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SELECTIVITY OF β-ADRENOCEPTOR AGONISTS AND ANTAGONISTS ON BRONCHIAL, SKELETAL, VASCULAR AND CARDIAC MUSCLE IN THE ANAESTHETIZED CAT

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1 The potencies of fifteen β-adrenoceptor agonists of widely differing chemical structures were compared with that of (−)-isoprenaline on bronchial muscle, soleus muscle, blood pressure and heart rate in the anaesthetized cat. The β-adrenoceptor antagonist potencies of propranolol and practolol were determined against (−)-isoprenaline in the same model.

2 (−)-Isoprenaline was the most potent agonist and its action was essentially unselective. Thus, on all four parameters the minimal effective dose was 0.003–0.01 μg/kg and maximal or near maximal responses were produced by 0.3–1 μg/kg. Trimetoquinol was also an essentially unselective agonist.

3 For thirteen of the remaining fourteen agonists, potency was similar on bronchial muscle, soleus muscle and blood pressure but significantly lower on heart rate.

4 The remaining agonist — AH 7616 (4-hydroxy-α^1-[[(1-methyl-3,3-diphenyl-propyl)amino]-methyl]-m-xylene-α^1,α^3-diol, acetate) – was also significantly less potent on heart rate than on the other parameters; in addition, it was clearly less potent on soleus muscle and blood pressure than on bronchial muscle when 5-hydroxytryptamine (5-HT) was used to induce bronchospasm. However, when acetylcholine was used instead of 5-HT the potency of AH 7616 on bronchial muscle, soleus muscle and blood pressure was very similar. AH 7616 may therefore possess a specific 5-HT antagonist action in addition to its β-adrenoceptor agonist action.

5 The fifteen test agonists were longer acting than (−)-isoprenaline and this was particularly true of trimetoquinol and soterenol.

6 The β-adrenoceptor antagonist potency of propranolol was almost identical on bronchial muscle, soleus muscle and blood pressure and very slightly lower on the heart. Practolol was 10–12 times more potent on the heart than on bronchial muscle, soleus muscle and blood pressure.

7 These findings suggest that it may not be possible to separate the bronchodilating and tremor-enhancing properties of β-adrenoceptor agonists. The results with agonists and antagonists are in accord with Lands' dual β-adrenoceptor sub-classification.

Introduction

β_2-Adrenoceptor agonists such as salbutamol (Cullum, Farmer, Jack & Levy, 1969) and terbutaline (Bergmann, Persson & Wetterlin, 1969) can produce effective bronchodilatation in man with relatively little accompanying cardiac stimulation (Legge, Gaddie & Palmer, 1971), but enhancement of physiological tremor has been noted as a side-effect, particularly after oral administration (Freedman, 1971; Legge *et al.*, 1971; Minette, 1971; Epstein, Barnard & Zsotér, 1973).

The enhancement of physiological tremor is thought to be mediated directly through β-adrenoceptors located in skeletal muscle (Marsden, Foley, Owen & McAllister, 1967; Marsden & Meadows, 1968); thus, β-adrenoceptor agonists decrease the degree of fusion and tension developed during submaximal tetanic contractions of the slow contracting motor units in human muscle (Marsden & Meadows, 1970). The same effects are produced in slow contracting skeletal muscle from animals, for example cat soleus muscle (Bowman & Zaimis, 1958). Bowman & Nott (1970) suggested therefore that the cat soleus would provide a useful model for forecasting tremor-enhancing potential in man. Using this preparation the skeletal muscle β-adrenoceptor has been classified as a β_2-type, like that in bronchial muscle (Bowman & Nott, 1970). From studies with a limited number of β-adrenoceptor agonists and antagonists it was concluded that differentiation of the β_2-adrenoceptors in these two tissues might not be possible (Bowman & Nott, 1970; Bowman & Rodger, 1972).

The present paper describes the results of a wide-

ranging study of this problem. The effects of 16 β-adrenoceptor agonists and of two β-adrenoceptor antagonists on bronchial muscle, soleus muscle, blood pressure and heart rate have been evaluated in the anaesthetized cat. The results provide further information on two inter-related questions: first, the possibility of separating the tremor-enhancing and bronchodilating actions of β-adrenoceptor agonists; and second, the validity of the dual β-adrenoceptor classification of Lands and co-workers (Lands, Arnold, McAuliff, Luduena & Buzzo, 1967).

Methods

Fasted adult cats of either sex (2.1–3.3 kg body weight) were anaesthetized with α-chloralose (80 mg/kg i.v.) after induction with a halothane (3%), nitrous oxide (3 l/min), oxygen (2 l/min) mixture. Sodium pentobarbitone (3–6 mg/kg i.v.) was given as necessary to suppress spontaneous respiration. Bilateral vagotomy was carried out in all animals to minimize the effects of autonomic reflexes on bronchodilator and positive chronotropic responses to β-adrenoceptor agonists (Boissier, Advenier, Giudicelli & Viars, 1971; Vaughan Williams, Bagwell & Singh, 1973). Rectal temperature was monitored and maintained at 37 ± 1°C by means of a heated table (Palmer, London). All drugs were injected through a cannulated jugular vein. All parameters were recorded on a Devices (type M–4) pen recorder.

