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The selectivity of β-adrenoceptor antagonists on isoprenaline-induced changes in heart rate, blood pressure, soleus muscle contractility and airways function in anaesthetized cats

L.G. Letts[1], D.P. Richardson, Diana M. Temple & L.R. Williams[2]

Department of Pharmacology, University of Sydney, Australia 2006

1 The β-adrenoceptor antagonist activity of propranolol, metoprolol, atenolol and butoxamine in anaesthetized cats has been measured and compared with the activity of four synthetic phenylethanolamine derivatives.

2 The effects of isoprenaline on four parameters in the anaesthetized cat: heart rate, blood pressure, soleus muscle contractility and airway reactance, were measured and the modification of the isoprenaline dose-response relation by each of the antagonist drugs assessed.

3 Parallel shifts in log dose-response curves for isoprenaline were caused by propranolol for all parameters, by metoprolol and atenolol for each parameter except blood pressure, and butoxamine for each except soleus muscle and heart rate.

4 Selectivity of action of the antagonists between different organs was measured by comparing DR10 values, computed from isoprenaline dose-ratios.

5 Propranolol was the most potent antagonist and showed slight selectivity of action on soleus muscle compared with heart. Atenolol and metoprolol were approximately equipotent and were cardioselective at low doses only. Butoxamine was the least potent antagonist and possessed non-β-adrenoceptor effects on the parameters measured.

6 Each of the new compounds, 4′-bromo-2′-methyoxy-N-isopropyl phenylethanolamine, the 4′-chloro- and 4′-methyl analogues, and 4′-methoxy-N-*t*-butyl phenylethanolamine, was a potent antagonist but did not exhibit any selectivity of action.

7 The results suggest no clear separation of β-adrenoceptors into β_1- and β_2- subclasses in organs of the cat. There is no apparent separation of β-adrenoceptor-mediated effects on skeletal muscle and airways.

Introduction

Lands, Arnold, McAuliff, Luduena & Brown (1967) proposed the division of β-adrenoceptors into a β_1 type (mediating an increase in heart rate) and a β_2 type (mediating bronchodilatation and a decrease in diastolic blood pressure). The decrease in fusion and tension developed during submaximal tetanic contractions of slow contracting skeletal muscle has also been ascribed to activation of β_2-adrenoceptors (Bowman & Nott, 1970). This sub-division has been useful in describing drugs with selective actions on tissues and organs mediated by β-adrenoceptors, for example β_1-adrenoceptor antagonists such as practolol (Dunlop & Shanks, 1968), atenolol (Barrett, Carter, Fitzgerald, Hull & Le Count, 1973) and metoprolol (Ablad, Carlsson & Ek, 1973) which are used in the treatment of angina pectoris and hypertension and β_2-adrenoceptor agonists such as salbutamol (Brittain, Farmer, Jack, Martin & Simpson, 1968) and terbutaline (Bergman, Persson & Wetterlin, 1969) which are used for the treatment of reversible airway obstruction e.g. asthma.

Comparison of the data obtained in animal experiments is difficult because of differences in the methods used by various authors and because of

[1]Present address: Merck Frosst Canada Inc., C.P./P.O. 1005, Pointe Claire-Dorval, Quebec H9R 4P8, Canada.

[2]Present address: School of Chemistry, Macquarie University, North Ryde, Australia 2113.

inherent difficulties of measuring selectivity *in vivo* (Furchgott, 1972) where pharmacokinetic and physiological reflex factors may interfere with quantitative assessments. It was decided to compare quantitatively, using the same methods for each compound, the β-adrenoceptor-mediated actions of several therapeutically available compounds in cat soleus muscle, airways and cardiovascular system *in vivo*. These results would then be used to compare the organ selectivity (Kenakin & Beck, 1980) of action of some new β-adrenoceptor antagonists (Williams, Lap, Lim, Temple, Easson & Letts, 1978). The results would also be useful in measuring organ selectivity of action and separation of β_1- and β_2-adrenoceptor mediated actions *in vivo* in cats. The possibility of separating the tremor-enhancing and bronchodilator actions of β-adrenoceptor agonists was also investigated.

Methods

Adult cats of unknown age and of either sex weighing 2.2 to 3.8 kg were anaesthetized with α-chloralose (80 mg kg^{-1}) plus sodium pentobarbitone (6 mg kg^{-1}) injected intraperitoneally. They were then prepared in the following way:

Soleus muscle preparation

The left soleus muscle was prepared in essentially the manner described by Bowman & Nott (1970). The cat was laid prone on a heated table and the left hind limb 'rigidly fixed' in a horizontal position by means of drills through the femur and the tibia and fibula. A skin incision was made from the level of the Achilles tendon to the popliteal fossa and the soleus muscle was dissected free from the neighbouring gastrocnemius and plantaris muscles. The tendon of insertion of the soleus muscle was cut and attached to a Grass force transducer (model FT 10 C). The skin flaps were raised to form a pool which was filled with liquid paraffin oil maintained at 37°C by means of a small heating lamp placed under the surface of the oil. A further incision was made above the popliteal space. The sciatic nerve was severed and small, shielded bipolar electrodes were placed around the peripheral end. The nerve was stimulated with rectangular pulses 100 μs in duration at the rate of 8 pulses per s for 1 s, once every 10 s. Voltages used were twice the voltage required to elicit a maximum twitch (6–20 V). Resting tension (40–100 g) was adjusted to give an optimal evoked twitch tension (Bowman & Nott, 1970) at the start of each experiment, and was maintained constant thereafter.

Airways function

The method used was a modification of the method described by Rodger (1974). Each cat was artificially ventilated with a respirator (Harvard apparatus) at a rate of 28 breaths min^{-1} with an air volume of about 14 ml kg^{-1}. Transpulmonary pressure was measured with a Statham differential pressure transducer (Model PM 5), one input of which was connected to a 14 g needle (via a short length of polythene tubing) and inserted at the left fifth or sixth intercostal space into the intrapleural space, leaving a 1 ml pneumothorax. The other input of the transducer was connected to the side-arm of the tracheal cannula by a second polythene tube of identical length and bore to the first. Airflow was measured with a mesh screen pneumotachograph (Mercury Electronics; Model F10L) connected to a second Statham differential pressure transducer (Model PM5). Electrical integration of the resulting air flow signal gave a measure of tidal volume.

Airway function was monitored by computing the airway reactance. Airway reactance (cm water l^{-1}), the reciprocal of total dynamic compliance, was computed by electronically identifying the points of zero flow during each respiratory cycle. The total transpulmonary pressure change (cmH_2O) between these two instants divided by the corresponding air volume change (l) enabled a breath by breath monitoring of airway function.

