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THE α- AND β-ADRENOCEPTOR BLOCKING POTENCIES OF LABETALOL AND ITS INDIVIDUAL STEREOISOMERS IN ANAESTHETIZED DOGS AND IN ISOLATED TISSUES

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1 The antagonist potencies of labetalol and each of its four stereoisomers have been compared at α_1-, β_1- and β_2-adrenoceptors in anaesthetized dogs and in isolated tissues.
2 The **RR** stereoisomer is a potent, non-selective antagonist at β-adrenoceptors but has only weak α_1-adrenoceptor blocking activity.
3 The **SR** stereoisomer was the most potent antagonist at α_1-adrenoceptors, and it also had similar potency as an antagonist at β-adrenoceptors.
4 The α- and β-adrenoceptor blocking profile of the **RS** stereoisomer is intermediate between that of the **RR** and **SR**, but the **SS** stereoisomer is a relatively weak antagonist at both α- and β-adrenoceptors.
5 It is concluded that, although most of the α_1-adrenoceptor blocking activity of labetalol is attributable to the **SR** stereoisomer and nearly all of its β-adrenoceptor blocking activity resides in the **RR** stereoisomer, each of the stereoisomers contributes to the overall pharmacological profile of labetalol.

Introduction

Labetalol, an antihypertensive agent that exerts competitive α- and β-adrenoceptor blocking activity, (Brittain & Levy, 1976; Mehta & Cohn, 1977), contains two asymmetric centres, and thus four stereoisomers exist. The labetalol used for animal and human investigations is a mixture of equal proportions of the four stereoisomers. Each of the stereoisomers has been stereospecifically synthesized from **R**- and **S**-phenylisobutylamine (Clifton, Collins, Hallet, Hartley, Lunts & Wicks, 1982) and their individual contributions to the α- and β-adrenoceptor blocking actions of labetalol have been determined.

A preliminary account of these findings has been presented to the British Pharmacological Society (Brittain, Drew & Levy, 1981).

Methods

Anaesthetized dogs

The α- and β-adrenoceptor blocking properties of labetalol and its stereoisomers were determined in anaesthetized dogs as described in detail by Daly, Flook & Levy (1975) and Kennedy & Levy (1975). In brief, beagle dogs of either sex, (7–11 kg) were anaesthetized with thiopentone (25 mg/kg i.v) and barbitone sodium (250 mg/kg i.p.). Dogs were artificially respired with room air via a cuffed endotracheal tube using a Palmer 'Ideal' respiration pump; tidal volume and stroke rate were adjusted to maintain blood P_{O_2}, P_{CO_2} and pH within normal limits (i.e. 70–110 mm Hg, 35–45 mm Hg and 7.35–7.45 respectively). Blood chemistry was measured using an ABL-1 acid-base laboratory (Radiometer: Copenhagen). Body temperature was maintained at 37°C using a thermostatically controlled heating blanket. Aortic pressure was measured with a Bell and Howell pressure transducer (Type 4-442-0001 or 4-327-L221) connected to a polythene cannula placed in the abdominal aorta via a femoral artery; heart rate was derived electronically from the pressure pulse by means of a Devices type 4521 instantaneous rate meter. Blood pressure and heart rate were recorded on a Devices MX6 chart recorder. Drugs were administered intravenously via a cannula in a femoral vein. Before experimentation both vagus nerves were sectioned in the neck in order to minimize cardiovascular reflexes. In each experiment phenylephrine or isoprenaline was injected intravenously to stimulate vascular α_1-adrenoceptors, or vascular and cardiac β-adrenoceptors, respectively. In each experiment dose-response curves to one of

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these agonists were repeated until responses became constant. Then labetalol, or one of its stereoisomers, was injected intravenously and the dose-response curve to phenylephrine or isoprenaline was repeated 15 min later. Further doses of antagonist were injected on a cumulative dose basis. The straight-line portions of the phenylephrine or isoprenaline dose-response curves obtained before and after each antagonist dose were compared at 2 or 3 points and an average agonist dose-ratio was determined. The results were plotted according to the method of Arunlakshana & Schild (1959) and, for each experiment, the dose of antagonist required to cause a ten fold shift to the right in the agonist dose-response curve (DR_{10}) was calculated.

Isolated tissues

The pA_2 values for labetalol and its stereoisomers were determined at α_1-, β_1- and β_2-adrenoceptors in isolated tissues. The effects of the stereoisomers at α_2-adrenoceptors were not examined because it has already been shown that labetalol, itself, is a very weak antagonist at α_2-adrenoceptors (Blakeley & Summers, 1977; Drew 1978). The tissues chosen were (i) rabbit aortic strips for α_1-adrenoceptors (Docherty, Constantine & Starke, 1981), (ii) guinea-pig left atria for β_1-adrenoceptors (Lands, Arnold, McAuliff, Luduena & Brown, 1967; Kaumann, Birnbaumer & Wittmann, 1978) and (iii) guinea-pig trachea for β_2-adrenoceptors (Lands *et al.*, 1967); although this tissue is reported to contain a subpopulation of β_1-adrenoceptors (Furchgott, 1976) it is our experience that responses to isoprenaline are mediated predominantly via β_2-adrenoceptors.