Tracheal pressure

Cats were artificially respired with room air by positive pressure ventilation with a Palmer Ideal pump. The stroke volume was 13 ml/kg body weight and the pump rate 28 strokes/minute. Intra-tracheal pressure (mmHg; 1 mmHg = 1.333 mbar) was measured from a side-arm of the tracheal cannula by the method of Dixon & Brodie (1903), modified by the use of an Ether (type UP1, ± 10″ WG) pressure transducer. In each cat the dose of 5-hydroxytryptamine (5-HT) required to produce an increase in resting intra-tracheal pressure of approximately 50% was determined; this varied between 2 and 20 μg/kg. This dose of 5-HT was injected every 10 min until constant bronchoconstrictor responses were obtained, and thereafter at 10 min intervals throughout the experiment. The lungs were inflated by occluding the pump outlet for three expirations following the peak of the bronchoconstrictor response to 5-HT in order to prevent atelectasis.

Soleus muscle

The left soleus muscle was prepared essentially as described by Bowman & Nott (1970). The sciatic nerve was stimulated through shielded bipolar platinum electrodes with rectangular pulses of 50 μs duration at twice the voltage required to elicit a maximal twitch (normally 10–25 V) delivered from a Farnell physiological stimulator (Farnell Instruments, Wetherby) *via* a radio-frequency isolation unit (type PI/U). Submaximal tetanic contractions (6–10 Hz) were elicited for 1 s every 10 s and isometric muscle tension recorded using an Ether (type UFI, 32 oz) strain gauge attached to the tendon of the soleus muscle. Skin flaps were raised to form a pool containing a mixture of equal amounts of warm liquid paraffin BP and 0.9% w/v NaCl solution (saline) maintained at 37 ± 1°C by means of a heating lamp and thermocouple. The strain gauge output was displayed on a Telequipment (type S51-A) oscilloscope at high gain so that resting tension could be accurately monitored. Resting tension was adjusted to give an 'optimal evoked twitch tension' (Bowman & Nott, 1970) at the start of each experiment and was maintained constant thereafter. This procedure is designed to ensure that contractions are comparable from experiment to experiment (Buller, Eccles & Eccles, 1960). The resting tension required varied between 40 and 110 g in individual experiments.

Cardiovascular system

Arterial blood pressure was monitored from a common carotid artery and recorded by means of a Consolidated Electrodynamics pressure transducer (type 4-327-L221). Heart rate was recorded using a Devices instantaneous ratemeter (type 2750) triggered by the pulse pressure.

Experimental procedure

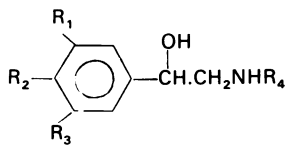
In all experiments tracheal pressure, soleus muscle contractions, arterial blood pressure and heart rate were recorded simultaneously. Completed preparations were left to stabilize for 60 min before use.

Studies with agonists

(−)-Isoprenaline was used as the reference drug in each experiment. (−)-Isoprenaline and the test β-adrenoceptor agonist were administered alternately, beginning with doses selected for each drug to produce near-threshold responses and using 3-fold increases in dose in each case. Not more than four dose-levels of each drug were administered; the interval between doses was at least 30 min and 6–8 h were required to complete the experiments. This experimental protocol was adopted so as to avoid the desensitization which has been observed with β-adrenoceptor agonists, particularly on blood pressure responses (Daly, Farmer & Levy, 1971). The β-adrenoceptor agonists were injected 1 min before 5-HT challenge and then

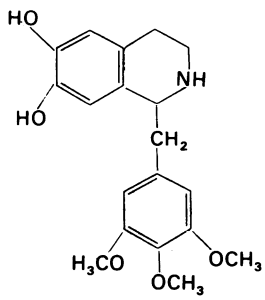
further 5-HT doses given until constant responses were again obtained. The largest inhibitory effect was always observed at the 1 min interval. Log dose-effect curves, using the maximum observed responses, were constructed for percentage inhibition of 5-HT-induced bronchoconstriction, for decrease in tension developed during a submaximal tetanus (g), for decrease in diastolic blood pressure (mmHg) and for increase in heart rate (beats/minute). Equipotent doses for (−)-isoprenaline and test β-adrenoceptor agonist were calculated in each experiment from about the mid-point of the straight-line portion of dose-effect curves. In practice, this corresponded to 50% inhibition of the 5-HT response, 200–400 g decrease in soleus muscle tension, 20–40 mmHg decrease in diastolic blood pressure and 20–40 beats/min increase in heart rate.

Table 1 Chemical structures of the β-adrenoceptor agonists studied



	R_1	R_2	R_3	R_4
Catecholamines				
Isoprenaline	OH	OH	H	$CH(CH_3)_2$
Resorcinol analogues				
Orciprenaline	OH	H	OH	$CH(CH_3)_2$
Fenoterol	OH	H	OH	$CH(CH_3)CH_2$—⟨⟩—OH
Terbutaline	OH	H	OH	$C(CH_3)_3$
Saligenin analogues				
AH 3021	CH_2OH	OH	H	$CH(CH_3)_2$
Salbutamol	CH_2OH	OH	H	$C(CH_3)_3$
AH 4553	CH_2OH	OH	H	$CH(CH_3)CH_2$—⟨⟩—OH
Salmefamol	CH_2OH	OH	H	$CH(CH_3)CH_2$—⟨⟩—OCH_3
AH 10013	CH_2OH	OH	H	$CH(CH_3)CH_2$—⟨⟩(OCH_3)
AH 7616	CH_2OH	OH	H	$CH(CH_3)CH_2CH$(⟨⟩(OCH_3))
AH 12561	CH_2OH	OH	H	$CH(CH_3)CH_2$—⟨⟩—$O(CH_2)_2OC_2H_5$
Other compounds				
AH 4941	$CH_2NHCONH_2$	OH	H	$C(CH_3)_3$
AH 4644	CH_2NHCOH	OH	H	$C(CH_3)_3$
AH 4325	$CH_2NHSO_2CH_3$	OH	H	$C(CH_3)_3$
Soterenol	$NHSO_2CH_3$	OH	H	$CH(CH_3)_2$