A submaximal and constant level of increased airway reactance (200–300%) was produced by the intravenous infusion (IMED infusion pump, model 922) of 5-hydroxytryptamine (5-HT) through a cannulated femoral vein (2.5 to 30 μg kg^{-1} min^{-1}). Bilateral vagotomy was carried out to eliminate vagally-mediated reflex alterations in pulmonary and cardiac function.

Cardiovascular system

Arterial blood pressure was measured by means of a polythene cannula inserted into the right carotid artery and connected to a pressure transducer (Statham, model P23A). Heart rate was measured by a biotachometer (Grass model 7P4D) triggered with the blood pressure signal.

Studies with β-adrenoceptor drugs

The drugs were injected through a cannula in the brachial vein. The effects of test drugs on heart rate, blood pressure and soleus muscle contractility were measured simultaneously in one series of experiments, and on airway reactance in another series

because the intravenous infusion of 5-HT produced cardiovascular responses despite bilateral vagotomy. After preparations of the cat were complete a submaximal dose of isoprenaline was administered and the animal was left to stabilize for at least 45 min before use.

In experiments involving measurement of airway reactance an initial infusion of 5-HT was made and the infusion rate adjusted to produce a constant increase in airway reactance (200–300%). Once established, this 5-HT infusion rate was kept constant for the remainder of the particular experiment. The 5-HT infusions took 10 to 15 min to produce a stable increase in airway reactance. The infusions of 5-HT lasted approximately 30 min; 5 min after termination of each infusion, the lungs were inflated (by increasing the air volume on the respirator by a factor of 3) to prevent atelectasis.

In all experiments isoprenaline was used as the reference agonist drug. The isoprenaline was administered as bolus doses in a cumulative fashion as described by Nott & Raper (1972) and Rodger (1974). Log dose-response curves were constructed for the percentage decreases in tension developed during a submaximal tetanus, diastolic blood pressure, 5-HT-induced increase in airway reactance and the percentage increase in heart rate.

For each parameter in each animal the quantitative analysis of antagonist action was measured by first establishing a cumulative dose-response curve to intravenous bolus doses of isoprenaline as described. This process was then repeated 5 min after the intravenous administration of each of three or more successively increasing doses of antagonist. Dose ratios (DR) for isoprenaline (i.e. ratio of dose of isoprenaline producing half maximal effect in the presence of antagonist to the dose producing the equivalent effect in the absence of antagonist) were determined from the ED_{50} values. Antagonist potency was evaluated by means of Arunlakshana & Schild (1959) plots of log (DR − 1) against the negative logarithm of the molar dose of antagonist. A least squares method of regression analysis was used; slopes and doses required to produce DR10 (i.e. a 10 fold shift of the curve to the right) were determined.

Drugs

The drugs used, which were donated by the manufacturers, were: 5-hydroxytryptamine creatinine sulphate (Sigma), isoprenaline sulphate (Burroughs Wellcome), propranolol hydrochloride and atenolol (ICI), metoprolol tartrate (Astra), butoxamine hydrochloride (Burroughs Wellcome), N-isoproply-2-methoxy-4′-methyl phenylethanolamine hydrochloride, and its 4′-Cl, 4′-Br and N-*t*.butyl analogues (Williams *et al.*, 1978).

Statistical methods

The ED_{50} values are geometric means with 95% confidence limits (Fleming, Westfall, De La Lande & Jellet, 1972). The DRs are arithmetic means ± standard errors (s.e.). The difference between mean values was analysed with Student's *t* test for non-correlated values. Parallelism of log dose-

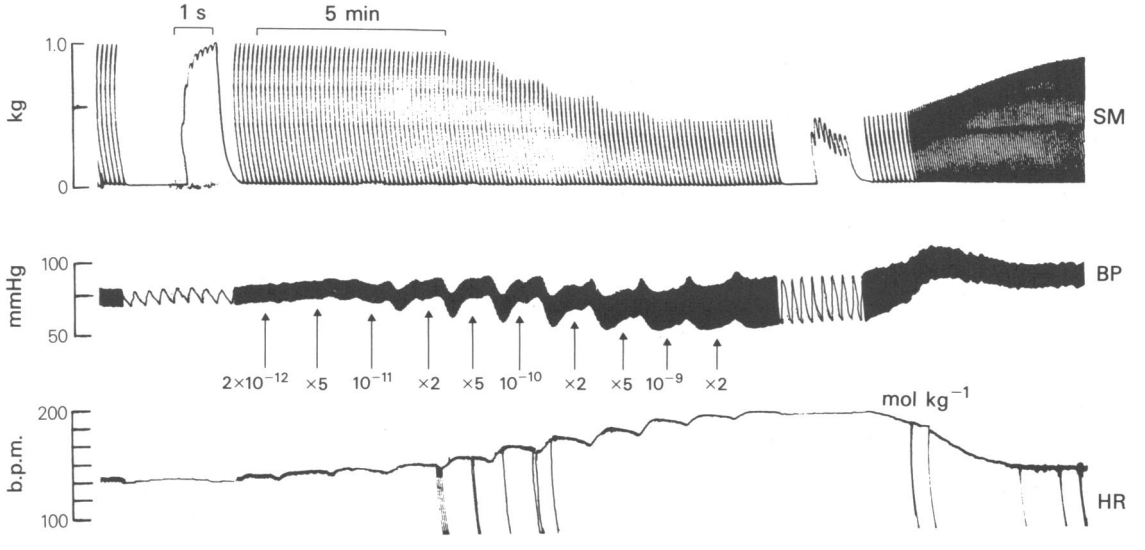

Figure 1 The effects of cumulative intravenous bolus doses of isoprenaline (mol kg^{-1}) on the simultaneous recordings of heart rate (HR), blood pressure (BP) and soleus muscle contractility (SM) in an anaesthetized cat.