In all experiments carried out *in vitro*, tissue responses were measured isometrically using Dynamometer UF1 2 oz strain gauges, and were displayed on a Devices MX 6 chart recorder. Agonist responses were expressed as a percentage of the maximum attainable, and the EC_{50} refers to that concentration required to cause 50% of the maximum effect.

Rabbit aortic strip

Thoracic aortic arteries were removed from rabbits killed by stunning and exsanguination. The aortae were cut spirally into strips as described by Furchgott & Bhadrakom (1953) and each strip was cut into four approximately equal pieces. Each piece of aorta was suspended in Krebs solution, at 37°C, under an initial tension of 0.3–0.5 g. The Krebs solution contained cocaine (3×10^{-5}M), corticosterone (4×10^{-5}M) and propranolol (1×10^{-6}M) to block uptake$_1$, uptake$_2$ and β-adrenoceptors, respectively.

Approximately 30–60 min after setting up the preparations a cumulative concentration-response curve to noradrenaline was established in each of the four preparations. Two or three more concentration-response curves were established in each preparation until approximately constant responses to noradrenaline were obtained. Intervals of about 1 h were allowed to elapse between successive concentration-response curves. Graded doses of the antagonist were then administered to three of the preparations whilst the fourth was left untreated as a time control preparation. After 45 min the concentration-response curves to noradrenaline were re-established in the presence of the antagonist, and in the untreated preparation.

The response to each concentration of noradrenaline in the absence, and in the presence, of antagonist was expressed as a percentage of the maximum response attainable. Agonist dose-ratios were then determined using EC_{50} values obtained before and after the addition of the antagonist and, after correction had been made for any spontaneous shift in the noradrenaline concentration-response curve obtained in the control preparation (Apperley, Humphrey & Levy, 1976), the pA_2 for each antagonist was calculated according to the method of Arunlakshana & Schild (1959).

Guinea-pig left atria

Guinea-pigs (Duncan Hartley derived; 200–400 g) were killed by cervical dislocation and the left atria were rapidly excised and set up on an electrode block in Krebs solution at 32°C as described by Blinks (1966). The initial load on the atria was 0.5 g. The cardiac muscle was stimulated directly, via the platinum electrodes milled flush with the surface of the electrode block, at a frequency of 1 Hz, using just supra-threshold pulses of 1 ms duration, delivered from a Palmer CFP 8048 stimulator. After a 30 min equilibration period, a concentration-response curve to the inotropic effect of cumulative additions of isoprenaline was obtained.

Four such preparations were run simultaneously, and when constant responses to isoprenaline had been obtained in all of them, graded doses of the antagonist were administered to three of the preparations, whilst the fourth was left untreated, as a control preparation. The concentration-effect curve to isoprenaline was redetermined in each preparation 45 min later. Agonist dose-ratios, measured at the EC_{50} level, in treated preparations were corrected for the spontaneous change in agonist sensitivity seen in the untreated preparation and the results obtained were used to derive the antagonist pA_2 value as described previously.

In these experiments no additions were made to the Krebs solution because isoprenaline is a very

weak substrate for uptake$_1$ (Furchgott 1967) and uptake$_2$ is relatively unimportant in this tissue (Furchgott, 1972).

Guinea-pig tracheal strips

Guinea-pigs (Duncan Hartley derived; 150–400 g) were stunned by a blow on the head and exsanguinated. Four transverse strips, each 2–3 cartilaginous rings wide, were removed from adjacent areas of the cervical trachea and mounted under an initial tension of 1 g in Krebs solution at 37°C as described by Coburn & Tomita (1973). The Krebs solution contained corticosterone (4×10^{-5} M) to inhibit uptake$_2$.

Tissues were contracted with methacholine (0.3 μg/ml; 1.87×10^{-6} M). When a stable contraction had developed, isoprenaline was administered to each preparation in a cumulative-concentration schedule to relax the tissue. When concentration-response curves to isoprenaline had become constant, the pA_2 value for labetalol or one of its stereoisomers was determined as described in the preceding two sections. The antagonist contact time was 45 min.

Effects on relative refractory period (negative dromotropic effects)

The technique used is essentially the same as that described by Dawes, (1946). Left atria were removed from guinea-pigs (Duncan Hartley derived, 200–400 g) and mounted in contact with two small electrodes on a perspex block and placed in Krebs solution at 32°C. The atria were stimulated at 1 Hz, 1 ms at 2.5–5 V continuously for 30–45 min. The stimulation frequency was then increased slowly but progressively until the atria could no longer follow the stimulation frequency. This procedure was repeated at intervals of 30 min until the maximum driving frequency was reproducible on three successive occasions. The antagonist under test was administered and the maximum driving frequency was established 30 min after administration of each dose. The concentration of each antagonist required to reduce maximum driving frequency by 50% was measured.