Trimetoquinol



Studies with antagonists

Log dose-effect curves for (−)-isoprenaline were determined on each parameter before and from 15 min after administration of propranolol or practolol. The (−)-isoprenaline dose-effect curves took 1.5–2 h to complete. The antagonist was given at intervals of 2–2.5 h in a cumulative dosing schedule. Three or four dose-levels of antagonist were tested in each cat.

The results were analysed by the method of Arunlakshana & Schild (1959). In each experiment and for each parameter, log [(−)-isoprenaline dose-ratio-1] was plotted against log dose antagonist (mg/kg). The regression line was fitted by eye. The slope of the regression and the dose of antagonist to produce an isoprenaline dose-ratio of 10 (DR_{10}) were estimated.

The direct effects of propranolol and practolol on 5-HT-induced bronchoconstriction, soleus muscle contractions, blood pressure and heart rate were also assessed in these experiments.

Table 2 Potency estimates for 15 β-adrenoceptor agonists relative to (−)-isoprenaline on bronchial muscle, soleus muscle, blood pressure and heart rate in the anaesthetized cat

		Mean equipotent dose			
	No. of experiments	*Inhibition of 5-HT-induced bronchospasm*	*Decrease in soleus muscle tension*	*Decrease in diastolic blood pressure*	*Increase in heart rate*
AH 4325	4	1.9 (1.1–3.7)	4.5 (2.4–8.3)	5.5 (2.6–11.6)	12.3 (9.4–16.1)
Fenoterol	5	2.5 (1.2–5.1)	2.9 (1.5–5.6)	2.1 (1.3–3.5)	6.3 (2.9–13.8)
AH 4553		2.6 (1.3–5.3)	5.3 (3.1–9.1)	5.3 (3.3–8.7)	10.9 (4.9–23.8)
Trimetoquinol	4	2.9 (1.9–4.5)	3.4 (2.5–4.6)	4.5 (1.6–13.3)	4.1 (2.4–6.9)
Soterenol	4	3.6 (1.7–7.8)	8.1 (5.9–11.2)	5.2 (4.0–6.8)	33.9 (15.6–73.7)
AH 12561	4	4.1 (1.9–8.7)	4.2 (1.8–9.9)	5.4 (4.7–6.2)	17.7 (5.2–60.5)
Salmefamol	4	4.4 (2.1–9.2)	10.3 (4.7–22.3)	7.6 (5.2–11.0)	25.9 (11.2–60.3)
Salbutamol	9	8.9 (6.4–12.3)	9.3 (7.9–11.0)	9.4 (6.6–13.6)	27.0 (19.7–37.2)
AH 4644	4	10.5 (8.9–12.5)	11.6 (8.1–16.6)	10.8 (5.9–19.5)	43.3 (30.2–62.0)
Terbutaline	5	13.6 (7.8–23.6)	23.2 (14.2–37.8)	19.7 (9.2–42.2)	112.7 (73.4–172.8)
AH 4941	4	16.6 (8.7–31.8)	29.7 (19.9–44.2)	33.5 (16.3–69.2)	74.2 (39.2–140.3)
AH 7616	4	25.7 (10.5–62.9)	125.7 (77.2–204.5)	105.4 (43.9–253.3)	871.0 (608.7–1246.5)
AH 3021	4	26.5 (10.9–64.5)	31.2 (19.8–49.2)	24.4 (8.5–70.6)	123.4 (58.9–258.2)
Orciprenaline	6	30.3 (22.1–41.7)	35.8 (27.1–45.8)	44.8 (30.7–65.5)	73.4 (44.3–121.4)
AH 10013	4	49.4 (34.5–70.6)	61.2 (20.9–179.7)	59.6 (46.5–76.4)	220.0 (171.7–281.8)

Results expressed as mean equipotent dose [(−)-isoprenaline = 1] with 95% confidence limits in brackets.

Statistical analysis

Equipotent doses quoted in the text and tables are geometric means with 95% confidence limits in brackets unless otherwise stated. Statistical comparisons were made using Students *t*-test and differences were considered to be significant when $P < 0.05$.