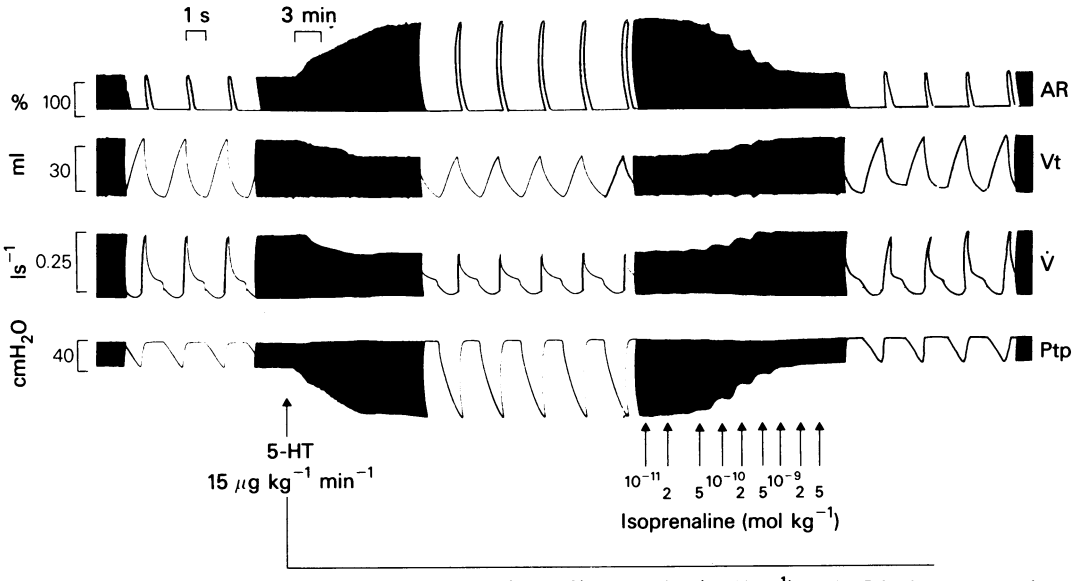

Figure 2 The effects of cumulative intravenous bolus doses of isoprenaline (mol kg^{-1}) on the 5-hydroxytryptamine (5-HT)-induced increases in airway reactance (AR) where Vt = tidal volume; V = flow; Ptp = transpulmonary pressure. AR = Ptp/Vt symbols for transpulmonary pressure ÷ volume.

response curves was tested by subjecting responses between 16 and 84% of maximal to a least squares regression analysis, and comparing the slopes using Student's *t* test for non-correlated values. The criterion for statistical significance was $P<0.05$.

Results

In three control experiments the determination of four cumulative dose-effect curves to isoprenaline for each parameter (Figures 1 and 2), repeated at 45 min intervals, varied less than 3 fold and were reproducible.

A representative experiment using propranolol is illustrated in Figure 3 and the results using various antagonists are given in Table 1.

Propranolol

Propranolol (0.1 to 2 μmol kg^{-1}) produced parallel shifts to the right of the isoprenaline log dose-response curve for each parameter. The slope values for the regression analyses (log (DR − 1) vs −log [propranolol dose]) for each parameter (Figure 4a) were not significantly different from each other. The results indicate that propranolol is approximately twice as potent for antagonism of isoprenaline-induced effects on blood pressure, and three times as potent on soleus muscle, compared with its antagonism of isoprenaline-induced increase in heart rate.

Metoprolol

In five cats, metoprolol (0.5, 2 and 10 mol kg^{-1}) produced parallel shifts to the right of the dose-response curve for the isoprenaline-induced increases in heart rate. In the same experiments, in three cats 0.5 μmol kg^{-1} metoprolol produced no effect and in two cats produced a shift only in the dose-response curve for the isoprenaline-induced vasodepressor actions. In one cat, none of the doses of metoprolol affected the isoprenaline-induced vasodepressor actions. In four cats, for the isoprenaline-induced decreases in soleus muscle contractility, metoprolol 0.5 μmol kg^{-1} did not produce a parallel shift in the isoprenaline dose-response curve. Although the threshold doses for the actions of isoprenaline were blocked, the maximum doses remained unchanged, which resulted in an increase in the slope of the isoprenaline dose-response curve. Metoprolol 2 and 10 μmol kg^{-1} produced parallel shifts to the right of the control isoprenaline dose-response curve in all five cats for its actions on soleus muscle.

In four cats metoprolol (1, 4 and 20 μmol kg^{-1}) produced parallel shifts to the right of the isoprenaline dose-response curves for its actions on airways reactance.

Each of the slope values for each parameter had a significant linear correlation (Figure 4b). The only slopes that differed significantly were those for the actions of metoprolol on antagonism of the isoprenaline-induced chronotropic and airway reac-

