions may be drawn on the relationship between chemical structure and potency and selectivity of the drugs on these two tissues *in vitro*. All the compounds, including those described by O'Donnell (1972), have also been examined on an isolated *in vitro* preparation of the guinea-pig hind-limb. The aim was to see whether the potent tracheal relaxants in the series were also always potent vasodilators (β_2) in preparations from the same species.

Methods

Guinea-pig isolated trachea and atria

Four-ring tracheal chains were prepared from adult female guinea-pigs weighing from 500-700 g as described by Chahl & O'Donnell (1967). The preparations were set up in Krebs solution (composition, 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) containing ascorbic acid (200 μg/ml) and aerated with 95% O_2 and 5% CO_2 at 37°C and were allowed to gain tone spontaneously until a steady level was reached (30 to 60 minutes). Relaxations were recorded isotonically with a modified Statham 10B strain gauge and a pen recorder. Drugs were administered in small volumes by an Agla micrometer syringe and cumulative concentration-response lines were obtained. Each successive dose was twice the previous dose and was added when the response to the preceding dose had reached a steady state. At the end of the determination of each line a concentration of isoprenaline producing a maximum relaxation of the preparation was added to the bath. Responses are expressed as a percentage of this maximum response.

Spontaneously beating isolated atria from the same adult female guinea-pigs were set up in Krebs

solution containing ascorbic acid (200 μg/ml) at 29-30° C. Atrial beats were recorded with a post-office counter activated by a mercury contact. Drugs were added by the cumulative method with two-fold dose increments. A contact time of 2 min for isoprenaline and 2.5 min for the other compounds was allowed so that equilibrium was reached before counting commenced. Atrial rate was obtained by taking 30 s counts at 1 min intervals. The mean of three consecutive counts was used. The response to each concentration of drug was the increase in atrial rate compared with the resting rate in the absence of any agonist. At the completion of each cumulative concentration-response line the maximum increase in rate was produced by a supramaximal concentration of isoprenaline and each response was expressed as a percentage of that maximum.

Concentration-response lines to both isoprenaline and test compound were always obtained on each preparation and, to allow for any variation in sensitivity with time, the order of addition of the two drugs was randomized between experiments. On many preparations a further concentration-response line to the test compound was then obtained in the presence of either propranolol (10^{-8} M, 60 min contact time) or cocaine (10^{-5} M, 30 min contact time).

For each preparation the cumulative bath concentration of drug on a logarithmic scale was plotted against response, expressed as a percentage of the maximum response to isoprenaline. The log molar concentration giving 50% of the maximum response (log EC_{50}) was interpolated from this line. Mean log EC_{50}s ± standard errors for each test compound and isoprenaline were then calculated from the log EC_{50} values from 5 preparations from different animals.

Guinea-pig isolated perfused femoral artery

Isolated femoral arteries from adult female guinea-pigs weighing 500-700 g were perfused with Krebs solution at 37° C by the method used by de la Lande & Rand (1965) for the rabbit ear artery. Perfusion rate was constant, ranging from 8-10 ml/min in different experiments. Changes in resistance of the artery resulted in changes in perfusion pressure which were recorded with a Statham P23AC pressure transducer and pen recorder.

Guinea-pig isolated perfused hind-limb

Single isolated hind-limbs of female guinea-pigs weighing 500-700 g were skinned and perfused through a cannula inserted into the external iliac artery. The cannula was filled with heparin to minimize clotting and small side branches of the artery were tied off so that the perfusing fluid passed into the femoral artery. The limb was perfused, at constant rate of flow using a roller pump, with Krebs solution containing an elevated Ca^{++} concentration (3.3 mM) at 37° C and bubbled with 95% O_2 and 5% CO_2. Changes in the resistance of the blood vessels resulted in changes in the perfusion pressure which were recorded with a Statham P23AC pressure transducer and pen recorder. The rate of flow ranged from 9-15 ml/min in different experiments but remained constant in any one experiment. The presence of 3.3 mM Ca^{++} in the Krebs solution raised the perfusion pressure to 40-70 mmHg. At this perfusion pressure vasodilator responses to isoprenaline and the test compounds were observed. The compounds were also tested after a single dose of propranolol (1 or 2×10^{-8} mol) which increased the vasodilator ED_{50} of isoprenaline at least 10 fold.

All drugs (in volumes of 0.025-0.2 ml) were injected into the perfusion fluid immediately proximal to the preparation. Isoprenaline and up to three test compounds were examined on each preparation. Responses to three or four different doses of each drug were obtained and a dose-response line to isoprenaline determined between that to each test compound. For each compound, log dose (mol) was plotted against response (mmHg). The log dose giving a response equal to 50% of the maximum vasodilator response to isoprenaline (log ED_{50}) was interpolated from this line. For all the compounds the mean log ED_{50} from preparations from 3 different animals was calculated. The mean log ED_{50} for isoprenaline in the same 3 experiments was also calculated.