Drugs

The drugs used were: acetylcholine chloride (British Drug Houses); 5-hydroxytryptamine creatinine sulphate (British Drug Houses); pentobarbitone sodium (Abbott) and α-chloralose (Hopkin & Williams). The β-adrenoceptor agonists used were: (−)-isoprenaline bitartrate dihydrate (Ward-Blenkinsop); orciprenaline sulphate (Boehringer Ingelheim); fenoterol hydrobromide (Boehringer Ingelheim); terbutaline sulphate (Draco); AH 3021 hydrochloride; salbutamol base; AH 4553 anisic acid monohydrate; salmefamol base; AH 10013 base; AH 7616 acetate; AH 12561 citrate; AH 4941 fumarate; AH 4644 formamide acetate; AH 4325 acetate (Allen & Hanburys Research); soterenol hydrochloride (British Drug Houses) and trimetoquinol base (Tanabe Seiyaku). With the exception of (−)-isoprenaline all the β-adrenoceptor agonists were racemic mixtures. The chemical structures of the β-adrenoceptor agonists are shown in Table 1. The β-adrenoceptor antagonists used were (±)-practolol and (±)-propranolol hydrochloride (Imperial Chemical Industries).

Stock solutions were freshly prepared for each experiment in saline. Dilutions were stored on ice in a light-proof box. Ascorbic acid (20 μg/ml) was added to solutions of (−)-isoprenaline to inhibit oxidation. Doses are expressed in terms of the base equivalent.

Results

Agonists

Each of the 16 agonists investigated decreased 5-HT-induced bronchoconstriction, decreased the tension developed during submaximal tetanic contractions of soleus muscle, decreased diastolic blood pressure and increased heart rate in a dose-related manner. The slopes of the dose-effect curves for the 15 test agonists were parallel to those for (−)-isoprenaline on all four parameters. The effects of each drug were antagonized by propranolol (0.5 mg/kg).

Potency estimates for the test agonists relative to (−)-isoprenaline are given in Table 2. (−)-Isoprenaline was the most potent agonist in the series and its action was essentially unselective. On all four parameters the minimal effective dose for (−)-isoprenaline was 0.003–0.01 μg/kg and maximal or near maximal responses were produced by 0.3–1 μg/kg. The other agonists can be divided into three distinct categories of selectivity profile. The first category contains only trimetoquinol which, like (−)-isoprenaline, was essentially unselective. The second category contains 13 of the remaining 14 agonists. For each agonist potency was significantly lower on heart rate than on bronchial muscle, soleus muscle and blood pressure. There were only small and less consistent differences in potency between the three latter tissues (see Table 2). Part of an experiment comparing AH 4644 (one of the agonists in this category) with (−)-isoprenaline is illustrated in Figure 1 and dose-effect curves for the complete experiment are shown in Figure 2.

AH 7616 is the only agonist in the third category. As with the category two agonists it was significantly less potent on heart rate than on the other parameters, but was also significantly less potent on soleus muscle and blood pressure than on bronchial muscle (see Table 2). Because of this unique profile of selectivity, further experiments were carried out with AH 7616 in which acetylcholine (20–50 μg/kg) was used as the spasmogen instead of 5-HT. AH 7616 was 138 (72–263, $n=4$) times less potent than (−)-isoprenaline against acetylcholine-induced brochoconstriction. This estimate is significantly different from that against 5-HT but is very similar to those on soleus muscle and blood pressure (see Table 2). AH 7616 may, therefore, possess a specific 5-HT antagonist action in addition to its β-adrenoceptor agonist action.

The data in Table 2 were analysed further by means of correlation coefficients and regression coefficients. If the *rank order* of β-adrenoceptor agonist potency for the series is the same on two parameters then the correlation coefficient will be 1; if, in addition, the *equipotent doses* on the two parameters are the same then the regression coefficient will be 1. AH 7616 was excluded from the analysis because of the possibility of an additional action on bronchial muscle distorting the estimate of β-adrenoceptor agonist potency. Correlation coefficients and regression coefficients based on the results obtained with the other agonists are summarized in Table 3. There is a significant correlation for rank order of potency for each pair of parameters compared. This is apparent also from an inspection of Table 2; for example, as potency against 5-HT-induced bronchoconstriction decreases, potency on the other three parameters also tends to decrease. In contrast, the regression coefficients fall into two distinct groups. In Group 1, regression coefficients are close to 1, whereas in Group 2 they are much larger than 1. The reason for this difference is that Group 2 consists of comparisons of heart rate with the other three parameters and, as described above, 13 of the 15 agonists used in the analysis were significantly less potent on heart rate than on the other parameters.