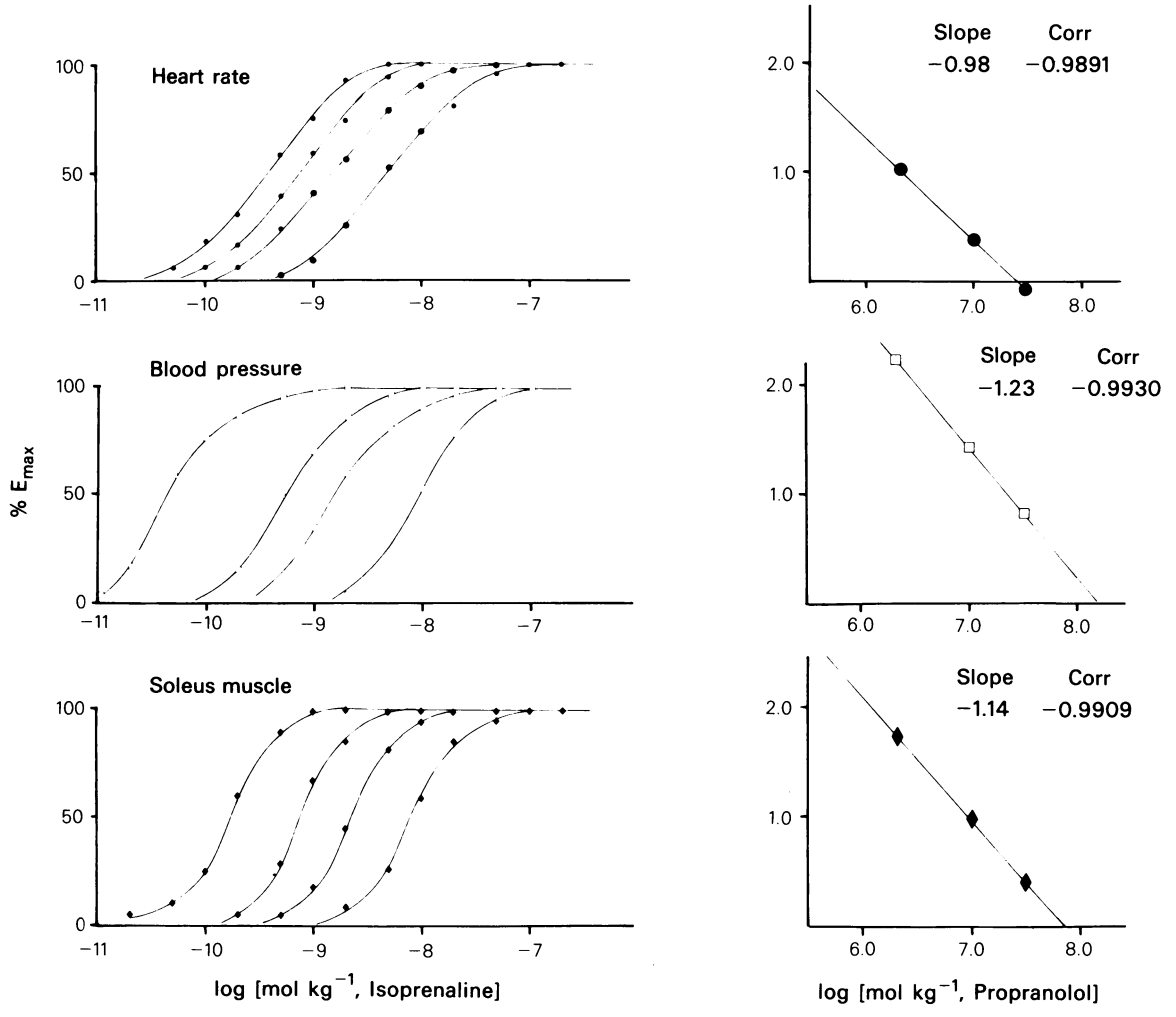

Figure 3 Log dose-response curves to cumulative doses of isoprenaline (mol kg^{-1} i.v.) on the simultaneous measurements of heart rate, blood pressure and soleus muscle contractility in an anaesthetized cat. Also illustrated are the least squares regression plots of log (DR − 1) against the negative molar antagonist dose (mol kg^{-1} i.v.) of propranolol for each of these parameters. Slope: of least squares regression of log (DR − 1) on negative [propranolol mol kg^{-1} i.v.]. Corr: regression correlation coefficient.

tance changes. A consequence of these non-parallel regression lines for the actions of metoprolol on these parameters is reflected in measurement of selectivity of action. As illustrated in Figure 4b, a low dose of metoprolol (0.1–0.5 μmol kg^{-1}) would preferentially block isoprenaline-induced chronotropic increases before affecting the isoprenaline-induced changes on the other parameters. As the dose of metoprolol increases, however, there is a decrease in the degree of preferential block of isoprenaline-induced chronotropic effects compared to the vascular, soleus muscle and airway reactance effects. Although the doses of metoprolol producing a 10 fold shift for the isoprenaline-induced changes on each parameter are illustrated in Table 1, this by no means reflects the absolute degree of selectivity of action of metoprolol.

Atenolol

In five cats atenolol (0.1 to 20 μmol kg^{-1}) produced parallel shifts to the right for the isoprenaline-induced increases in heart rate. In the same experiments, in two cats, 0.1 μmol kg^{-1} produced no effects for the isoprenaline-induced changes in diastolic blood pressure. In two other cats, all the doses produced parallel shifts to the right of the dose-response

Table 1 Activity of antagonists of isoprenaline on heart rate, blood pressure, soleus muscle and airway reactance responses in anaesthetized cats

	Heart rate	*Blood pressure*	*Soleus contractility*	*Airway reactance*
Propranolol				
DR	0.26 ± 0.06	0.14 ± 0.06	0.08 ± 0.02	0.14 ± 0.03
Slope	−1.12 ± 0.10	−1.12 ± 0.17	−1.10 ± 0.20	−1.33 ± 0.04
(*n*),selectivity	(5), 1.00	(3), 0.54	(5), 0.31*	(3), 0.54
Metoprolol				
DR	1.91 ± 0.05	5.48 ± 3.34	5.34 ± 1.41	3.90 ± 0.23
Slope	−0.81 ± 0.10	−1.14 ± 0.28	−0.92 ± 0.20	−1.80 ± 0.08
(*n*),selectivity	(5), 1.00	(4), 3.06	(5), 2.80*	(4), 1.62
Atenolol				
DR	1.79 ± 0.48	1.21(0.70, 1.73)	10.8 ± 3.22	2.25 ± 0.40
Slope	−0.67 ± 0.06	−0.92(−0.78, −1.07)	−0.80 ± 0.12	−1.03 ± 0.11
(*n*),selectivity	(4), 1.00	(2), 0.68	(4), 6.03*	(4), 1.26
Butoxamine				
DR	220 ± 177	7.65 ± 1.54	6.73 ± 0.27	4.31 ± 0.46
Slope	0.34 ± 0.30	−1.65 ± 0.18	−2.01 ± 0.11	−1.29 ± 0.19
(*n*),selectivity	(3), 1.00	(5), 0.03	(5), 0.03	(3), 0.02
(1)				
DR	0.66 ± 0.15	0.17 ± 0.03	0.17 ± 0.03	
Slope	−1.08 ± 0.15	−1.05 ± 0.12	−1.09 ± 0.10	N/A
(*n*),selectivity	(5), 1.00	(5), 0.26*	(5), 0.26*	
(2)				
DR	1.03 ± 0.05	0.76 ± 0.15	0.56 ± 0.10	0.24 ± 0.04
Slope	−1.07 ± 0.07	−1.08 ± 0.14	−1.90 ± 0.23	−1.18 ± 0.14
(*n*),selectivity	(4), 1.00	(3), 0.74	(4), 0.54*	(3), 0.23*
(3)				
DR	0.65 ± 0.21	0.52 ± 0.11	0.40 ± 0.07	
Slope	−1.04 ± 0.16	−1.07 ± 0.14	−1.22 ± 0.08	N/A
(*n*),selectivity	(4), 1.00	(5), 0.80	(4), 0.61	
(4)				
DR	0.63 ± 0.13	0.18 ± 0.07	0.14 ± 0.02	0.07 ± 0.002
Slope	−1.22 ± 0.15	−1.23 ± 0.29	−1.28 ± 0.09	−1.31 ± 0.18
(*n*),selectivity	(5), 1.00	(5), 0.29*	(5), 0.22*	(4), 0.11*

(1) = 2′-methoxy-4′-bromo-N-isopropyl-phenylethanolamine
(2) = 2′-methoxy-4′-methyl-N-isopropyl-phenylethanolamine
(3) = 2′-methoxy-4′-chloro-N-isopropyl-phenylethanolamine
(4) = 2′-methoxy-4′-methyl-N-butyl-phenylethanolamine
DR: dose of antagonist (μmol kg^{-1}, i.v.) to produce an isoprenaline dose-ratio of 10.
Slope: of least squares regression from plots of log [isoprenaline dose-ratio − 1] on log [antagonist dose, (mol kg^{-1}, i.v.)].
Selectivity: DR (blood pressure, soleus contractility, airway reactance) + DR (heart rate).
n: number of cats *$P < 0.05$.

curve for the vasodepressor actions of isoprenaline. In these cats, 0.1 μmol did not affect the isoprenaline dose-response curve for the decreases in soleus muscle contractility, however, the remaining doses (0.5, 20 μmol kg^{-1}), in all five cats, produced parallel shifts to the right. For its actions on isoprenaline-induced decreases in airway-reactance, atenolol (0.5 to 20 μmol kg^{-1}) in four cats produced parallel displacements to the right of the isoprenaline dose-response curve.