Krebs solution

The composition of the Krebs solution in all these experiments was (mmol/l): Na^+ 143.4, K^+ 5.9, Mg^{2+} 0.6, Ca^{2+} 1.3, Cl^- 124.5, $H_2PO_4^-$ 1.2, $SO4^{2-}$ 0.6, $HCO^-{}_3$ 25 and glucose 11.1. The Krebs solution in the organ baths and in the reservoir was bubbled continuously with 95% O_2 and 5% CO_2.

Drugs used

The following drugs were used: cocaine hydrochloride (Macfarlan Smith), corticosterone (Sigma), (−)-isoprenaline bitartrate dihydrate (Ward Blenkinsop Ltd), labetalol hydrochloride (Allen & Hanburys), methacholine chloride (Sigma), (−)-noradrenaline bitartrate (Koch-Light), (−)-phenylephrine hydrochloride (Koch-Light) and propranolol hydrochloride (I.C.I.).

The individual stereoisomers of labetalol (Figure 1) were prepared either as the hydrochloride salt, or as the free base by members of the Chemistry Research and Development Departments, Glaxo Group Research Ltd.

The effects of the stereoisomers, either hydrochloride or free base, were tested in anaesthetized dogs, but only the free bases were used in isolated tissue experiments. All drugs except the stereoisomer bases were dissolved shortly before use in 0.9% w/v NaCl solution (saline) or distilled water; the bases were mixed with an equimolar quantity of maleic acid (10 mg enantiomer base: 3.5 mg maleic acid) and dissolved in distilled water. The doses of all drugs mentioned in the text are expressed in terms of the free base.

Results

Anaesthetized dogs

The mean resting blood pressures and heart rates in the different groups of dogs, before antagonist administration, are shown in Table 1. Labetalol, and each of the stereoisomers, injected intravenously, reduced resting blood pressure and heart rate. Because no difference could be seen between the results obtained with the hydrocloride salts and the free bases, results were pooled for each individual stereoisomer (Figure 2).

Labetalol (0.1–10 mg/kg) and its **RR**-isomer (0.03–30 mg/kg) were similar in potency at reducing blood pressure, although the **RR**-isomer was slightly more potent than labetalol at low doses (0.03–0.1 mg/kg). The **RS**-isomer (0.3–10 mg/kg) and the **SR**-isomer (0.3–10 mg/kg) were approximately equipotent, and 2–3 times less potent than labetalol. The **SS**-isomer (0.1–30 mg/kg) was 3–4 times less potent than labetalol. Labetalol caused a dose-dependent reduction in heart rate; at low doses the **RR**-isomer (0.03–0.3 mg/kg) was similar in potency to labetalol but the dose-response curve to higher doses of the **RR**-isomer became flattened. At low doses the **RS**-isomer (0.3–1 mg/kg) was less potent than labetalol at reducing heart rate but was approximately equipotent at higher doses. The dose-

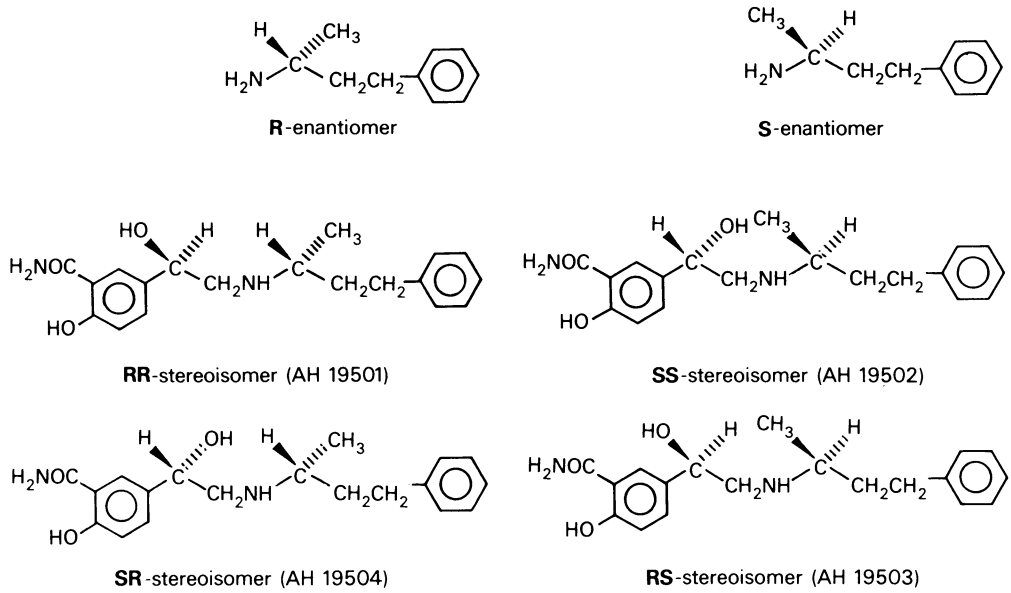

Figure 1 The stereospecific synthesis of the individual stereoisomers of labetalol.

response curves to the **SS**- and the **SR**-isomer were parallel to that of the **RS**-isomer, but these compounds were approximately 3 and 5 times less potent, respectively, than the **RS**-isomer.