Expression of results

Potency. On the tracheal and atrial preparations, the potency of the compounds has been expressed as the negative value of the mean log EC_{50} ± s.e. On the hind-limb preparation, potency of the compounds has been expressed as the negative value of the mean log ED_{50} ± s.e. Thus, a high figure represents high potency.

Selectivity. For some of the compounds examined the negative mean log EC_{50} on trachea was significantly greater than that on atria, that is, the compound showed selectivity for the trachea. The difference between the negative mean log EC_{50}s on the two preparations was obtained. The antilog of this difference has been defined as the selectivity ratio. A figure greater than 1 represents

selectivity for trachea and less than 1 selectivity for atria.

Relative potency. The potency (as defined above) of each compound was compared with that of isoprenaline in the same group of preparations and a figure for relative potency of each compound, compared with isoprenaline as 100, obtained on trachea, atria and hind-limb.

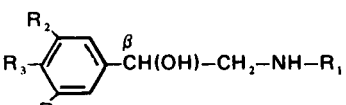
Drugs

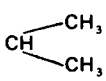
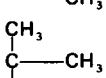
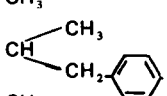
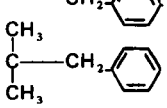
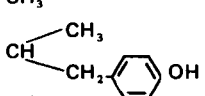
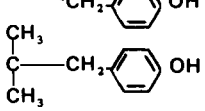
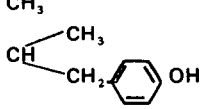
The chemical structures of the compounds examined are shown in Table 1. The compounds used were: Cc 24 base (mixture of two racemates, Philips-Duphar); Cc 25 benzoate (mixture of two racemates, Philips-Duphar); (±)-Cc 33 base (Philips-Duphar); (±)-Cpa 25 acetate (Philips-Duphar); (±)-isoprenaline sulphate (Burroughs-Wellcome); (±)-Me 454 base (Boehringer-Ingelheim); (±)-Me 506 hydrobromide (Boehringer-Ingelheim); (±)-orciprenaline sulphate (Boehringer-Ingelheim); (±)-terbutaline hydrochloride (Me 501, Boehringer-Ingelheim); (±)-tertiary-butyl noradrenaline (Sterling-Winthrop); Th 1161 hydrochloride (mixture of two racemates, Boehringer-Ingelheim); Th 1165a hydrobromide (high m.p. racemate, m.p. 228-230° C, Boehringer-Ingelheim).

Other drugs used were: cocaine hydrochloride (D.H.A.); histamine acid phosphate (B.D.H.); (−)-noradrenaline acid tartrate (Sigma); phentolamine methanesulphonate (Regitine, Ciba); (±)-propranolol hydrochloride (I.C.I.).

All drugs were obtained as pure powders except for phentolamine which was obtained as a solution in ampoules. The sympathomimetic amines were made up in 0.01 N HCl to give stock solutions of 10^{-2} M. Dilutions were made in Krebs solution containing ascorbic acid (0.2 mg/ml) and kept on ice during the determination of the dose-response line.

Table 1 Compounds examined for their potency on β-adrenoceptors.



R_1 N-*alkyl substituent group*	R_2, R_3 = OH, R_4 = H *Catechols*	$R_2 R_4$ = OH, R_3 = H *Resorcinols*
	Isoprenaline	Orciprenaline
	Tertiary-butyl noradrenaline (*t*-butyl NA)	Terbutaline
	Cc 24	Th 1161*
	Cc 33	Not examined
	Cc 25*	Th 1165a*
	Me 454	Me 506*
	Cpa 25 (no β-OH group)	Not examined

Compounds with an asterisk were examined only on the isolated hind-limb. Data for activity of these compounds on isolated trachea and atria are from O'Donnell (1972).

Statistical Analyses

The measure of variation of the mean quoted is the standard error. Student's t test was used to assess the significance of the difference between two mean (neg. log EC_{50}s). Tests of significance on pairs of differences were made by applying a t-test using a total weighted average variance.

Results

Potency on trachea and atria

Six of the compounds illustrated in Table 1 were examined on tracheal and atrial preparations in this study, t-butyl noradrenaline (t-butyl NA), Cc 24, Cc 33, Me 454, Cpa 25 and terbutaline. One of these, Cpa 25, which lacks a hydroxyl group on the β-carbon atom in the side-chain, is discussed separately. The potencies on trachea and atria of the other six compounds listed in Table 1 have previously been described (O'Donnell, 1972), but data on isoprenaline and orciprenaline were also obtained in this study.