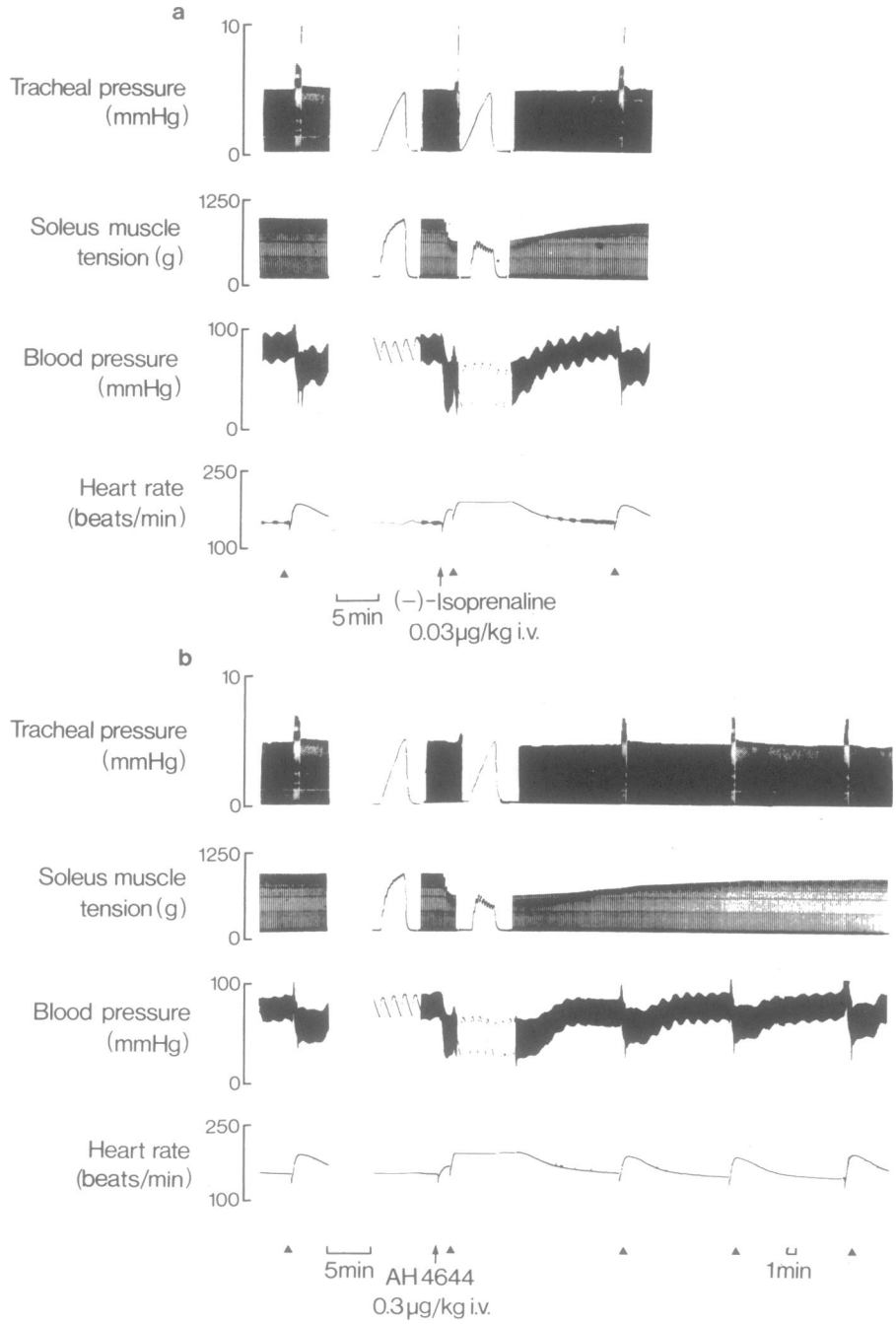


Figure 1 Anaesthetized cat, 2.2 kg ♂. Effects of (a) (−)-isoprenaline and (b) AH 4644 on 5-HT-induced bronchoconstriction, submaximal tetanic contractions of the soleus muscle, arterial blood pressure and heart rate. In each trace the paper speed was temporarily increased from 5 mm/min to 10 mm/sec before administration and at the peak effect of the β-adrenoceptor agonist to compare the degree of fusion of the soleus muscle contractions. (▲) 5-HT (5 μg/kg i.v.). The 5-HT-induced bronchoconstriction was accompanied by an increase in heart rate and a decrease in blood pressure. Note that AH 4644 had less effect on heart rate than (−)-isoprenaline at an equipotent bronchodilating dose (increases of 19 and 30 bts/min respectively), that AH 4644 was longer acting than (−)-isoprenaline and that for both AH 4644 and (−)-isoprenaline the duration of action on lung was shorter than on soleus muscle.

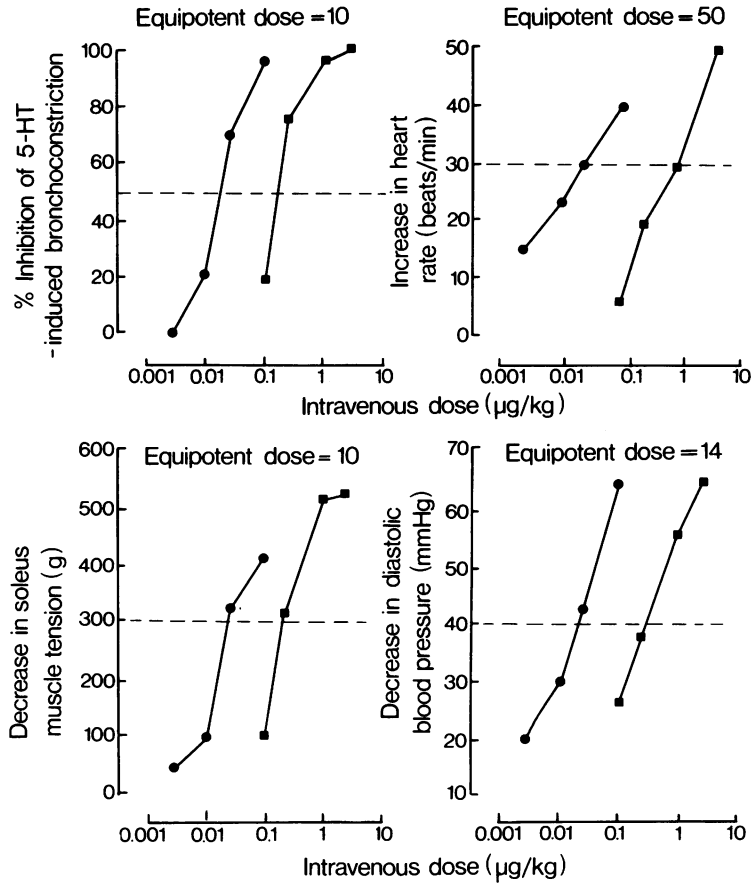


Figure 2 Anaesthetized cat, 2.2 kg ♂. Comparison of the potencies of (−)-isoprenaline (●) and AH 4644 (■). Equipotent doses ((−)-isoprenaline = 1) were calculated at ————. Part of this experiment is illustrated in Figure 1.