Labetalol and its stereoisomers produced parallel, dose-dependent shifts to the right in the vasopressor response curve to phenylephrine, and the positive chronotropic and vasodilator-response curves to isoprenaline. The α- and β-adrenoceptor blocking potencies of labetalol and its stereoisomers are shown in Table 2. As has been described previously, labetalol is equipotent at blocking the cardiac β_1- and vascular β_2-adrenoceptors, but is some 10–30 times less potent at blocking vascular α_1-adrenoceptors (Brittain & Levy, 1976). In contrast, The **RR**-isomer was more potent than labetalol at blocking β_1- and β_2-adrenoceptors but was much weaker at blocking α_1-adrenoceptors. In contrast the **SR**-isomer was slightly more potent than labetalol at blocking α_1-adrenoceptors but was 20–40 times *less* potent than labetalol as a β-adrenoceptor antagonist; indeed the **SR**-isomer was, itself, almost equipotent at blocking α- and β-adrenoceptors. The **RS**-isomer was 7 to 10 times less potent than labetalol at blocking β-adrenoceptors and was a weak α_1-adrenoceptor antagonist. The **SS**-isomer was a relatively weak antagonist at α- and β-adrenoceptors.

The slopes of the Schild plots for labetalol and its stereoisomers against isoprenaline at β_1- and β_2-adrenoceptors were generally close to unity, which is consistent with competitive antagonism at these receptor sites; this was also true for the **RR**-, **SS**- and

Table 1 The resting blood pressures and heart rates of barbitone-anaesthetized dogs before treatment with labetalol or one of its stereoisomers

Antagonist	n	*Systolic blood pressure* (mmHg)	*Diastolic blood pressure* (mmHg)	*Heart rate* (beats/min)
Labetalol	12	150±4	101±4	135±6
RR-isomer	12	141±5	94±3	153±6
SS-isomer	9	149±4	102±2	161±7
RS-isomer	8	140±3	96±4	152±4
SR-isomer	10	147±4	101±4	139±6

All values are presented as mean ± s.e.mean; values were measured after constant responses to the agonist had been obtained, and immediately before antagonist administration.

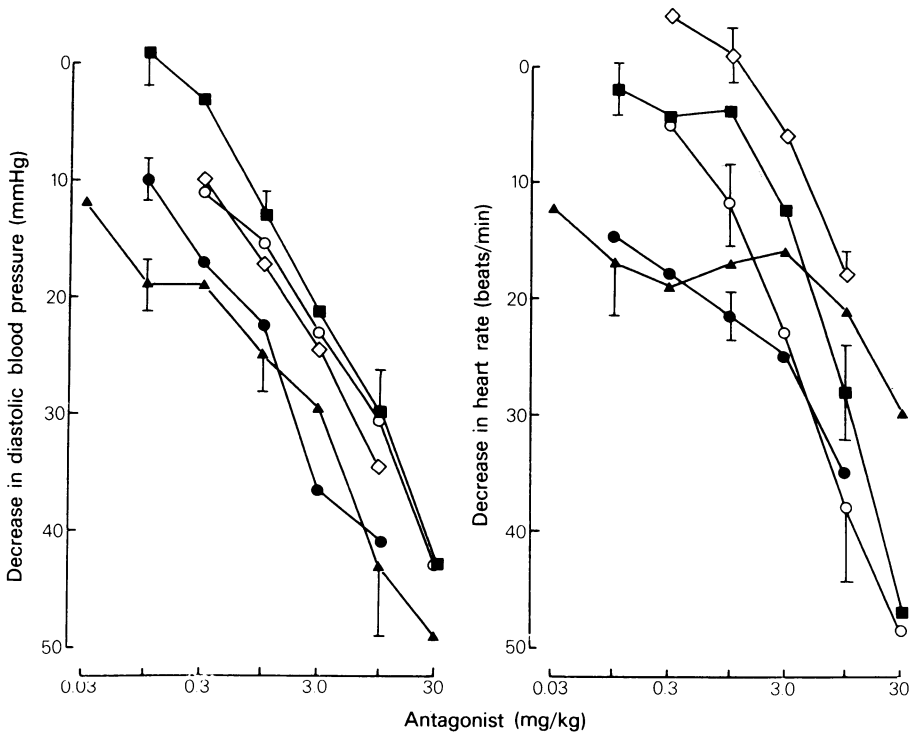

Figure 2 The effects of labetalol (●), **RR**-isomer (▲), **SS**-isomer (■), **RS**-isomer (○) and **SR**-isomer (◇) on resting blood pressure and heart rate in barbitone-anaesthetized beagle dogs. Results are expressed as mean values and vertical bars show s.e.mean.