The compounds examined in the present study all produced a concentration-dependent relaxation of the trachea and the same maximum relaxation as isoprenaline; they did not affect the sensitivity of the trachea to isoprenaline. Although the response of the trachea to these compounds tended to be slower than that to isoprenaline the log concentration-response lines were parallel. On atria the compounds produced a concentration-dependent positive chronotropic effect. The log concentration-response lines were parallel to that of isoprenaline and the compounds did not affect the sensitivity of the atria to isoprenaline. The same maximum increase in heart rate as with isoprenaline was achieved except after terbutaline where it was only 72-90% of the isoprenaline maximum response.

On both trachea and atria, propranolol (10^{-8} M) caused a parallel shift of the log concentration-response lines for all the compounds to a higher concentration range with no change in maximum response. On neither preparation were the compounds potentiated by cocaine (10^{-5} M).

Table 2 summarizes the potencies of 10 compounds on trachea and atria including those described previously (O'Donnell, 1972) which are included so that comparisons can be made between drugs. Of the compounds in the present study, 2 were more potent than isoprenaline on trachea, t-butyl NA ($P < 0.01$) and Me 454 ($P < 0.001$). Cc 33 and terbutaline were less potent than isoprenaline ($P < 0.05$ and $P < 0.001$) respectively) and Cc 24 was not different from isoprenaline. On atria all these compounds were less potent than isoprenaline ($P < 0.001$) except for Me 454 which did not differ from isoprenaline.

Selectivity for trachea

The selectivity ratios (see methods section) for the 10 compounds are summarized in Table 3. All the resorcinol compounds were selective for trachea and each one was more selective than the corresponding catechol compound possessing the same N-alkyl substituent group.

The order of selectivity of the compounds was the same in the catechol and resorcinol series when related to the N-alkyl substituent group. The most selective compounds possessed a t-butyl substituent and the greatest selectivity was achieved if a p-hydroxy phenyl group was also substituted in the t-butyl group (e.g. Me 454, Me 506). Compounds with a p-hydroxyphenyl group substituted in the N-*iso*propyl group (Cc 25, Th 1165a) also showed selectivity which was absent (Cc 24) or less marked (Th 1161) if the p-hydroxy group was not present.

Isolated perfused femoral artery

On this preparation vasodilatation could be demonstrated only as a physiological antagonism of constrictor responses to histamine. A vasodilator activity of acetylcholine could be revealed in this way, but not of isoprenaline. It was concluded, therefore, that the guinea-pig femoral artery did not contain a significant population of β-adrenoceptors. The artery constricted in response to noradrenaline (5×10^{-10} to 5×10^{-9} mol) and the responses were blocked by a dose of phentolamine (1.5×10^{-8} mol). The femoral artery preparation was used to ascertain whether the compounds possessed significant α-adrenoceptor stimulant activity which, if present, might be expected to modify the vasodilator potency on the whole limb.

Vasoconstriction was obtained with isoprenaline (5×10^{-7} to 2×10^{-6} mol) but none of the test compounds caused vasoconstriction in doses from 2.5×10^{-10} mol to 2×10^{-6} mol. In the highest doses used some of the compounds (Cc 24, Cc 33, Me 454, and Me 506) appeared to exhibit α-adrenoceptor blocking activity in that they caused a reversible block of the responses to subsequent doses of noradrenaline without affecting the constrictor responses to either histamine or K^+.

Potency on hind-limb

Isoprenaline (5×10^{-11}-1×10^{-9} mol) produced a dose-dependent fall in perfusion pressure of the

Table 2 Potencies of compounds compared with isoprenaline on trachea, atria and hind-limb.