As well as differences in selectivity profile there were differences in time to peak effect and in the duration of action of the agonists investigated. Peak effect for 14 of the agonists was attained at 60–100 s on soleus muscle and 20–40 s on blood pressure and heart rate. Trimetoquinol and soterenol differed slightly in that peak effect was attained at 80–150 s on soleus muscle and 30–60 s on blood pressure and heart rate. The experimental design precluded precise assessment of time to peak effect on bronchial muscle. All of the test agonists were longer acting than (−)-isoprenaline and this was particularly true of trimetoquinol and soterenol. In addition, for any given agonist there were differences in duration of action between tissues. For example, the duration of the response on bronchial muscle was shorter than that on soleus muscle. This is shown for (−)-isoprenaline and AH 4644 in Figure 1.

Antagonists

In preliminary experiments, dose-effect curves for (−)-isoprenaline were obtained on bronchial muscle, soleus muscle, blood pressure and heart rate at 1.5 h intervals over a period of 6 hours. The sensitivity of individual cats ($n=4$) to (−)-isoprenaline varied by less than 2-fold over this period.

Effects of propranolol and practolol on sensitivity to (−)-isoprenaline

The results are summarized in Table 4. Propranolol (0.03, 0.1, 0.3 and 1 mg/kg), caused parallel displacements to the right of the (−)-isoprenaline dose-effect curves on all four parameters. The antagonist potency of propranolol was almost identical on bronchial muscle, soleus muscle and blood pressure

but very slightly lower on the heart. Practolol (3, 10 and 30 mg/kg), also caused parallel displacements to the right of the (−)-isoprenaline dose-effect curves on all four parameters but, in contrast to propranolol, was 10–12 times more potent on the heart than on bronchial muscle, soleus muscle and blood pressure. There was no significant difference in the antagonist potency of practolol on the latter three parameters.

Partial agonist activity of practolol

Practolol (3 mg/kg), increased heart rate by 20 beats/min (range 10–29 beats/min, $n=6$), the response returning to control levels in 60–80 minutes. Administration of propranolol (5 mg/kg), during the practolol tachycardia resulted in a prompt return to control levels or below. A typical experiment is illustrated in Figure 3a. Higher doses of practolol (10–100 mg/kg) had little or no effect on heart rate, but the 30 mg/kg dose produced a slight reduction and the 100 mg/kg dose a marked reduction in soleus muscle contractions lasting for 3–8 minutes. Propranolol (5 mg/kg), had no effect on, or potentiated the soleus muscle response to practolol (Figure 3b). Thus, it would appear that the effect of practolol on heart rate arises from its partial agonist action, whereas its effect on soleus muscle does not.

Propranolol had no detectable partial agonist activity in doses up to 1 mg/kg.

Discussion

In this study the actions of a wide variety of β-adrenoceptor agonists and of two β-adrenoceptor antagonists have been determined in a single species simultaneously on bronchial muscle, soleus muscle, blood pressure and heart rate. The cardiovascular and soleus muscle actions of three of the agonists – salbutamol, orciprenaline and trimetoquinol – have been described previously (Bowman & Nott, 1970; Apperley & Daly, 1972; Houston & Rodger, 1974). Our results agree closely with those of the previous reports.

Our study was designed to provide information on two inter-related questions. First, is it possible to separate the bronchodilating and tremor-enhancing actions of β-adrenoceptor agonists? Our results show that sensitivity on bronchial and soleus muscle is similar for each of the 16 agonists investigated. Corresponding studies in the anaesthetized cat with the β-adrenoceptor agonists rimiterol (Bowman & Rodger, 1972), MJ 9184-1 (Gwee, Nott, Raper & Rodger, 1972) and isoetharine (Rodger, 1973)

Table 3 Correlation coefficients and regression coefficients for β-adrenoceptor agonist activity in the anaesthetized cat

Comparison	*Correlation coefficient*	*Regression coefficient*
Group 1		
Bronchial muscle *v* soleus muscle	0.98*	1.20 (1.06–1.35)
Bronchial muscle *v* blood pressure	0.96*	1.21 (0.99–1.43)
Soleus muscle *v* blood pressure	0.98*	1.00 (0.87–1.14)
Group 2		
Heart *v* bronchial muscle	0.97*	4.05 (3.02–5.08)
Heart *v* soleus muscle	0.99*	3.38 (2.67–4.09)
Heart *v* blood pressure	0.91*	3.01 (1.94–4.09)

Values estimated from data in Table 2. 95% confidence limits in brackets. AH 7616 omitted.
Bronchial muscle – inhibition of 5-HT-induced bronchoconstriction. Soleus muscle – decrease in tension developed during sub-maximal tetanic contraction. Blood pressure – decrease in diastolic blood pressure. Heart – increase in heart rate.
* Significant correlation ($P<0.001$).
Group 1 regression coefficients are not significantly different from one another.
Group 2 regression coefficients are not significantly different from one another.
Group 1 regression coefficients are significantly different from Group 2 regression coefficients.

