The α - and β -adrenoceptor blocking activities of labetalol and its **RR-SR** (50 : 50) stereoisomers

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1 We compared the α_1 -, α_2 - and β_1 -adrenoceptor blocking potencies of labetalol with those of its two stereoisomers (**RR** and **SR**) in pithed rats and in homogenized rat cerebral cortex and heart.

2 In pithed rats, labetalol and the **RR-SR** combination were given orally either at doses of 25 and 50 mg kg⁻¹ body wt. or intravenously at doses of 1 and 5 mg kg⁻¹ body wt. Prazosin 4 and 20 μ g kg⁻¹ body wt. and propranolol 1 and 5 mg kg⁻¹ body wt., were given intravenously for comparison studies of potency at α_1 - and β_1 -adrenoceptors, respectively. Effects were studied before and after i.v. administration of either phenylephrine (at doses which increased the mean arterial pressure by approximately 80 mmHg) or isoprenaline (at doses that increased heart rate by approximately 100 beats min⁻¹).

3 In pithed rats, labetalol and the **RR-SR** combination antagonized, in a dose-dependent manner, the pressor effect of phenylephrine ($P < 0.05$) and the chronotropic effect of isoprenaline ($P < 0.05$). Following both oral and intravenous dosing, the **RR-SR** combination was twice potent as labetalol in terms of α_1 - and β_1 -adrenoceptor antagonism at equivalent doses.

4 Labetalol and the enantiomers lacked affinity at α_2 -adrenoceptors while at α_1 -adrenoceptors the order of potency was prazosin $\gg$ **RR-SR** > labetalol. At β_1 -adrenoceptors, the affinity of the compound **RR-SR** was about 3 times that of labetalol.

5 As labetalol is a mixture of active (**RR** and **SR**) and inactive (**SS** and **SR**) enantiomers (in terms of α - and β -receptor actions), the combination of **RR** and **SR** may be a valuable substitute for labetalol in the treatment of systemic hypertension. Although the potential for non-specific side effects (common to all four enantiomers) could be expected to be diminished, recent reports by postmarketing surveillance indicate that the **RR** isomer (dilevalol) can induce liver toxicity. Interestingly, labetalol is devoid of this effect; whether the combination of **RR** and **SR** enantiomers could be of clinical importance warrants further investigation.

Keywords: Labetalol; labetalol **RR-SR** isomers; adrenoceptors; arterial pressure; heart rate; radioligand binding

Introduction

Labetalol, an antihypertensive agent that possesses competitive α_1 - and β_1 -adrenoceptor antagonist activity, contains two asymmetric centres, and thus four stereoisomers exist. The formulation used clinically and experimentally is a mixture of equal proportions of the four stereoisomers **RR**, **SR**, **SS**, and **RS**. The α - and β -adrenoceptor antagonist potencies of the individual stereoisomers differ considerably such that most of the α_1 -blocking activity of labetalol is attributable to the **SR** isomer, whereas most of the β_1 -adrenoceptor blocking activity is attributable to the **RR** isomer (dilevalol), while the **SS** and **RS** isomers are devoid of α_1 - and β_1 -antagonist activity (Baum *et al.*, 1981; Brittain *et al.*, 1982; Chrisp & Goa, 1990; Sybertz *et al.*, 1981).

Since non-specific drug toxicity is commonly independent of chirality (Materson *et al.*, 1989; Shoenberger *et al.*, 1989; Chrisp & Goa, 1990), it can be expected that each enantiomer contributes equally to labetalol's potential for side effects (such as dizziness, dyspepsia, diarrhoea and nausea, the latter two being dose-related). However, this is not always true and the **RR** isomer (dilevalol) has recently been withheld from the market due to liver toxicity. We do not know whether the combination of the **RR** and **SR** isomers may represent a valuable substitute for both labetalol and dilevalol. Therefore, we have characterized the α_1 - and β_1 -blocking activity of these enantiomers in combination.