RS-isomers against phenylephrine at vascular α_1-adrenoceptors, but the slopes for labetalol or the **SR**-isomer against phenylephrine were invariably less than unity.

Isolated tissues

Rabbit aortic strips In spirally cut strips of rabbit aorta, labetalol ($0.3-3.0 \times 10^{-5}$M), the **SS**-isomer ($0.3-3.0 \times 10^{-5}$M) and the **SR**-isomer ($0.91-9.1 \times 10^{-6}$M) caused concentration-dependent, parallel shifts to the right in the noradrenaline concentration-response curve; maximal responses were not reduced. The mean pA_2 values for these compounds are shown in Table 3. The mean slopes of the Schild plots are also shown. With the exception of one experiment using the **SS**-isomer and one using the **SR**-isomer, the slopes of the regressions from individual experiments were always less than unity. Nevertheless, the results show that labetalol and its **SR**-isomer are more potent antagonists than the **SS**-isomer at α_1-adrenoceptors; however, there was little difference between the potencies of labetalol and of its **SR**-isomer. The labetalol-induced antagonism of the contractile responses to noradrenaline in the rabbit aorta is illustrated in Figure 3; also shown, for comparison, is its antagonism of the positive inotropic responses to isoprenaline in guinea-pig atria (see below).

The **RR**- and **RS**-isomers were tested at a concentration of 3×10^{-5}M only; higher concentrations were not used in order to avoid producing non-specific inhibition of the contractile responses to noradrenaline. Under these conditions, in three experiments the **RR**-isomer caused a 29.2, 7.6 and 6.7 fold shift (mean = 14.5) to the right in the noradrenaline concentration-response curve. In three other experiments the **RS**-isomer caused a 10.8, 5.3 and 8.5 fold shift (mean = 8.2) in the curve to noradrenaline.

It is possible to calculate the pA_2 for the antagonist from the effects of a single concentration according to the method described by Schild (1949). However, this assumes that the slope of the plot is unity. Because the results obtained with labetalol and the **SS**- and **SR**-isomers suggested that this was unlikely, the pA_2 values for the **RR**- and **RS**-isomers were calculated graphically from the mean shifts obtained in the presence of 3×10^{-5} M of each stereoisomer, and assuming a slope of 0.86 (the mean of the regressions obtained from all the experiments with labetalol, the **SS**- and the **SR**-isomers). The estimated pA_2 values

Table 2 α- and β-Adrenoceptor blocking potencies of labetalol and its stereoisomers in anaesthetized dogs

Antagonist		*Phenylephrine-induced increase in blood pressure (α_1-adrenoceptors)*			*Isoprenaline-induced increase in heart rate (β_1-adrenoceptors)*			*Isoprenaline-induced decrease in blood pressure (β_2-adrenoceptors)*	
	n	DR_{10}	Slope	n	DR_{10}	Slope	n	DR_{10}	Slope
Labetalol	6	8.9 (6.2–12.7)	0.76 (0.69–0.83)	6	0.34 (0.27–0.43)	1.18 (1.09–1.28)	6	0.24 (0.13–0.45)	1.14 (0.87–1.5)
RR-isomer	5	58 * (43–78)	1.03 (0.88–1.21)	7	0.15 (0.13–0.18)	1.10 (1.0–1.2)	7	0.11 (0.06–0.19)	1.23 (1.05–1.43)
SS-isomer	4	22.6 (13.4–38.1)	1.08 (0.79–1.49)	5	9.86 (5.46–17.8)	0.98 (0.64–1.49)	5	< 10	–
RS-isomer	3	39.3 (19.7–78.5)	1.38 (0.96–1.99)	5	2.23 (1.19–4.19)	1.19 (1.01–1.41)	5	2.58 (1.76–3.79)	1.43 (1.11–1.85)
SR-isomer	5	5.11 (2.70–9.67)	0.75 (0.70–0.81)	5	7.95 (3.97–15.9)	1.01 (0.85–1.20)	5	11.31 (3.88–32.95)	0.95 (0.54–1.67)

DR_{10} = dose of antagonist (mg/kg) required to produce an agonist dose-ratio of 10. * Obtained by extrapolation.
Slope = regression of Arunlakshana & Schild (1959) plot. *n* = number of experiments. Results expressed as geometric mean (and 95% confidence limits).