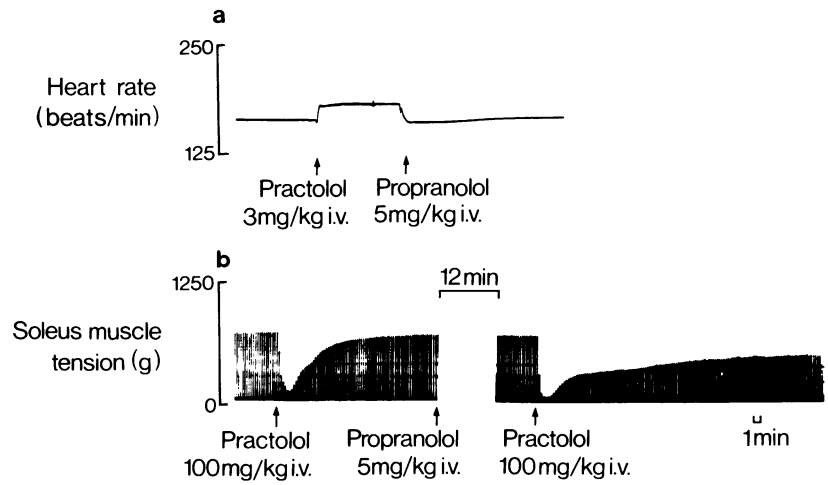


Figure 3 Anaesthetized cat, 3 kg ♂. The effect of practolol on (a) heart rate and (b) soleus muscle contractions. Note that propranolol (5 mg/kg i.v.) blocked the increase in heart rate to practolol but potentiated the soleus muscle response.

produced the same finding. If the cat soleus muscle preparation is a predictive model for enhancement of physiological tremor in man, as has been suggested (Bowman & Nott, 1970) then all of the β-adrenoceptor agonists examined to date would be expected to enhance physiological tremor and cause bronchodilatation over the same dose-range when administered systematically. It is noteworthy that in the two studies reported in man in which this question has been examined in detail, the bronchodilator actions of salbutamol, terbutaline and rimiterol were accompanied by enhanced physiological tremor (Watson & Richens, 1974; Marlin & Turner, 1975). We therefore agree with the previous conclusions (Bowman & Nott, 1970; Bowman & Rodger, 1972) that it may prove impossible to separate the bronchodilating and tremor-enhancing properties of β-adrenoceptor agonists except by giving the drugs by inhalation.

One agonist in our series, AH 7616, did appear at first sight to differentiate bronchial and soleus muscle β-adrenoceptors but it was shown subsequently that a more likely explanation of the observed selectivity is the presence of an additional 5-HT-antagonist action. The combination of potent β-agonist and 5-HT antagonist actions in one drug appears to be novel. It is, however, difficult to envisage any advantage for AH 7616 over conventional β-adrenoceptor agonists in the treatment of asthma since it is unlikely that 5-HT contributes to the underlying bronchospasm.

The second question is: how do our results relate to the problem of the sub-classification of β-

Table 4 Antagonist activity of propranolol and practolol against bronchial muscle, soleus muscle, blood pressure and heart rate responses to (−)-isoprenaline in the anaesthetized cat

Antagonist	*Inhibition of 5-HT-induced broncho-constriction*		*Decrease in tension of submaximal tetanus of soleus muscle*		*Decrease in diastolic blood pressure*		*Increase in heart rate*	
	DR_{10}	*Slope*	DR_{10}	*Slope*	DR_{10}	Slope	DR_{10}	*Slope*
Propranolol ($n=6$)	0.13 (0.08–0.19)	1.07 (0.9–1.3)	0.13 (0.07–0.3)	1.04 (0.9–1.1)	0.11 (0.07–0.2)	1.04 (0.9–1.1)	0.23 (0.18–0.3)	1.06 (0.8–1.3)
Practolol ($n=6$)	25.2 (10.8–58.8)	0.96 (0.7–1.2)	22.4 (14.2–35.4)	1.08 (0.9–1.4)	27.8 (23.7–32.5)	0.96 (0.9–1.1)	2.3 (1.6–3.2)	0.9 (0.8–1.0)

DR_{10} – dose of antagonist (mg/kg i.v.) to produce an isoprenaline dose-ratio of 10.
Slope – of regression obtained from plot of log (isoprenaline dose-ratio − 1) on log antagonist dose (mg/kg i.v.).
95% confidence limits are in brackets. n=no. of cats.