All slopes had a significant linear correlation. A comparison of the slope values of the regression analysis (Figure 4c) for the action of atenolol against each of the four isoprenaline-induced effects indicates a similarity of action as was described with metoprolol. Comparing the slope values of each parameter there is a significant difference between the slope value for the actions of atenolol on isoprenaline-induced changes in airway reactance and heart rate with each of the other parameters.

The combined regression plots for the action of atenolol on the isoprenaline-induced effects for each

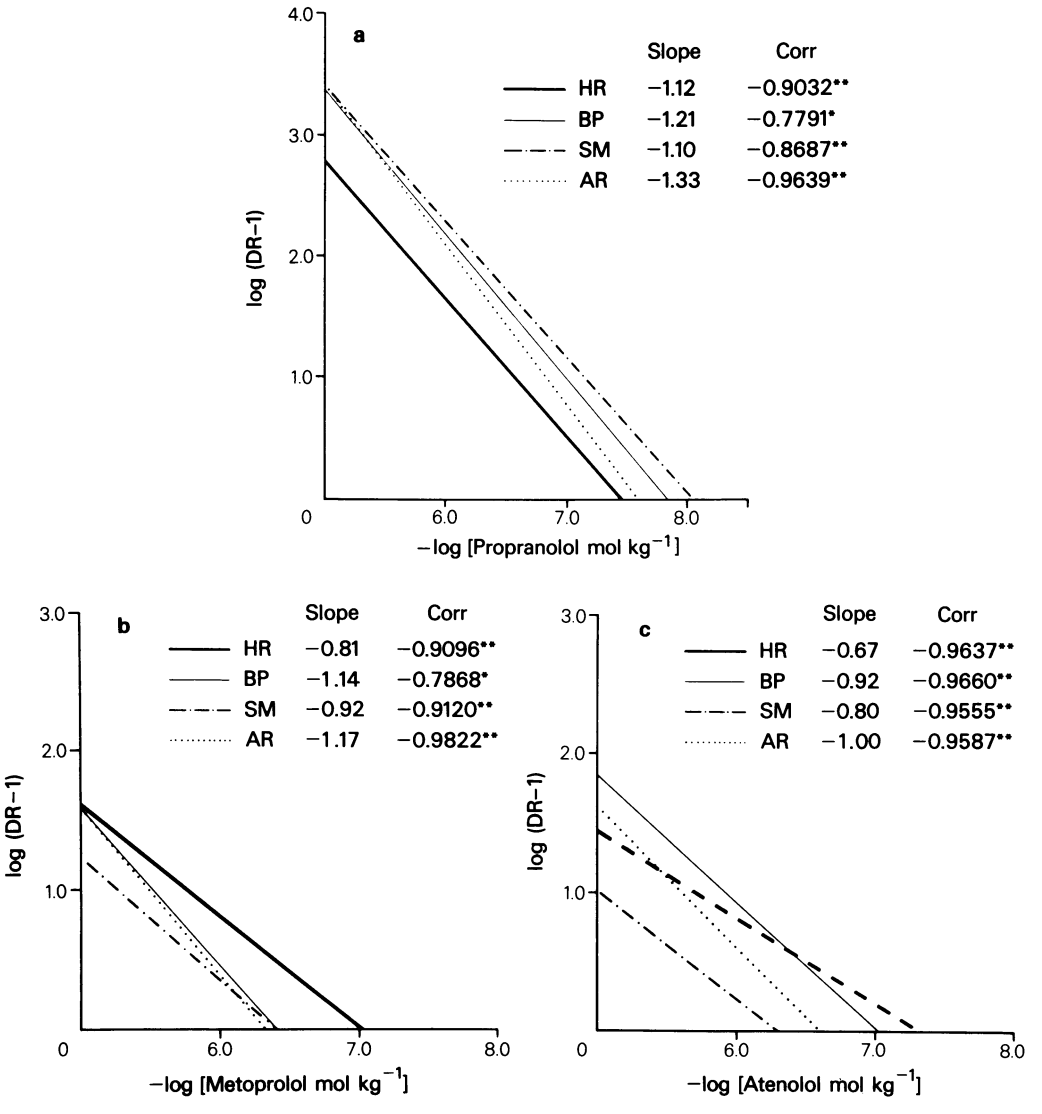

Figure 4 The antagonist actions of (a) propranolol (b) metoprolol and (c) atenolol (all mol kg^{-1}, i.v.) on the isoprenaline-induced changes in heart rate (HR), blood pressure (BP), soleus muscle contractility (SM) and airway reactance (AR) in anaesthetized cats.
Slope: of least squares regression of log (DR − 1) on negative log [antagonist mol kg^{-1} i.v.]
Corr: regression correlation coefficient
*$P<0.05$; **$P<0.01$.

parameter are illustrated in Figure 4c. Atenolol (0.1–0.5 μmol kg^{-1}) preferentially antagonized the isoprenaline-induced changes in heart rate and blood pressure compared to the isoprenaline-induced changes in airway reactance and soleus contractility. As the dose of atenolol progressively increased, the antagonism in the isoprenaline-induced actions on each parameter correspondingly increased. However, there is a concomitant and progressive decrease in the degree of preferential antagonism for the isoprenaline-positive chronotropic effects. In a further series of experiments, cardiac sympathectomy was performed by crushing both stellate ganglia. The results with atenolol in these three cats were not significantly different.

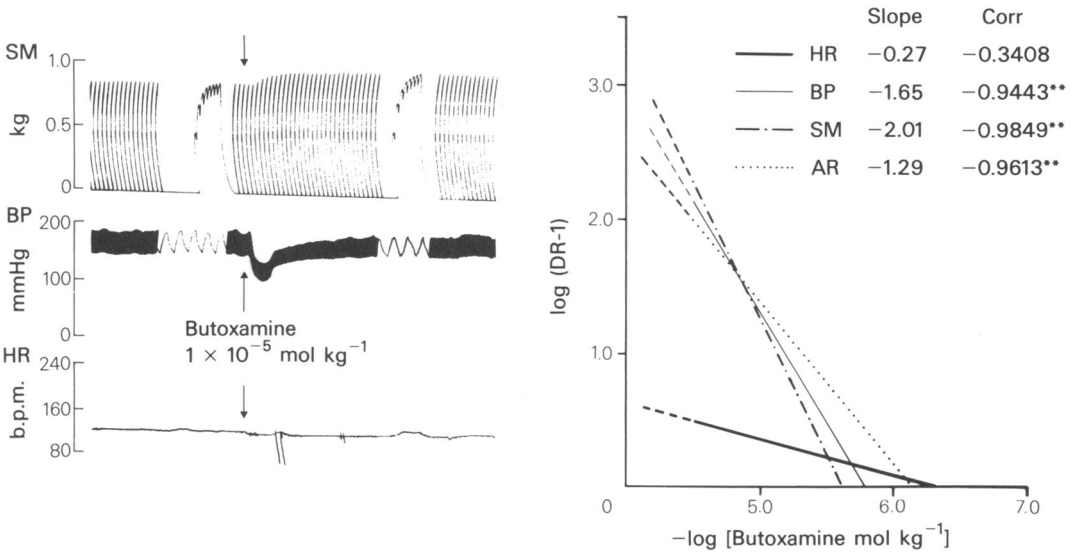

Figure 5 The effects of butoxamine (1×10^{-5} mol kg^{-1} i.v.) on the simultaneous recordings of soleus muscle contractility (SM), blood pressure (BP) and heart rate (HR) and the least squares regression plots for the antagonist action of butoxamine on isoprenaline-induced changes in heart rate (HR), blood pressure (BP) soleus muscle contractility (SM) and airway reactance (AR) in anaesthetized cats. Slope: of least squares regression of log (DR − 1) on negative logarithm [Butoxamine mol kg^{-1} i.v.]. Corr: regression correlation coefficient
**$P < 0.01$