Table 3 α- and β-Adrenoceptor blocking potencies of labetalol and its stereoisomers in isolated tissues

Antagonist		*Rabbit aortic strip* (α_1-adrenoceptors)*			*Guinea-pig left atrium** (β_1-adrenoceptors)*			*Guinea-pig tracheal strip** (β_2-adrenoceptors)*	
	n	pA_2	Slope	n	pA_2	Slope	n	pA_2	Slope
Labetalol	6	6.99 (6.27–7.25)	0.80 (0.74–0.86)	6	7.68 (7.20–8.16)	1.17 (0.94–1.39)	6	7.40 (7.02–7.78)	1.07 (0.90–1.24)
RR-isomer	3	5.87 (e)	–	6	8.26 (8.06–8.74)	1.19 (1.06–1.32)	5	8.52 (8.21–8.83)	0.93 (0.78–1.08)
SS-isomer	6	5.98 (5.76–6.19)	0.86 (0.70–1.01)	4	6.43 (e)	–	4	< 6.0 (e)	–
RS-isomer	3	5.5 (e)	–	3	6.97 (e)	–	4	6.33 (e)	–
SR-isomer	8	7.18 (6.94–7.42)	0.94 (0.81–1.06)	4	6.37 (e)	–	4	< 6.0 (e)	

pA_2 and slope calculated as described by Arunlakshana & Schild (1959).
n = number of experiments; e = pA_2 estimated from shift produced in the presence of 300 ng/ml (9.1×10^{-7}M) of antagonist. Results expressed as mean (and 95% confidence limits). Agonist used was *noradrenaline or **isoprenaline.

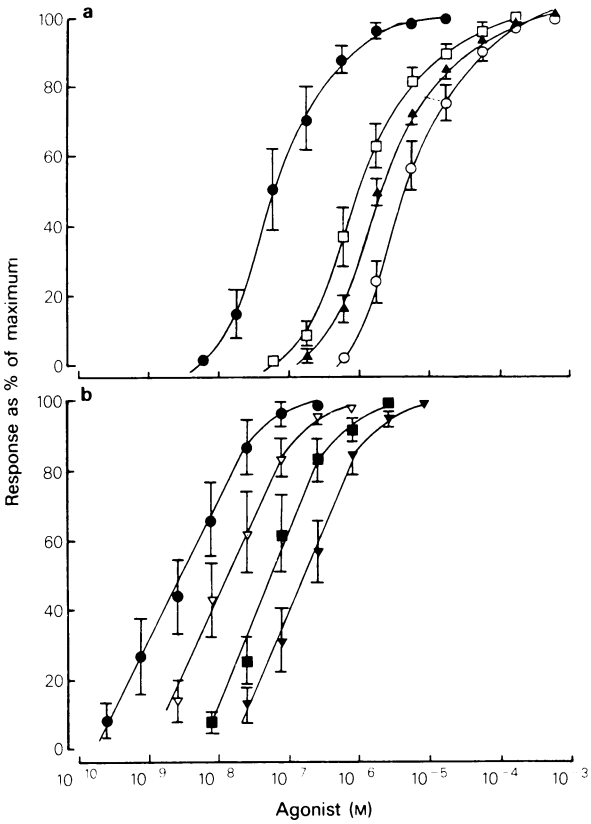

Figure 3 (a) The contractile responses of rabbit isolated aortic strips to noradrenaline before (●) and after 45 min contact with 0.3 (□), 0.91 (▲), or 3.0 (○) × 10^{-5}M labetalol ($n = 6$). (b) the positive inotropic responses of guinea-pig isolated left atria to isoprenaline before (●) and after 45 min contact with labetalol 0.91 (▽), 3.0 (■) and 9.1 (▼) × 10^{-7}M labetalol ($n = 6$). Agonist responses in (a) and (b) are expressed as a percentage (mean) of the maximum attainable; vertical lines show s.e.mean.

for the **RR**- and **RS**-isomers calculated in this way are also shown in Table 3.

In four experiments the pA_2 for the **SR**-isomer was calculated using phenylephrine, in place of noradrenaline, as the agonist. The pA_2 for the **SR**-isomer in these experiments was 7.10 (7.07–7.12); the slope of the regression was 0.99 (0.77–1.27). Thus, the pA_2 value for the **SR**-isomer against phenylephrine was almost identical to that obtained against noradrenaline (7.18).

Guinea-pig atria In guinea-pig isolated left atria labetalol ($0.91–9.1 \times 10^{-7}$M) and its **RR**-isomer ($0.3–3 \times 10^{-7}$M) caused concentration-dependent parallel shifts to the right in the isoprenaline-induced positive inotropic response curves. Maximum responses to isoprenaline were unaffected. The pA_2 values for labetalol and the **RR**-isomer are shown in Table 3. As anticipated from the results obtained in the anaesthetized dogs, the **RR**-isomer was more potent than labetalol at blocking β_1-adrenoceptors. The **SS**-, **RS**- and **SR**-isomers were tested at a single concentration of 9.1×10^{-7}M.