Name of compound	*Negative log EC_{50} ± s.e. (Molar concentration)*				*Negative log ED_{50} ± s.e. (Dose in mol)*	
	Trachea		*Atria*		*Hind-limb*	
	Compound	*Isoprenaline*	*Compound*	*Isoprenaline*	*Compound*	*Isoprenaline*
t-Butyl NA	8.93 ± 0.11** (5)	8.26 ± 0.15 (5)	7.92 ± 0.04*** (5)	8.61 ± 0.05 (5)	10.45 ± 0.22 (3)	10.20 ± 0.21 (3)
Cc 24	7.93 ± 0.06 (5)	7.99 ± 0.10 (5)	8.00 ± 0.10*** (5)	8.66 ± 0.05 (5)	10.14 ± 0.15 (3)	9.70 ± 0.08 (3)
Cc 33	8.00 ± 0.08* (5)	8.27 ± 0.06 (5)	7.73 ± 0.07*** (5)	8.70 ± 0.04 (5)	10.05 ± 0.10 ((3)	9.70 ± 0.14 (3)
Cc 25†	9.76 ± 0.14*** (8)	8.29 ± 0.11 (8)	9.04 ± 0.20 (8)	8.70 ± 0.04 (8)	10.99 ± 0.16* (3)	10.17 ± 0.19 (3)
Me 454	9.43 ± 0.16*** (5)	8.37 ± 0.08 (5)	8.33 ± 0.19 (5)	8.56 ± 0.16 (5)	10.94 ± 0.18 (3)	10.20 ± 0.21 (3)
Orciprenaline	7.03 ± 0.06*** (5)	7.99 ± 0.05 (5)	6.56 ± 0.06*** (5)	8.49 ± 0.07 (5)	8.21 ± 0.26** (3)	10.19 ± 0.17 (3)
Terbutaline	7.09 ± 0.05*** (5)	7.60 ± 0.05 (5)	5.43 ± 0.08*** (5)	8.87 ± 0.06 (5)	8.43 ± 0.22** (3)	10.20 ± 0.21 (3)
Th 1161†	7.03 ± 0.10*** (5)	7.90 ± 0.11 (5)	6.07 ± 0.12*** (5)	8.37 ± 0.04 (5)	7.53 ± 0.18*** (3)	9.64 ± 0.14 (3)
Th 1165a†	8.65 ± 0.04*** (32)	8.06 ± 0.05 (29)	7.28 ± 0.05*** (28)	8.45 ± 0.04 (27)	9.54 ± 0.37 (3)	10.00 ± 0.26 (3)
Me 506†	7.94 ± 0.11 (6)	8.03 ± 0.06 (6)	6.21 ± 0.11*** (6)	8.63 ± 0.07 (6)	8.98 ± 0.28* (3)	10.12 ± 0.21 (3)

Number of preparations is in brackets. Results on trachea and atria for the compounds marked (†) are from O'Donnell (1972); isoprenaline results for these compounds are data previously obtained by O'Donnell (unpublished).

Asterisks indicate significant differences between the potency of the compound and that of isoprenaline: * $0.05 > P > 0.01$; ** $0.01 > P > 0.001$; *** $P < 0.001$.

Table 3 Effect of changing the *N*-alkyl substituent group on the selectivity of catechol and resorcinol compounds for trachea compared with atria.

N-alkyl substituent group	*Name of compound*		*Difference between neg. log EC_{50}s for trachea and atria ± s.e.*		*Comparison bet. resorcinol and catechol (t value)*	*Selectivity ratio trachea : atria (antilog difference)*	
	Catechols	*Resorcinols*	*Catechols*	*Resorcinols*		*Catechols*	*Resorcinols*
$C(CH_3)_2-CH_2-$  $-OH$	Me 454	Me 506	1.10 ± 0.25** (5, 5)†	1.73 ± 0.16*** (6, 6)	3.60**	12.6	51.7
$C(CH_3)_3$	*t*-butyl NA	Terbutaline	1.01 ± 0.11*** (5, 5)	1.66 ± 0.09*** (5, 5)	5.43***	10.2	45.7
$CH(CH_3)CH_2-$  $-OH$	Cc 25	Th 1165a	0.72 ± 0.25* (8, 8)	1.37 ± 0.06*** (32, 28)	6.95***	5.2	25.5
$C(CH_3)_2CH_2-$ 	Cc 33	–	0.27 ± 0.11* (5, 5)	–	–	1.9	–
$CH(CH_3)CH_2-$ 	Cc 24	Th 1161	−0.07 ± 0.12 (5, 5)	0.96 ± 0.16*** (5, 5)	8.38***	0.85	9.1
$CH(CH_3)_2$	Iso	Orciprenaline	−0.60 ± 0.06*** (35, 35)	0.47 ± 0.09*** (5, 5)	12.44***	0.25	3.0

† Number of tracheal and atrial preparations used. Differences between negative log EC_{50}s for trachea and atria are derived from Table 2. A positive value indicates that the compound was more potent on trachea than atria, a negative value that it was more potent on atria than trachea.
Asterisks indicate significant differences: * $0.05 > P > 0.01$; ** $0.01 > P > 0.001$; *** $P < 0.001$.