Butoxamine

In one cat, butoxamine (0.1 to 2 μmol kg^{-1}) had no effect on the isoprenaline log dose-response curve for heart rate. In four other cats 2 μmol kg^{-1} was without effect, whereas 10 μmol kg^{-1} did produce a parallel shift to the right of the isoprenaline log dose-response curve. In two of these cats a dose of 30 μmol kg^{-1} was lethal and a dose of 50 μmol kg^{-1} was lethal in the remaining two. In the same experiments, butoxamine 2, 10 and 30 μmol kg^{-1} produced a parallel shift to the right of the log dose-response curves for both the isoprenaline-induced decreases in diastolic blood pressure as well as the isoprenaline-induced decreases in soleus muscle contractility. For its actions on airway reactance butoxamine (2 and 10 μmol kg^{-1}) in three cats produced a shift to the right of the log dose-response curve for the isoprenaline-induced effects. Butoxamine (30 μmol kg^{-1}) was lethal in the same three cats.

The slope values for the actions of butoxamine on isoprenaline-induced changes in diastolic blood pressure, soleus muscle contractility and airway reactance have significant linear correlations. The slope values for airway reactance and soleus muscle contractility are significantly different.

In general, butoxamine exhibited a narrow dose range in which to investigate its antagonist action since for each parameter doses below 2 μmol kg^{-1} did not shift the log dose-response curves for the isoprenaline-induced effects, whereas doses of 30–50 μmol kg^{-1} were lethal. The same doses that produced the measurable shifts of the isoprenaline curves, by themselves, exhibited marked effects. Butoxamine 2 and 10 μmol kg^{-1} produced a marked decrease in blood pressure, on increase in soleus contractility and an increase in airway reactance (Figure 5) and a 30 μmol kg^{-1} butoxamine produced an immediate drop to zero of the resting blood pressure and the animals died within 2 min. In two cats the direct effects of butoxamine were not blocked by prior administration of propranolol.

2′-Methoxy-4′-bromo-N-isopropyl-phenylethanolamine

At doses ranging from 0.05 to 1 μmol kg^{-1}, this compound produced parallel shifts to the right of the isoprenaline-induced log dose-response curves for heart rate, blood pressure and soleus contractility; its actions were not investigated on airway reactance. In five cats, bolus intravenous doses (0.05, 0.2 and 1 μmol kg^{-1}) produced an immediate and sustained increase of the heart rate of 18 ± 2, 20 ± 3 and 11 ± 2 b.p.m. respectively ($n = 5$). The same doses

produced transient decreases of 5±2, 6±1 and 3±1 mmHg ($n=5$) of the resting blood pressure values, but had no effects on soleus contractility. The slope values for the regression analyses for each of the parameters tested had a significant linear correlation and there were no significant differences between each of the slope values (Table 1). The DR10 values of 0.17 μmol kg^{-1} for blood pressure and soleus contractility were significantly different from DR10 value for heart rate and the compound therefore exhibits a significant four fold selectivity of action for blood pressure and soleus contractility compared with heart rate.

2'-Methoxy-4'-chloro-N-isopropyl-phenylethanolamine

This compound (at 0.05 to μmol kg^{-1}) produced parallel shifts to the right of the isoprenaline-induced log dose-response curves for the parameters heart rate, blood pressure and soleus contractility; its actions were not investigated on airway reactance. In four cats, intravenous doses of 0.05, 0.2 and 1 μmol kg^{-1} of the compound produced rapid and sustained increase in the heart rate of 24±3, 22±3 and 12±3 b.p.m. respectively. In the same cats isoprenaline produced an increase of 62±3 b.p.m. The same doses of this compound produced small transient decreases of the resting diastolic blood pressure 2±1, 4±3 and 8+4 mmHg respectively. Isoprenaline induced a decrease of 79±13 mmHg in the same animals. The slope values for the regression analyses for each parameter had a significant linear correlation and there were no significant differences between each of the slope values (Table 1). There were no significant differences between the DR10 values for each of the slope values (Table 1), nor were there significant differences between the DR10 values for each of the parameters investigated, so that no selectivity of action was apparent.

2'-Methoxy-4'-methyl-N-isopropyl-phenylethanolamine

This compound, at doses from 0.1 to 5 μmol kg^{-1}, produced parallel shifts to the right of the isoprenaline-induced log dose-response curves for each of the parameters. In four cats, bolus intravenous doses of 0.1, 0.5 and 2 μmol kg^{-1} of this compound produced an immediate and sustained increase in the heart rate of 14±7, 17±3 and 21±4 b.p.m. respectively. In the same animals, isoprenaline produced a mean increase of 73±5 b.p.m. The same doses produced no effect on soleus contractility, but they produced transient decreases of 3±2, 3±2 and 18±8 mmHg respectively ($n=4$) of the resting diastolic blood pressures. Bolus intravenous doses of 0.2, 1 and 5 μmol kg^{-1} produced 10±10, 31±8 and 33±22% increases in airway reactance ($n=3$). The slope values for the regression analyses for each parameter had a significant linear correlation (Table 1) and there were no significant differences between each of the slope values. A comparison of DR10 values (Table 1) showed the DR10 values for soleus contractility and airway reactance were significantly different from the heart rate DR10 value ($0.01<P<0.02$, 6 d.f.; $P<0.001$, 5 d.f. respectively). The compound therefore showed a significant selectivity of action when soleus contractility and blood pressure were compared with heart rate. The DR10 values for soleus contractility and airway reactance were significantly different ($0.02<P<0.05$, 5 d.f.).