Higher concentrations were avoided because preliminary testing showed that the maximum inotropic response to isoprenaline was depressed by 3×10^{-6} M of antagonist. The **SS**-isomer (9.1×10^{-7} M), caused a 3.3, 1.7, 6.8 and 2.1 fold shift (mean = 3.5) in the isoprenaline-response curve in four preparations. The **RS**-isomer caused the curve to be shifted 11.4, 12.8 and 4.3 fold (mean = 9.5) in three experiments, and the **SR**-isomer produced a 5.2, 2.0, 2.3 and 3.1 fold shift (mean = 3.2) in four other experiments. Because the slopes of the Schild plots for labetalol and its **RR**-isomer versus isoprenaline were not significantly different from unity the pA_2 for the **SS**-, **SR**- and **RS**-isomers were calculated using the mean shifts caused by each of the antagonists at a concentration of 9.1×10^{-7} M as described by Schild (1949). The mean results are shown in Table 3.

Guinea-pig tracheal strip As in atria, labetalol and its **RR**-isomer competitively antagonized the responses to isoprenaline in strips of guinea-pig trachea contracted with methacholine (Table 3). The shifts produced by the **SS**-isomer at a concentration of 9.1×10^{-7} M were 1.07, 1.07, 1.69 and 1.35 fold (mean = 1.3); by the **RS**-isomer were 2.41, 4.11, 4.61 and 1.69 fold (mean = 3.2) and by the **SR**-isomer were 1.17, 1.6, 3.15 and 1.23 fold (mean = 1.8). The estimated pA_2 values for these antagonists (assuming a slope of unity) are also shown in Table 3.

Maximum driving frequency in guinea-pig right atria Labetalol and all the stereoisomers caused a concentration-dependent reduction in the maximum stimulation frequency that guinea-pig atria could follow. The threshold concentration for each drug was usually $3 \times 10^{-6}–3 \times 10^{-5}$ M; the concentrations required to reduce maximum driving frequency by 50% are given in Table 4. Over the same time period untreated preparations showed very little change in responsiveness.

Discussion

The results of the experiments described here demonstrate that each of the stereoisomers of labetalol

Table 4 Negative dromotropic effects of labetalol and its stereoisomers in guinea-pig isolated left atria

Antagonist	n	*– log molar concentration of antagonist required to reduce maximum driving frequency by 50%*
Labetalol	4	4.15 (4.61–3.69)
RR-isomer	4	4.07 (4.70–3.44)
SS-isomer	4	4.32 (4.44–4.18)
RS-isomer	4	3.94 (4.35–3.53)
SR-isomer	4	4.10 (4.10–3.91)

Results are expressed as mean (and 95% confidence limits).

block α_1-, β_1- and β_2-adrenoceptors to varying degrees, but that they have widely differing pharmacological profiles. The results obtained with labetalol in anaesthetized dogs confirm those previously reported by Brittain & Levy (1976); thus labetalol is equipotent at blocking cardiac β_1- and vascular β_2-adrenoceptors, and is approximately 10–30 times less potent at blocking vascular α_1-adrenoceptors. The results obtained with the stereoisomers indicate that most of the α_1-adrenoceptor blocking activity of labetalol is attributable to the **SR**-isomer and, to a lesser extent, to the **SS**-isomer, whereas most of the β-adrenoceptor blocking activity is attributable to the **RR**-isomer and, to a lesser extent, to the **RS**-isomer. When examined individually it is clear that the **RR**-isomer is a potent and equally effective antagonist at β_1- and β_2-adrenoceptors, but has only weak α-adrenoceptor blocking activity. However, the **SR**-isomer is similar in potency at blocking α- and β-adrenoceptors, and the **SS**- and **RS**-isomers are relatively weak antagonists at α- and β-adrenoceptors.

The α- and β-adrenoceptor blocking studies carried out in isolated tissues broadly confirm the findings made in anaesthetized dogs and the studies on the negative dromotropic effect of the antagonists reveal that the 'membrane stabilizing' action of labetalol and its stereoisomers only occurs at concentrations greatly in excess of those required to block α- and β-adrenoceptors. The pA_2 values obtained for labetalol, itself, in the present experiments are slightly lower than previously reported (Brittain & Levy, 1976). This may be explained, at least in part, by the fact that no correction was made for spontaneous changes in tissue sensitivity towards the agonists in the previous study. The current values fit more closely with those recently obtained by other workers. Thus the pA_2 quoted for labetalol at α_1-adrenoceptors in the dog saphenous vein is 7.05 (Humphrey, 1978), and the values obtained in rabbit (Dage, Cheng & Woodward, 1981) and guinea-pig (Nakagawa, Shinamoto, Nakazawa & Inai, 1980) aorta are 6.65 and 6.77 respectively. Dage *et al.* (1981) reported a pA_2 of 7.67 for labetalol at β-adrenoceptors in guinea-pig right atria and Nagawa *et al.* (1980) obtained values of 7.62 and 7.84 in guinea-pig left and right atria respectively; the latter authors also quoted a pA_2 of 7.63 in guinea-pig trachea, which is close to the value obtained in the present experiments.