In this study, we attempted to establish whether the **RR-SR** combination possesses greater potency for blockade at α_1 - and β_1 -receptors than labetalol. We took a 50:50 combination of the two active enantiomers (**RR-SR**) and compared the α_1 -blocking activity with that of labetalol and prazosin (measured as the ability to inhibit the increase in arterial pres-

sure following phenylephrine) and the β_1 -blocking activity to that of labetalol and propranolol (measured as the ability to inhibit the increase in heart rate following isoprenaline). We also studied the *in vitro* binding of labetalol and the **RR-SR** (50:50) on α_1 - and α_2 -adrenoceptors in the rat cerebral cortex and with β_1 -adrenoceptors in the rat heart by radioligand binding techniques.

Methods

Animals

Male CD-COBS rats (300–400 g body wt., Charles River, Italy) were used for all experiments. Rats were fasted overnight and allowed to drink water freely. All animals received humane care in accordance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 80-23, revised 1978).

In vivo studies

Rats were anaesthetized with ether and pithed according to the technique described by Gillespie & Muir (1967). Pancuronium bromide and atropine were injected intravenously at a dose of 0.2 mg kg⁻¹ body wt. and 1 mg kg⁻¹ body wt., respectively. The animals were ventilated with room air, at a stroke volume of approximately 8 ml kg⁻¹ body wt. and at a rate of 55 strokes min⁻¹, after vagotomy and tracheostomy. Ventilation was adjusted to maintain arterial blood gases at physiological levels. Body temperature was maintained with a heating lamp. The carotid artery was cannulated for arterial

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blood pressure monitoring (Gould P23 ID, U.S.A.) and blood sampling for gas analysis. The femoral vein was cannulated for drug dosing. The electrocardiogram was recorded via standard limb leads (Battaglia Rangoni Polygraph, Bologna, Italy).

The drug solutions were made fresh daily. For oral dosing, labetalol (25 and 50 mg kg⁻¹ body wt.) and the RR-SR combination (25 and 50 mg body wt.), were dissolved in 0.4% carboxymethylcellulose. For the intravenous dosing, atropine sulphate (1 mg kg⁻¹), labetalol hydrochloride (25 and 50 mg kg⁻¹ body wt.), RR-SR (50:50) hydrochloride (25 and 50 mg kg⁻¹ body wt.), propranolol hydrochloride (1 and 5 mg kg⁻¹ body wt.) and prazosin hydrochloride (4 and 20 µg kg⁻¹ body wt., with 5% w/v sucrose) were dissolved in distilled water; phenylephrine hydrochloride (0.1–10 µg kg⁻¹ body wt.), isoprenaline hydrochloride (0.005–5 µg kg⁻¹ body wt., with 0.1% w/v ascorbic acid) and pancuronium bromide (200 µg kg⁻¹ body wt., brought to pH = 4 with acetic acid) were dissolved in saline. The concentration of each drug is expressed as the free base.

Five groups of animals (5–6 rats/group) were used for oral administration studies. Rats were given 25 and 50 mg kg⁻¹ body wt. of either labetalol or RR-SR, by gavage, 1 h before surgical preparation, with the control group receiving the same volume of the vehicle (0.4% carboxymethylcellulose). Following surgical preparation, the animals were allowed to stabilize for 20–30 min. Following this time, phenylephrine, at increasing doses (from 0.1 to 10 µg kg⁻¹ body wt.) was given at intervals of 10 min until an increase in mean arterial blood pressure of approximately 80 mmHg was achieved. Isoprenaline, at increasing doses (from 0.005 to 5 µg kg⁻¹ body wt.), was then given at intervals of 10 min until an increase in heart rate of 100 beats min⁻¹ was reached.

Nine groups of animals (3–5 rats/group) were used for intravenous administration studies. Following the surgical preparation, the animals were allowed to stabilize for 20–30 min and then rats were given 1 or 5 mg kg⁻¹ body wt., of labetalol, 1 or 5 mg kg⁻¹ body wt. of the RR-SR, or 4 or 20 µg kg⁻¹ body wt. of prazosin intravenously; with two control groups receiving 1 ml kg⁻¹ body wt. of distilled water or saline. After a 15–30 min interval, phenylephrine (5 µg kg⁻¹ body wt.) or isoprenaline (0.5 µg kg⁻¹ body wt. given over 60 s) was administered.