adrenoceptors? Lands' classification is widely used, but several workers have found difficulty in accommodating their results within a simple β_1/β_2 adrenoceptor framework (see Furchgott, 1972 and Daly, Flook & Levy, 1975 for references), and Barrett (1973) described Lands' classification as 'an over simplification that is neither valid nor useful'. There are two standard procedures for receptor classification, namely the determination of relative potencies in a series of agonists and determination of the potencies of competitive antagonists (Furchgott, 1972); both have been used in the present study. When compared with (−)-isoprenaline, 13 of the 15 agonists examined were clearly less potent on cardiac muscle than on bronchial, soleus or vascular muscle. Practolol showed the opposite selectivity, being some 10–12 times more potent as an antagonist on cardiac muscle than on bronchial, soleus or vascular muscle. These complementary findings provide strong support for the dual β-adrenoceptor concept of β_1-adrenoceptors in cardiac muscle and β_2-adrenoceptors in bronchial, soleus and vascular muscle. Furthermore, the similar potency of each drug, whether agonist or antagonist, in the latter three tissues points to the homogeneity of the β_2-adrenoceptor sub-type. This evidence is particularly striking when it is recalled that the experiments were carried out in intact animals and therefore under conditions which could give rise to differences in potency even though the receptors involved were identical (Furchgott, 1972; Daly *et al.*, 1975). We conclude, therefore, that Lands' classification is both valid and useful, at least in the four tissues examined. This conclusion is in line with those of previous investigations carried out in this laboratory with β-adrenoceptor agonists and antagonists in anaesthetized dogs (Daly *et al.*, 1971, 1975) and guinea-pigs (Apperley & Levy, 1975). We cannot, of course, discount the possibility that further differentiation of β-adrenoceptors might be achieved with novel β-adrenoceptor agonists or antagonists, perhaps through combination with as yet unidentified different exosites in the different tissues (Brittain, Jack & Ritchie, 1970).

Several other points worthy of comment emerge from our study. Lands' classification was derived from data obtained with a series of catecholamines (Lands *et al.*, 1967). Because criticisms of the classification arose from results obtained with non-catecholamine β-adrenoceptor agonists or with β-adrenoceptor antagonists it has been suggested that adrenoceptor classification be undertaken primarily with catecholamines and that non-catecholamine agonists or antagonists are useful only in a corroborative role (Arnold & McAuliff, 1971; Arnold, 1972; Grana, Lucchelli & Zonta, 1974). However, no such restriction need be applied to our results; Lands' classification is supported by data obtained with non-catecholamine β-adrenoceptor agonists and with β-adrenoceptor antagonists.

The existence of sub-types of β-adrenoceptor was indicated in the present study by comparisons of *relative* potency in the agonist series (regression coefficient analysis, Table 3), but not by comparisons of *rank order* of potency (correlation coefficient analysis, Table 3). O'Donnell & Wanstall (1974) also found this to be so for analogues of isoprenaline and orciprenaline on guinea-pig atria, trachea and hind limb vasculature. Thus, comparison of rank order of potency, first used to differentiate α- and β-adrenoceptors (Ahlquist, 1948) and then β_1- and β_2-adrenoceptors (Lands *et al.*, 1967) is not a completely reliable method for differentiating receptors; its success appears to depend on the particular agonists used.

Other workers have found that the selectivity of β_2-adrenoceptor agonists is lower in the cat than in other laboratory species, for example the guinea-pig (Bowman & Rodger, 1972; Rodger, 1973; Houston & Rodger, 1974; Davey, Malta & Raper, 1974). Our results are consistent with this conclusion. Furthermore, the β_2-adrenoceptor agonists salbutamol and MJ 9184-1 are weak partial agonists compared to isoprenaline in guinea-pig isolated cardiac muscle (Farmer, Kennedy, Levy & Marshall, 1970; Davey *et al.*, 1974) but are potent full agonists in cat isolated cardiac muscle (Cornish & Miller, 1975; Davey *et al.*, 1974). These findings led to the suggestion that β-adrenoceptors in cat and guinea-pig may be different (Davey *et al.*, 1974) but an alternative explanation, consistent with the available evidence, can be envisaged. There is no reason to suppose that the relationship between stimulus, as defined by Stephenson (1956), and final response is the same for all organs, or for the same organ in different species. If there are significant differences in this regard then comparison of the relative potency of agonists with unequal efficacies (Stephenson, 1956) would reveal differences even though the receptors concerned were identical (Furchgott, 1972; Jenkinson, 1973). In particular, an agonist with a low efficacy would be relatively less potent in a tissue in which a high stimulus was necessary for threshold response than one in which a low stimulus was necessary. Salbutamol and MJ 9184-1 must have lower efficacies than isoprenaline in cardiac muscle, since they are partial agonists in guinea-pig atria. This is also true for several of the other β_2-adrenoceptor agonists examined in our study (unpublished observations). The species differences could therefore be explained by postulating that the stimulus necessary for threshold response is higher in guinea-pig than in cat cardiac muscle. Two further observations, both involving practolol, are in accord with this idea. First, practolol possesses weak partial agonist activity. This activity, however, is much more obvious in cat than in guinea-pig cardiac muscle (Kaumann, A.J. & Blinks, J.R., personal communication). Second, the antagonist potency of a drug is determined only by its affinity for

the receptor and should therefore be independent of differences in the stimulus-response relationship. Hence, it is instructive to compare β-adrenoceptor antagonist potency in guinea-pig and cat cardiac muscle. These data are available for practolol and show that the pA_2 against isoprenaline in guinea-pig cardiac muscle (6.76, Drew & Levy, 1972) is virtually identical with that in cat cardiac muscle (6.55, Cornish & Miller, 1975), indicating that the β-adrenoceptors involved are the same. Thus, our hypothesis has the advantage of reconciling apparently conflicting results with β-adrenoceptor agonists and antagonists.

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