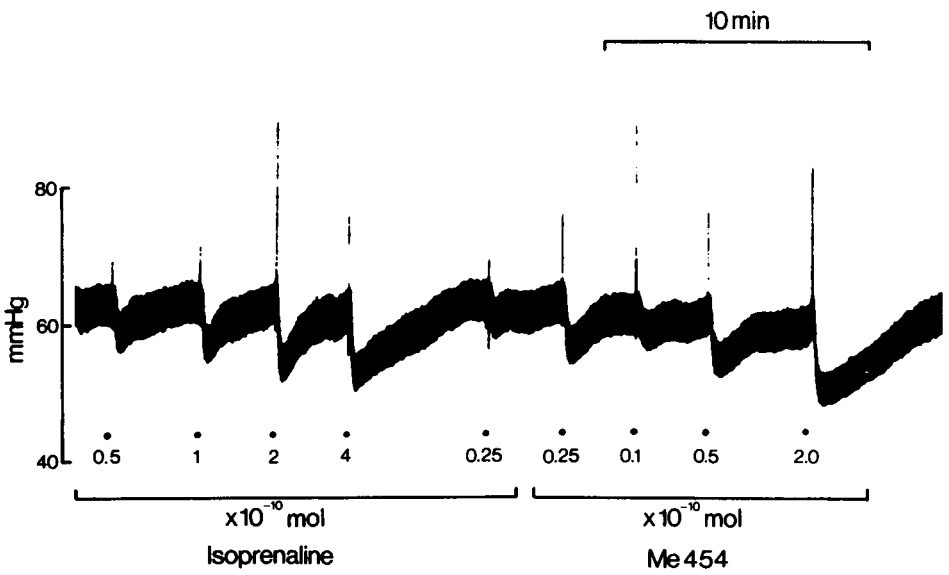


Fig. 1 Guinea-pig isolated hind-limb preparation. Perfusion at 37° C at 15 ml/min with Krebs solution containing 3.3 mM Ca^{++} and ascorbic acid (200 μg/ml), bubbled with 95% O_2 : 5% CO_2. Decreases in perfusion pressure (vasodilatation) were produced by isoprenaline and Me 454 administered in volumes from 0.025 to 0.2 ml.

hind-limb (vasodilatation). All the compounds in Table 2 also caused a decrease in hind-limb perfusion pressure. Vasodilator responses to isoprenaline and Me 454 are shown in Figure 1. The vasodilator potencies of the compounds are shown in Table 2. Although all the catechol compounds tended to be more potent than isoprenaline, the difference was significant only for Cc 25 ($P < 0.05$). The resorcinol compounds were less potent than isoprenaline although for Th 1165a the difference was not significant. The hind-limb responses to isoprenaline and all the test compounds could be blocked by propranolol (1 or 2×10^{-8} mol).

Relative potencies

A direct comparison between the potencies of the test compounds on trachea or atria and that on the hind-limb could not be made. This was because in tracheal and atrial experiments actual bath concentrations of drug were known allowing calculation of EC_{50}s, whereas in the hind-limb experiments only the dose of drug administered was known and thus ED_{50}s were calculated. Also, paired tracheal and atrial preparations taken from the same guinea-pig were used, whereas the hind-limb experiments were carried out on a different group of animals. To circumvent this problem the potencies of all the test compounds were directly related to that of isoprenaline since this had been included as a reference drug in all experiments. The relative potency results gave a figure on each tissue whereby each catechol was compared to isoprenaline and by which each resorcinol compound could be compared to orciprenaline. They also enabled the potency of each compound on the three tissues to be compared. The latter comparisons are dependent upon isoprenaline itself being a non-selective agonist since it is arbitrarily assigned a figure of 100 on each tissue. Isoprenaline showed a small, but significant, selectivity for atria ('cardio-selectivity') in the experiments (Table 2). Comments have only been made on differences between relative potencies which are large enough for this small isoprenaline difference to be negligible.

The figures in Table 4 show that, on trachea, the catechol compounds tended to be more potent than isoprenaline and the resorcinol compounds more potent than orciprenaline. In contrast, on atria, the catechol compounds (except Cc 25) were less potent than isoprenaline and the resorcinol compounds (except Th 1165a) less potent than orciprenaline. As a result of these trends, there was clear separation between the relative potency figures on trachea and atria for most compounds

Table 4 Relative potencies of compounds (isoprenaline = 100) on trachea, atria and hind-limb.

Name of compound	*Difference between neg. log EC_{50}s or ED_{50}s of compound and iso ± s.e.*			*Comparison bet. trachea and hind-limb (t value)*	*Relative potency (isoprenaline = 100)*		
	Atria	*Trachea*	*Hind limb*		*Atria*	*Trachea*	*Hind-limb*
Isoprenaline					100	100	100
t-butyl NA	−0.69 ± 0.05	0.67 ± 0.14	0.25 ± 0.30	1.85	20	468	178
Cc 24	−0.66 ± 0.11	−0.06 ± 0.06	0.44 ± 0.17	3.72**	22	87	275
Cc 33	−0.97 ± 0.08	−0.27 ± 0.10	0.35 ± 0.17	4.88***	11	54	224
Cc 25	0.34 ± 0.20	1.47 ± 0.18	0.82 ± 0.25	3.25**	219	2950	661
Me 454	−0.23 ± 0.25	1.06 ± 0.18	0.74 ± 0.28	1.54	59	1150	550
Orciprenaline	−1.93 ± 0.09	−0.96 ± 0.08	−1.98 ± 0.31	5.56***	1.2	11	1.0
Terbutaline	−3.44 ± 0.10	−0.51 ± 0.07	−1.77 ± 0.30	7.32***	0.036	31	1.7
Th 1161	−2.30 ± 0.13	−0.87 ± 0.15	−2.11 ± 0.23	7.06***	0.50	13	0.78
Th 1165a	−1.17 ± 0.06	0.59 ± 0.06	−0.46 ± 0.45	7.43***	6.8	390	35
Me 506	−2.42 ± 0.13	−0.09 ± 0.13	−1.14 ± 0.35	5.21***	0.38	81	7.2