Exclusion criteria

Rats with cardiac arrhythmias caused by pithing (atrio-ventricular block) or isoprenaline (ventricular tachycardia) were excluded. Any animal that was excluded, was immediately replaced by another.

In vitro studies

For the receptor binding studies, drugs (hydrochloride salts) were dissolved fresh daily in distilled water giving concentrations ranging from 10⁻⁵ M to 10⁻¹⁰ M and added to incubation mixture in a volume of 10 µl.

α-Adrenoceptor binding was studied with crude membrane preparations from the rat cerebral cortex. Tissue was excised and homogenized in 50 volumes of 50 mM Tris HCl buffer (pH = 7.4) and centrifuged at 50 000 *g* for 10 min. Pellets were washed with Tris buffer, incubated for 15 min at 37°C and washed again with Tris buffer. Finally, pellets were resuspended in 100 volumes of Tris HCl 50 mM containing 0.1% ascorbic acid (pH = 7.7) and 10 µM pargyline. To evaluate α₁-binding, 0.35 nM [³H]-prazosin (specific activity of 76.6 Ci mmol⁻¹, NEN) plus 1 ml of tissue preparation were incubated for 30 min at 25°C, in the presence or absence of an excess (3 µM) of phentolamine to delineate non-specific binding (Greengrass & Bremner, 1979). To evaluate α₂-binding, 0.4 nM [³H]-clonidine (specific activity of 41.5 Ci mmol⁻¹, NEN) plus 1 ml of tissue preparation were incubated for 20 min at 25°C

in the presence or absence of an excess (100 µM) of noradrenaline to delineate non specific binding (Greenberg *et al.*, 1976).

β₁-Adrenoceptor binding was studied in heart homogenate by the technique of Baker & Potter (1980), with minor modifications (Gobbi *et al.*, 1988). The heart was homogenized in 20 volumes of 10 mM Tris HCl buffer (pH = 7.7). An equal volume of 1 M KCl was then added to the tissue preparation. After 10 min at 0°C, the sample was centrifuged for 10 min at 50 000 *g* three times, with an intermediate incubation for 10 min at 37°C. Prior to the last centrifugation the homogenate was passed through a 4 gauze layer to remove connective tissue elements. Finally, the pellet was resuspended in 80 volumes of 50 mM Tris HCl buffer (pH = 7.7) containing 0.1% ascorbic acid and 10 µM pargyline. One nM [³H]-dihydroalprenolol (specific activity of 102 Ci mmol⁻¹, NEN) was added to 1 ml of tissue preparation and incubated for 20 min at 25°C in the absence or presence of an excess (10 µM) of propranolol to delineate non-specific binding.

Incubations were terminated by vacuum filtration on Whatman GF/B (α-receptors) or GF/C glass fibre filters, followed by washing with 5 ml Tris buffer three times. The filter was counted in 10 ml Filter Count Solution using a liquid scintillator spectrometer (Beckman, LS-7500), at approximately 50% counting efficiency.

Inhibition curves were obtained by using 9–12 drug concentrations in triplicate. Data from the *in vitro* binding study were analysed by non-linear fitting programmes (Sacchi Landriani *et al.*, 1983) according to the 'logistic function' (De Lean *et al.*, 1978) calculated as follows:

$$y = \frac{a - d}{1 + \left(\frac{x}{c}\right)^b} + d$$

y = percent of ³H-ligand bound; *a* = minimum effect (maximum percentage of ³H-ligand binding); *d* = maximum effect (minimum percentage of ³H-ligand binding, non-specific binding); *x* = concentration of the displacing drug; *c* = molar concentration of the displacing drug giving 50% of the effect between *a* and *d* or IC₅₀; *b* = slope factor.