However, closer inspection of the data reveals differences between the results obtained in anaesthetized dogs and in isolated tissues. In particular, the α:β-adrenoceptor blocking ratios of labetalol and of its **SR**-isomer are smaller *in vitro* than *in vivo*, but this is not the case for the **RR**-, **SS**- or **RS**-isomers. The differences found with labetalol and its **SR**-isomer could be attributable either to an underestimation of their α-adrenoceptor blocking potency, or to an overestimation of their β-adrenoceptor blocking potency, in whole animals compared with isolated tissues. The former seems more likely, however, because the relative potencies of all the compounds in blocking β-adrenoceptors are closely comparable *in vitro* and *in vivo*. It must be concluded therefore that the difference in the α:β-adrenoceptor blocking potency ratios of labetalol and the **SR**-isomer stem from the estimation of their α-adrenoceptor blocking potencies. In view of the fact that the rabbit aortic strip is reputed to contain only α_1-adrenoceptors (Docherty *et al.*, 1981) it is unlikely that the antagonist potency estimates made in this tissue are at fault. The following explanation seems more probable. Although phenylephrine is regarded as a 'selective' α_1-adrenoceptor agonist (Starke, Endo & Taube, 1975; Drew, 1976) its selectivity is not absolute; indeed there is evidence that a small component of the pressor response to phenylephrine in pithed rats is mediated via vascular α_2-adrenoceptors (Flavahan & McGrath, 1981). The same is probably true in dogs, in which there are also vascular α_2-adrenoceptors (Constantine, Gunnel & Weeks, 1980). Thus, as the doses of phenylephrine are increased in order to overcome the α_1-adrenoceptor blockade exerted by

the antagonist, the vascular α_2-adrenoceptors will also be stimulated to a greater extent. Although the potencies of the individual stereoisomers at blocking the α_2-adrenoceptors have not been determined, labetalol itself, at a dose of 30 mg/kg (i.v.) produces only a 3 fold parallel shift to the right in the vasopressor response curve to the preferential α_2-adrenoceptor agonist 2-*N*,*N*-dimethylamino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene (Drew, 1980) in pithed rats (Drew, 1982). If, as seems possible, the **SR**-isomer is much less potent at blocking the vascular α_2- than the α_1-adrenoceptors, the α_2-adrenoceptor-mediated component of the phenylephrine-induced pressor responses will lead to an underestimation of the apparent α_1-adrenoceptor blocking potency of the **SR**-isomer, and also, therefore, of labetalol. The same factor could also account for the low slopes of the Schild plots obtained for labetalol and for the **SR**-isomer *in vivo*. Such an underestimation of α_1-adrenoceptor blocking potency would not occur with the other isomers if the separation between their α_1- and α_2-adrenoceptor blocking potencies was less marked because of their lower potency at blocking the α_1-adrenoceptor. In support of these arguments is the finding that the slope of the Schild plot for the highly selective α_1-adrenoceptor antagonist, prazosin (Cambridge, Davey & Massingham, 1977) against phenylephrine in anaesthetized dogs is less than unity, whereas that for the non-selective antagonist, phentolamine (Timmermans, Van Meel & Van Zwieten, 1980) *is* approximately unity (unpublished observations).

Despite the small quantitative differences between the α- and β-adrenoceptor blocking potencies of labetalol and its stereoisomers *in vitro* and *in vivo*, it is nevertheless clear that the adrenoceptor blocking profile of labetalol is not attributable to the properties of any individual stereoisomer; instead each of the stereoisomers contributes to the overall effects of labetalol. Bearing in mind the individual profiles of each of the stereoisomers, it is interesting to note that the stereoisomer that is primarily responsible for labetalol's β-adrenoceptor blocking activity (the **RR**-isomer) is as potent as, or more potent than labetalol at reducing blood pressure, and that both are more potent in this respect than the stereoisomer that exerts the greatest α-adrenoceptor blocking activity (the **SR**-isomer). Unpublished observations suggest that this is attributable to a fall in cardiac output, rather than peripheral resistance, in barbitone anaesthetized thoracotomized dogs in which there is high efferent sympathetic drive (Lokhandwala, Cavero, Buckley & Jandhyala 1973), although intrinsic sympathomimetic activity at vascular β-adrenoceptors may also be responsible in part, as suggested by Baum, Watkins, Sybertz, Vemulapalli, Pula, Eynon, Nelson, Vliet, Glennon & Moran (1981). A similar effect at cardiac β-adrenoceptors may account for the relatively flat dose-response curve of the **RR**-isomer on resting heart rate. Preliminary findings with the **RR**-isomer in human volunteers showed that it did not reduce resting supine blood pressure (G. Dixon, personal communication), which suggests that it behaved principally as a β-adrenoceptor antagonist.

In conclusion, it is clear that the therapeutic efficacy of labetalol in the treatment of hypertension stems from the combination of the pharmacological properties of the individual stereoisomers that constitute labetalol, rather than from any individual stereoisomer alone.

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