2'-Methoxy-4'-methyl-N-tert.butyl phenylethanolamine

The compound at doses ranging from 0.05 to 2 μmol kg^{-1} produced parallel shifts to the right of the isoprenaline-induced log dose-response curves for each of the parameters. In five cats, bolus intravenous doses of 0.05, 0.2 and 1 μmol kg^{-1} of this compound caused increases in the heart rate of 15±5, 17±8 and 12±5 b.p.m. respectively. Isoprenaline in the same animals increased the heart rate by 70±7 b.p.m. These doses produced no decrease in the resting diastolic blood pressure and negligible effects on soleus muscle contractility, and doses of 0.1, 0.5 and 2 μmol kg^{-1} increased airway reactance 35±28, 4±28 and 36±26% respectively ($n=3$). The slope values for the regression analyses for each parameter had a significant linear correlation and there were essentially no differences between each of the slope values (Table 1). The DR10 value for heart rate was significantly different from blood pressure ($0.001<P<0.02$, 8 d.f.), soleus contractility ($0.001<P<0.01$, 8 d.f.) and airway reactance ($0.001<P<0.01$, 7 d.f.).

Discussion

The results obtained with the β-adrenoceptor antagonists showed that all were capable of antagonizing isoprenaline-induced effects on heart rate, blood pressure, soleus muscle contractility and airways reactance.

In several cases interpretation of the data for analysis of the antagonist action of these drugs is complicated by the lack of similarity of slopes. This is particularly applicable with the slopes for heart rate and airway reactance of metoprolol, atenolol and butoxamine. The reasons for the differences in these slope values are speculative. However, several points

are evident. For the antagonism of isoprenaline-induced increases in heart rate, metoprolol and atenolol produced relatively low slope values, whereas relatively high slope values were obtained for the actions of butoxamine on the blood pressure and soleus muscle responses to isoprenaline. In all cases there was a significant linear correlation. As discussed by Furchgott (1972) and Triggle & Triggle (1976), if a drug does have supplementary actions such that it affects the storage, release or uptake of amines at sympathetic nerve endings or interferes with the extraneuronal uptake mechanisms, effects become apparent. Firstly, the slopes of the log dose-response curve for the agonists used should vary and secondly, plots of log (DR − 1) versus the negative log of the molar antagonist dose may result in slopes with values different from unity.

There is the possibility that the differences in slopes could be due to influences of homeostatic reflexes plus selective β-adrenoceptor blockade. As well, there is the possible influence of a central action of these antagonists. The results obtained for atenolol in cats whose cardiac autonomic reflexes were removed by acute sympathectomy showed the regression slope value for the heart rate parameter was again less than that for airways reactance and the regression again showed high linearity.

A possibility not investigated in this study is the co-existence of β_1- and β_2-sub-types of receptors in the same tissue. O'Donnell & Wanstall (1980) have reported that slope values for log (DR − 1) vs log antagonist plots obtained from *in vitro* experiments on a tissue which possessed both sub-types of receptors may vary from those expected when an agonist (such as isoprenaline) which is capable of interacting with both β_1- and β_2-adrenoceptors, is used to measure the antagonist potency of either a selective β_1- or β_2-adrenoceptor antagonist. The coexistence of β_1- and β_2-adrenoceptors in various organs of the cat has been reported, e.g. heart; Carlsson, Ablad, Brandstrom & Carlsson (1972), O'Donnell & Wanstall (1979), airways: Lulich, Mitchell & Sparrow (1976).

Nevertheless, several points emerge from the present study with these antagonists. Propranolol was the most potent of the antagonists studied and is exhibited a slight β_2-selectivity of action. This slight β_2-selectivity of action of propranolol agrees with the findings in the cat by Apperley, Daly & Levy (1976) and in the dog by Shanks (1966), Boisser, Advenier, Guidicelli & Viaro (1971), Vaughan Williams, Bagwell & Singh (1973), Hainsworth, Karim & Stoker (1974), Daly, Flook & Levy (1975), Harms & Spoelstra (1978) and Fitzgerald & O'Donnell (1970). Metoprolol and atenolol were approximately equipotent and produced similar patterns in the degree of their relative antagonist actions. Thus, while low doses of either antagonist would preferentially antagonize the effects of the isoprenaline-induced increases in heart rate compared to the isoprenaline-induced actions of blood pressure, soleus contractility and airway function, increasing the dose not only increased the degree of antagonism of all parameters, but decreased the preferential action on heart rate. This decrease in the degree of preferential antagonism of heart rate with increasing dose may offer an explanation for the reported lack of therapeutic efficacy of β_1-selective antagonists in man.

The antagonist action of butoxamine had a narrow dose range. A similar finding for the actions of butoxamine in the dog was reported by Daly *et al.* (1975); in the present study in the cat, the doses required to shift the isoprenaline dose-response curves also exhibited direct effects on the same parameters i.e. a decrease in blood pressure, an increase in the peak tension and degree of fusion of sub-tetanic contractions of the soleus muscle and an increase in airway reactance. Bowman & Nott (1970) have reported that butoxamine, in doses required to antagonize the actions of isoprenaline, augments the control contractions of the soleus muscle *in vivo* in cats. In the present study, although butoxamine preferentially shifted the isoprenaline dose-response curves for soleus muscle compared to those on heart rate if the direct actions of these doses of butoxamine are also considered, then the degree of preferential antagonism exhibited is not necessarily due to a selective β_2-adrenoceptor blockade. This preferential antagonism could be explained in terms of functional antagonism. The direct effects of butoxamine were not abolished by prior administration of propranolol, and were therefore not associated with β-adrenoceptor activation.

Each of the 4′-substituted-2′-methoxy phenethanolamines was a potent antagonist of the effects induced by isoprenaline. Each gave slope values which did not differ significantly from each other. Comparison of the relative antagonism of each compound, on each parameter, shows the 4′-chloro compound is essentially non-selective in its antagonism of the effects of isoprenaline. Each of the other 4′-substituted compounds gave results similar to those obtained for propranolol. Increasing the size of the amino-substituent from isopropyl to tert-butyl in the 4′-methyl substituted phenethanolamine increased the relative potency on each parameter without affecting the selectivity of action. A similar finding in cats was reported by Malta & Raper (1975) on the influence of an identical change in the amino-substitution on the β-adrenoceptor agonist activity of soterenol. In the present study the change in the amino substitution from isopropyl to tert-butyl tended to increase the slope values obtained for each parameter.

Thus the results obtained in this study with selective β_1- and β_2-adrenoceptor antagonists show no clear separation of an organ selectivity of action *in vivo* in cats and do not support the concept of a simple β_1/β_2-adrenoceptor classification described by Lands *et al.* (1967). Nor do they show the possibility of separation of tremor-enhancing and airways activity *in vivo*. The results are, however, compatible with the presence of both sub-types of β-adrenoceptors in the heart (Carlsson *et al.*, 1972).

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References

ABLAD, B., CARLSSON, E. & EK, L. (1973). Pharmacological studies of two new cardioselective adrenergic beta-receptor antagonists. *Life Sci.*, **12**, 107–119.