Binding affinities (*K_i*) for inhibitors were calculated from IC₅₀ according to the equation of Cheng & Prusoff (1973):

$$K_i = \frac{IC_{50}}{1 + \frac{[L]}{K_d}}$$

IC₅₀ = concentration of the drug giving 50% of radioligand binding inhibition at the site, obtained by the non-linear fitting of the experimental points according to the logistic function; [L] = concentration of the radioligand; *K_d* = affinity constant of the radioligand.

Values are expressed as mean ± variation coefficient (s.d. of the parameter). Student's *t* test between parameters was used in the *in vitro* study for comparison of affinity of RR-SR vs labetalol.

Measurement of haemodynamic variables

Arterial blood pressure and heart rate (derived from the ECG) were recorded throughout the experiment.

In the dose-response studies, 10 min intervals were imposed between each dose of phenylephrine or isoprenaline to allow haemodynamic variables to stabilize: plateau blood pressure and heart rate values were recorded and expressed as the pre-drug control value.

In rats given labetalol and RR-SR 50 mg kg⁻¹ orally, phenylephrine and isoprenaline at doses higher than 10 µg kg⁻¹ and 5 µg kg⁻¹ respectively, were required in order to obtain the maximal response on arterial blood pressure and heart rate (as defined earlier). However, at those doses toxic effects occurred (e.g., isoprenaline higher than 5 µg kg⁻¹ induced ventricular arrhythmias in 100% of the animals), precluding

maximum values being reached. Thus, results from dose-response studies of phenylephrine on blood pressure and isoprenaline on heart rate, have been evaluated in terms of the concentration of agonist ($\mu\text{g kg}^{-1}$ body wt.) that increased mean arterial blood pressure by 15 mmHg and heart rate by 35 beats min^{-1} .

Partial pressures of oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) were measured in the arterial blood (1 ml) from pithed rats randomly selected ($n = 4$) and were found to be in the physiological range ($P_{O_2} = 73 \pm 3$ mmHg, $P_{CO_2} = 43 \pm 2$ mmHg).

Arterial blood pressure and heart rate are expressed as mean $\pm$ s.e.mean. Analysis of variance was followed by Duncan's test for multiple comparison (Rocchetti & Recchia, 1982). Differences were considered statistically significant at $P < 0.05$.

Consideration of pharmacokinetics

Intravenous administration was chosen in order to limit the influence of absorption and first-pass metabolism. First-pass extraction by both the liver and the gut may result in differences in systemic availability for enantiomeric pairs of high extraction ratio drugs (Williams & Lee, 1985). Significant stereoselective hepatic extraction has been demonstrated for metoprolol (Lennard *et al.*, 1983) and propranolol (Walle & Walle, 1979).

Comparison between the *in vivo* and the *in vitro* studies

The concentration of antagonists in equilibrium with receptors after oral administration was assumed to be equivalent to the plasma concentration reported previously with identical doses (Martin *et al.*, 1976). To compare the results obtained *in vivo* with the binding affinities from the *in vitro* studies, we calculated the apparent dissociation constant (K_B) of the agonist-receptor complex from the oral dose-response study (Furchgott, 1972) as follows:

$$K_B = \frac{[B]}{\frac{[A']}{[A]} - 1}$$

B = plasma concentration of antagonist; $[A']$ and $[A]$ = concentrations of agonist giving an equal response in the presence and absence of the antagonist respectively, referred to as the dose-ratio.

The plasma concentration of the antagonist was calculated from the plasma elimination curve of labetalol described elsewhere (Martin *et al.*, 1976). In absence of published data, we assumed that the enantiomers are metabolized at the same rate.

Results

In vivo studies

Control values for arterial blood pressure and heart rate were similar in all the groups. Table 1 shows values of mean arterial blood pressure and heart rate in pithed rats pretreated with adrenoceptor blocking drugs, prior to phenylephrine and isoprenaline dosing. A significant reduction in arterial blood pressure was caused by i.v. RR-SR (50:50) compared with other treatments. On theoretical grounds, both labetalol and the RR-SR combination can be expected to decrease and increase blood pressure, respectively owing to their relative α - and β -selectivity.