Differences between negative log values for compound and isoprenaline were derived from Table 2. A positive value indicates that the compound was more potent than isoprenaline, a negative value that it was less potent. This is seen more clearly in the right hand columns where the differences are converted to relative potencies with isoprenaline expressed as 100.
Asterisks indicate significant differences between trachea and hind-limb: * $0.05 > P > 0.01$; ** $0.01 > P > 0.001$; *** $P < 0.001$.

Table 5 Potencies and relative potencies of Cpa 25 on trachea and atria compared with isoprenaline.

	Potency Neg. log EC_{50} ± s.e.		*Difference between neg. log EC_{50}s for trachea and atria ± s.e.*	*Selectivity ratio trachea : atria (antilog. difference)*
	Trachea	*Atria*		
Cpa 25	6.34 ± 0.07 (5)	6.37 ± 0.12 (5)	−0.03 ± 0.14	0.93
Isoprenaline	7.96 ± 0.06 (5)	8.76 ± 0.08 (5)	−0.80 ± 0.10***	0.16
Difference between neg. log EC_{50}s of Cpa 25 and isoprenaline	−1.62 ± 0.09***	−2.39 ± 0.14***		
Relative potency of Cpa 25 (isoprenaline = 100)	2.4	0.41		

*** Difference $P < 0.001$.

(even allowing for the small 'cardioselectivity' of isoprenaline referred to above) and this would be in accordance with the tracheal selectivity (Table 3) exhibited by most of the compounds. On hind-limb, as on trachea, the catechol compounds tended to be more potent than isoprenaline and the resorcinol compounds more potent than orciprenaline. The relative potencies on hind-limb differed significantly from those on trachea for all compounds except *t*-butyl NA and Me 454. The difference was particularly marked in the resorcinol compounds and the relative potencies of the resorcinol compounds were at least 10-fold lower on hind-limb than on trachea.

Compound Cpa 25

This compound is identical in structure to Cc 25 except that it lacks a hydroxyl group on the β-carbon atom. It was much less potent than isoprenaline on both trachea ($P < 0.001$) and atria ($P < 0.001$, Table 5). In contrast to Cc 25, the log EC_{50}s on trachea and atria were not different, that is, it was not selective for trachea (Table 5). With doses of Cpa 25 up to 5×10^{-7} mol there was no vasodilator response on the hind-limb whilst the ED_{50} for isoprenaline is between 6×10^{-11} and 2×10^{-10} mol. No constrictor response of the isolated artery occurred with doses of Cpa 25 up to 2×10^{-5} mol.

Discussion

In developing new β-adrenoceptor stimulants as potential bronchodilators for use in asthma, it is advantageous for compounds to have high potency combined with some degree of selectivity of action for β-adrenoceptors in the respiratory tract. If the classification of Lands *et al.* (1967a, b) is accepted then potent sympathomimetic bronchodilators (β_2) will also be potent vasodilators (β_2) although not necessarily potent cardiac stimulants (β_1). The subclassification of β-receptors by Lands and his co-workers was based on comparisons of *in vitro* with *in vivo* responses in a number of different species. Since it is now recognized that results can vary between species (Bowman & Rodger, 1972), the present study was carried out with *in vitro* preparations from only one species, the guinea-pig. The effect on tracheal β-adrenoceptor potency and on β-adrenoceptor selectivity of changing the catechol nucleus to resorcinol in amines with the same *N*-alkyl substituent group and of changing the *N*-alkyl substituent group in amines with either a catechol or resorcinol nucleus has been examined.

Without exception, each resorcinol compound was less potent than the corresponding catechol compound on trachea, atria and hind-limb. In both the catechol and resorcinol series, tracheal potency was increased if *N*-*iso*propyl was replaced by *N*-*t*-butyl or if a *p*-OH phenyl group was substituted on either the isopropyl or the *t*-butyl group. A phenyl group without the *p*-OH did not enhance potency. Similar relationships have been reported for a series of catechols examined for their bronchodilator activity against acetylcholine induced bronchoconstriction in guinea-pigs *in vivo* (Moed, Van Dijk & Niewand, 1955).