APPERLEY, G.H., DALY, M.J. & LEVY, G.P. (1976). Selectivity of beta-adrenoceptor agonists and antagonists on bronchial, skeletal, vascular and cardiac muscle in the anaesthetized cat. *Br. J. Pharmac.*, **48**, 340P.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BARRETT, A.M., CARTER, J., FITZGERALD, J.D., HULL R. & LE COUNT D. (1973). A new type of cardioselective adrenoceptor blocking drug. *Br. J. Pharmac.*, **48**, 340P.

BERGMAN, J., PERSSON, H. & WETTERLIN, K. (1969). Two new groups of selective stimulants of adrenergic beta-receptors. *Experienta*, **25**, 899–901.

BOISSIER, J.R., ADVENIER, C., GUIDICELLI, J.F. & VIARO, P. (1971). Studies on nature of bronchial beta-adrenoceptors. *Eur. J. Pharmac.*, **15**, 101–109.

BOWMAN, W.C. & NOTT, M.W. (1970). Actions of some sympathomimetic bronchodilator and beta-adrenoceptor blocking drugs on contractions of the cat soleus muscle. *Br. J. Pharmac.*, **38**, 37–49.

BRITTAIN, R.T., FARMER, J.B., JACK, D., MARTIN, L.E. & SIMPSON, N.T. (1968). α-[(t-butylamino)methyl]-4-hydroxy-m-xylene-α1,α3-diol (AH 3365): a selective beta-adrenergic stimulant. *Nature*, **219**, 862–863.

CARLSSON, E., ALBAD, B., BRANDSTROM, A. & CARLSSON, B. (1972). Differentiated blockade of the chronotropic effects of various adrenergic stimuli in the cat heart. *Life Sci.*, **11**, 953–958.

DALY, M.J., FLOCK, J.J. & LEVY, G.P. (1975). The selectivity of beta-adrenoceptor antagonists on cardiovascular and bronchodilator responses to isoprenaline in the anaesthetized dog. *Br. J. Pharmac.*, **53**, 173–181.

DUNLOP, D. & SHANKS, R.G. (1968). Selective blockade of adrenoceptive beta-receptors in the heart. *Br. J. Pharmac.*, **32**, 201–218.

FITZGERALD, J.D. & O'DONNELL, S.R. (1978). The antagonism by propranolol and α-methylpropranolol (ICI 77602) of vascular and cardiac responses to isoprenaline in anesthetized dogs. *Clin. exp. Pharmac. Physiol.*, **5**, 579–586.

FLEMING, W.W., WESTFALL, D.P., DE LA LANDE, I.S. & JELLETT, L.B. (1972). Log normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. *J. Pharmac. exp. Ther.*, **181**, 339–345.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Catecholamines. Handbook of Experimental Pharmacology*, XXXIII ed. Blaschko, H. & Muscholl, E. pp. 283–335. Berlin, Heidelberg, New York: Springer.

HARMS, H.H. & SPOELSTRA, A.J.G. (1972). Cardiac and bronchial beta-adrenoceptor antagonistic potencies of atenolol, metoprolol, acebutolol, practolol, propranolol and pindolol and the anaesthetised dog. *Clin. exp. Pharmac. Physiol.*, **5**, 53–59.

HAINSWORTH, R., KARIM, F. & STOKER, J.B. (1974). Blockade of peripheral vascular responses to isoprenaline by three beta-adrenoceptor antagonists in the anaesthetised dog. *Br. J. Pharmac.*, **51**, 161–168.

KENAKIN, T.P. & BECK, D. (1980). Is prenalterol (H133/80) really a selective beta-adrenoceptor agonist? Tissue selectivity resulting from differences in stimulus-response relationships. *J. Pharmac. exp. Ther.*, **213**, 406–413.

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

LETTS, L.G., RICHARDSON, D.P. & TEMPLE, D.M. (1979). The selectivity of some beta-adrenoceptor antagonists *in vivo* in cats. *Proc. Aust. Physiol. Pharmac. Soc.*, **10**, 194.

LULICH, K.M., MITCHELL, H.W. & SPARROW, M.P. (1976). The cat lung strip as an *in vitro* preparation of peripheral airways: a comparison of beta-adrenoceptor agonists, autacoids and anaphylactic challenge on the lung strip and trachea. *Br. J. Pharmac.*, **58**, 71–79.

MALTA, E. & RAPER, C. (1975). Influence of changes in amine substitution on the beta-adrenoceptor stimulant activity of soterenol and related compounds in the anaesthetized cat. *Clin. exp. Pharmac. Physiol.*, **2**, 259–263.

NOTT, M.W. & RAPER, C. (1972). The use of cumulative dose-response curves in potency comparisons of sympathomimetic amines on the cat soleus muscle. *Br. J. Pharmac.*, **44**, 589–591.

O'DONNELL, S.R. & WANSTALL, J.C. (1980). A study of some propranolol analogues for selective β-adrenoceptor antagonism using pA2 values on isolated trachea and atria from guinea-pig. *Br. J. Pharmac.*, **68**, 705–710.

O'DONNELL, S.R. & WANSTALL, J.C. (1979). The importance of choice of agonist in studies designed to predict beta-2: beta-1 adrenoceptor selectivity of antagonists from pA2 values on guinea-pig trachea and atria. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **308**, 183–190.

RODGER, I.W. (1974). Use of cumulative dose-response curves in potency comparisons of sympathomimetic amines on pulmonary resistance in anaesthetized cats. *Clin. exp. Pharmac. Physiol.*, **1**, 211–217.

SHANKS, R.G. (1966). The effect of propranolol on the cardiovascular responses to isoprenaline, adrenaline and noradrenaline in the anaesthetized dog. *Br. J. Pharmac. Chemother.*, **26**, 233–333.

TRIGGLE, C.R. & TRIGGLE, D.J. (1976). Structure and function of the synapse. In *Chemical Pharmacology of the Synapse*, ed. Triggle, D.J. & Triggle, C.R. pp. 1–127, London: Academic Press.

VAUGHAN WILLIAMS, E.M., BAGWELL, E.E. & SINGH, B.N. (1973). Cardiospecificity of beta-adrenoceptor blockade. A comparison of the relative potencies on cardiac and peripheral vascular beta-adrenoceptors of propranolol, of practolol and its orthosubstituted isomer and of oxprenolol and its parasubstituted isomier. *Cardiovasc. Res.*, **7**, 226–240.

WILLIAMS, L.R., LAP, B.V., LIM,. C.H., TEMPLE, D.M., EASSON, P.A. & LETTS, L.G. (1978). 2'-methoxyphenylethanolamines – potential beta-adrenergic blocking agents. *J. med. Chem.*, **21**, 1081–1084.

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