The dose-response effects of i.v. phenylephrine on mean arterial blood pressure and isoprenaline on heart rate in pithed rats pretreated orally with adrenoceptor blocking drugs, are shown in Figures 1 and 2, respectively. Labetalol and the RR-SR combination significantly inhibited ($P < 0.05$), in a dose-dependent manner, the effects of phenylephrine on

Table 1 Mean arterial blood pressure (MAP) and heart rate (HR) in pithed rats pretreated with adrenoceptor blocking drugs prior to i.v. phenylephrine and isoprenaline

Treatment	MAP (mmHg)	HR (beats min^{-1})
<i>Oral study</i> ($n = 5-6$)		
Control (vehicle 1 ml kg^{-1})	41 $\pm$ 1	333 $\pm$ 19
Labetalol (25 mg kg^{-1})	40 $\pm$ 1	326 $\pm$ 17
Labetalol (50 mg kg^{-1})	42 $\pm$ 2	344 $\pm$ 10
RR-SR (25 mg kg^{-1})	40 $\pm$ 3	357 $\pm$ 11
RR-SR (50 mg kg^{-1})	41 $\pm$ 4	359 $\pm$ 9
<i>Intravenous study</i> ($n = 3-5$)		
Control (water 1 ml kg^{-1})	46 $\pm$ 1	322 $\pm$ 10
Control (saline 1 ml kg^{-1})	48 $\pm$ 1	322 $\pm$ 18
Labetalol (1 mg kg^{-1})	46 $\pm$ 3	313 $\pm$ 23
Labetalol (5 mg kg^{-1})	47 $\pm$ 3	356 $\pm$ 14
RR-SR (1 mg kg^{-1})	33 $\pm$ 1*	337 $\pm$ 5
RR-SR (5 mg kg^{-1})	37 $\pm$ 4*	338 $\pm$ 7
Propranolol (1 mg kg^{-1})	52 $\pm$ 1	334 $\pm$ 8
Propranolol (5 mg kg^{-1})	48 $\pm$ 1	358 $\pm$ 5
Prazosin (4 $\mu\text{g kg}^{-1}$)	46 $\pm$ 2	334 $\pm$ 16
Prazosin (20 $\mu\text{g kg}^{-1}$)	47 $\pm$ 4	315 $\pm$ 7

Values are mean $\pm$ s.e.mean.

* $P < 0.05$ versus control.

the arterial blood pressure and of isoprenaline on heart rate compared with control. The doses of phenylephrine and isoprenaline that increased the mean arterial blood pressure by 15 mmHg and heart rate by 35 beats min^{-1} are shown in Table 2. The RR-SR (50:50) combination was more potent than labetalol, at equivalent doses, both at α_1 - and β_1 -adrenoceptors.

Changes in mean arterial blood pressure (ΔMAP) and heart rate (ΔHR) in pithed rats pretreated intravenously with adrenoceptor blocking drugs following single doses of phenylephrine (5 $\mu\text{g kg}^{-1}$) and isoprenaline (0.5 $\mu\text{g kg}^{-1}$) are shown in Table 3. Labetalol, RR-SR (50:50) combination, and prazosin significantly inhibited ($P < 0.05$), in a dose-dependent manner, the effects of phenylephrine on arterial blood pressure compared to control rats (Table 3). Labetalol, the RR-SR combination and propranolol, significantly inhibited ($P < 0.05$), in

Figure 1 Dose-response effects of intravenous phenylephrine on mean arterial blood pressure changes in rats given vehicle (Δ) ($n = 6$), labetalol 25 mg kg^{-1} ($\square$) ($n = 5$), labetalol 50 mg kg^{-1} ($\circ$) ($n = 5$), the RR-SR combination 25 mg kg^{-1} ($\blacksquare$) ($n = 5$) and RR-SR combination 50 mg kg^{-1} ($\bullet$) ($n = 5$) orally, 1 h prior to dose-response study.