Most of the compounds were not equipotent on trachea and atria since the substituent groups favouring tracheal potency did not necessarily favour atrial potency. Quantitative estimation of the degree of selectivity was made using the method of O'Donnell (1972), that is, the log EC_{50}s

of each compound on trachea and atria were compared. Under the conditions of our experiments, isoprenaline was not equipotent on trachea and atria. Other workers who provide quantitative data on the selectivity of β-receptor agonists have compared the potencies of compounds relative to isoprenaline on the different tissues (Farmer & Levy, 1968; Fogelman & Grundy, 1970). Our results were also analysed in terms of relative potency to isoprenaline. Because isoprenaline was not equipotent on trachea and atria the two methods of estimating trachea/atria selectivity gave different quantitative results. Relative potency, using isoprenaline as reference drug, tended to exaggerate the potential selectivity of a compound. This is clearly seen with the data for compound Cpa 25 (see Table 5). Comparison of the EC_{50}s on trachea and atria suggest that Cpa 25 is equipotent on the two tissues, that is, non-selective. On the other hand, the relative potency of Cpa 25 (compared to isoprenaline) was six times greater on trachea than atria, which could be interpreted as selectivity for trachea.

Although the quantitative estimate of selectivity differs according to which of the above methods is used, the same conclusions on the relationship between structure and tracheal selectivity amongst the compounds in this study were reached, irrespective of the method of analysis. All the resorcinol compounds were more selective than the corresponding catechol compounds; in both the catechol and resorcinol series the most selective compounds possessed an *N*-*t*-butyl, with or without a *p*-OH phenyl substituent; and substitution of a *p*-OH phenyl group, but not the phenyl group, in the *N*-*iso*propyl radical, conferred selectivity.

The only means whereby the potency of the compounds on trachea could be compared with that on the hind-limb was to use relative potencies (see results section). Although those *N*-alkyl substituents which favoured tracheal potency also favoured hind-limb potency, the relative potency values on these two tissues were not always quantitatively similar. In the resorcinol series the relative potencies on hind-limb were at least 10-fold lower than those on trachea, that is, these compounds appeared to be selective for trachea compared to hind-limb. If this apparent selectivity had simply reflected a difference in the potency of the reference compound (isoprenaline) on the two tissues, as described above for Cpa 25 on trachea and atria, then a similar selectivity would also have been seen among the catechol compounds. On the contrary, some of the catechol compounds had higher relative potencies on hind-limb than on trachea.

It is thus tempting to speculate that the resorcinol compounds distinguish between two different β-receptor types in trachea and blood vessels, especially since Levy (1973) recently suggested that the β-adrenoceptors in trachea, heart and blood vessels of dog were different. The two approaches used by early workers who subclassified β-receptors using data on the relative potencies of agonists were applied to our data to see whether this speculation was justified. Use of the Spearman rank order correlation coefficient used by Lands *et al.* (1967b) showed a significant correlation between trachea and hind-limb ($r_s = 0.81$, $P < 0.01$) suggesting no difference between the receptors in these two tissues. However, significant correlation was also found between trachea and atria ($r_s = 0.75$, $P < 0.01$), from which one could also conclude that tracheal and atrial receptors were not different. The latter conclusion is not supported by the large differences in EC_{50}s of the compounds, observed on trachea and atria, and is not in keeping with the postulation of Lands *et al.* (1967a) that cardiac and bronchodilator responses are mediated by different receptor types. Thus it is doubtful whether the high correlation between tracheal and hind-limb relative potencies obtained by this rank order analysis can be taken as adequate evidence for the receptors in these two tissues being the same. Comparison of the quantitative values of relative potencies of agonists, as used by Furchgott (1967), showed no similarity between trachea and atria, suggesting different receptors mediating these two responses. Some quantitative similarity between the relative potencies of the five catechol compounds on trachea and hind-limb was found. This supports the concept that β-adrenoceptors in trachea and hind-limb are similar. The 10-fold difference between the relative potency values on trachea and hind-limb for all the resorcinol compounds cannot be ignored. Before concluding that this difference reflects selectivity of the compounds for different receptors in the two tissues, one must consider whether or not it can be explained by factors unrelated to the nature of the β-receptor. For instance, an underestimate of the vasodilator relative potencies of the resorcinols could occur if the drugs possessed vasoconstrictor activity. Other agonists with a selectivity for β_2-receptors have been shown to have weak vasoconstrictor activity, probably mediated by α-adrenoceptors, e.g. soterenol (Dungan, Cho, Gomoll, Aviado & Lish, 1968), salbutamol and AQ 110 (Fogelman & Grundy, 1970); none of the resorcinol compounds constricted the guinea-pig femoral artery (which appears to possess only α-adrenoceptors) in doses equal to, or much higher than, those producing hind-limb vasodilatation.

Conversely, factors which might overestimate the relative potencies of the resorcinol compounds on trachea include (a) additional non-β-receptor relaxation of the trachea by the resorcinol compounds and (b) a site of loss in trachea with an affinity for the reference compound, isoprenaline, but not for the resorcinol compounds. Neither of these possibilities has been excluded in the present study. Nevertheless, the *in vitro* experiments carried out do suggest that compounds related to orciprenaline can be less potent as vasodilators than might be anticipated from their potency as tracheal relaxants. *In vivo* experiments with orciprenaline (Engelhardt, Hoefke & Wick, 1961) and Th 1165a (O'Donnell, 1970) would appear to support this observation.

A preliminary report of this work was presented to the 20th meeting of the Australian Physiological and Pharmacological Society, University of New South Wales, August, 1973. The work was supported by a grant from the National Health & Medical Research Council of Australia and this aid is gratefully acknowledged One of us (J.C.W.) is an N.H. & M.R.C. Research Officer. Our thanks are also due to Miss Christine Hunjas for excellent technical assistance, and to Dr G. Hall (Boehringer-Ingelheim, Australia), Dr J. van Dijk (Philips Duphar, Holland) and Dr S. Archer (Sterling Winthrop Research Institute, U.S.A.) for kindly providing the compounds investigated.

References

BOWMAN, W.C. & RODGER, I.W. (1972). Actions of the sympathomimetic bronchodilator, rimiterol (R798), on the cardiovascular, respiratory and skeletal muscle systems of the anaesthetized cat. *Br. J. Pharmac.*, **45**, 574-583.

CHAHL, L.A. & O'DONNELL, S.R. (1967). The interaction of cocaine and propranolol with catecholamines on guinea pig trachea. *Eur. J. Pharmac.*, **2**, 77-82.

CULLUM, V.A., FARMER, J.B., JACK, D. & LEVY, G.P. (1969). Salbutamol: a new, selective β-adrenoceptive receptor stimulant. *Br. J. Pharmac.*, **35**, 141-151.

de la LANDE, I.S. & RAND, M.J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol. med. Sci.*, **43**, 639-656.

DUNGAN, K.W., CHO, Y.W., GOMOLL, A.W., AVIADO, D.M. & LISH, P.M. (1968). Pharmacologic potency and selectivity of a new bronchodilator agent: Soterenol (MJ 1992). *J. Pharmac. exp. Ther.*, **164**, 290-301.

ENGELHARDT, A., HOEFKE, W. & WICK, H. (1961). Zur pharmakologie des sympathomimeticums 1-(3,5-dihydroxyphenyl)-1-hydroxy-2-isopropyl-amino-äthane. *Ärztl. Forsch.*, **11**, 521-525.

FARMER, J.B. & LEVY, G.P. (1968). New sympathomimetic amines: actions on catecholamine receptors. *Br. J. Pharmac.*, **34**, 698P.

FOGELMAN, F. & GRUNDY, H.F. (1970). A comparison of the cardiovascular actions of AQ 110 with those of isoprenaline and salbutamol *Br. J. Pharmac.*, **38**, 416-432.

FURCHGOTT, R.F. (1967). The pharmacological differentiation of adrenergic receptors. *Ann. N.Y. Acad. Sci.*, **139**, 553-570.

LANDS, A.M., ARNOLD, A., McAULIFF, J.P., LUDUENA, F.P. & BROWN, T.G., Jnr. (1967a). Differentiation of receptor systems activated by sympathomimetic amines. *Nature, Lond.*, **214**, 597-598.

LANDS, A.M., LUDUENA, F.P. & BUZZO, H.J. (1967b). Differentiation of receptors responsive to isoproterenol. *Life Sci.*, **6**, 2241-2249.

LEVY, B. (1973). The β-adrenoceptor blocking properties of the α-methyl analogues of propranolol and practolol in the anaesthetized dog. *Br. J. Pharmac.*, **49**, 514-526.

MOED, H.D., VAN DIJK, J. & NIEWIND, H. (1955). Synthesis of β-phenylethylamine derivatives. (iii) Bronchodilators. *Recl. Trav. chim. Pays-Bas. Belg.*, **74**, 919-936.

O'DONNELL, S.R. (1970). A selective β-adrenoceptor stimulant (Th 1165a) related to orciprenaline. *Eur. J. Pharmac.*, **12**, 35-43.

O'DONNELL, S.R. (1972). An examination of some β-adrenoreceptor stimulants for selectivity using the isolated trachea and atria of the guinea pig. *Eur. J. Pharmac.*, **19**, 371-379.

PERSSON, H. & OLSSON, T. (1970). Some pharmacological properties of Terbutaline (INN), 1-(3,5-dihydroxyphenyl)-2-(*t*-butylamino)-ethanol. A new sympathomimetic β-receptor-stimulating agent. *Acta med. scand.* suppl. **512**, 11-19.

(Received November 20, 1973)