Figure 2 Dose-response effects of intravenous isoprenaline on heart rate changes in rats given vehicle (Δ) ($n = 5$), labetalol 25 mg kg^{-1} ($\square$) ($n = 3$), labetalol 50 mg kg^{-1} ($\circ$) ($n = 3$), the RR-SR combination 25 mg kg^{-1} ($\blacksquare$) ($n = 3$) and RR-SR (50:50) 50 mg kg^{-1} ($\bullet$) ($n = 3$) orally, 1 h prior to dose-response study.

a dose-dependent manner, the effects of isoprenaline on heart rate, compared with control (Table 3).

In vitro studies

The affinities of labetalol and the RR-SR combination at α_1 -, α_2 - and β_1 -adrenoceptors are shown in Table 4. Both labetalol and the RR-SR combination had low affinity for α_2 -adrenoceptors and high affinity for α_1 -adrenoceptors (Table 4). The order of potency at the α_1 -adrenoceptor was prazosin > RR-SR (50:50) > labetalol. Affinity constants (K_i) at α_1 -adrenoceptors were different for labetalol and the RR-SR combination, the latter being about twice as potent as labetalol.

In Table 4 are also shown the results obtained from displacement of [^3H]-dihydroalprenolol at β_1 -adrenoceptors from the rat heart. Labetalol and the RR-SR combination displaced [^3H]-dihydroalprenolol with slope values approaching unity (as did propranolol). The RR-SR (50:50) isomers were significantly more active (approximately 2–3 times) than labetalol at cardiac β_1 -adrenoceptors.

Table 2 Doses of i.v. phenylephrine and isoprenaline required to increase the mean arterial blood pressure by 15 mmHg or heart rate by $35 \text{ beats min}^{-1}$ in pithed rats pretreated with adrenoceptor blocking drugs

Treatment	Phenylephrine ($\mu\text{g kg}^{-1}$ body wt.)	Isoprenaline ($\mu\text{g kg}^{-1}$ body wt.)
<i>Oral study (n = 5–6)</i>		
Control (vehicle 1 ml kg^{-1})	0.68 ± 0.10	0.01 ± 0.002
Labetalol (25 mg kg^{-1})	$1.72 \pm 0.14^*$	0.21 ± 0.05
Labetalol (50 mg kg^{-1})	$4.75 \pm 0.59^*$	$1.15 \pm 0.36^*$
RR-SR (25 mg kg^{-1})	$3.95 \pm 1.41^*$	$1.26 \pm 0.87^*$
RR-SR (50 mg kg^{-1})	$8.28 \pm 1.34^*$	$2.90 \pm 0.87^*$

Values are mean $\pm$ s.e.mean.

* $P < 0.05$ vs control.

Table 3 Changes in mean arterial blood pressure (ΔMAP) and heart rate (ΔHR) following phenylephrine ($5 \mu\text{g kg}^{-1}$) and isoprenaline ($0.5 \mu\text{g kg}^{-1}$) in pithed rats pretreated with adrenoceptor blocking agents

Treatment	ΔMAP (mmHg)	ΔHR (beats min^{-1})
<i>Intravenous study (n = 3–5)</i>		
Control (water 1 ml kg^{-1})	$+58 \pm 5$	$+139 \pm 7$
Labetalol (1 mg kg^{-1})	$+26 \pm 1^*$	$+43 \pm 7^*$
Labetalol (5 mg kg^{-1})	$+15 \pm 2^*$	$+16 \pm 3^*$
RR-SR (1 mg kg^{-1})	$+10 \pm 1^*$	$+21 \pm 2^*$
RR-SR (5 mg kg^{-1})	$+6 \pm 2^*$	$+5 \pm 1^*$
Control (saline 1 ml kg^{-1})	$+58 \pm 1$	$+122 \pm 13$
Propranolol (1 mg kg^{-1})	—	$+38 \pm 4^*$
Propranolol (5 mg kg^{-1})	—	$+9 \pm 1^*$
Prazosin ($4 \mu\text{g kg}^{-1}$)	$+19 \pm 3^*$	—
Prazosin ($20 \mu\text{g kg}^{-1}$)	$+7 \pm 4^*$	—

Values are mean $\pm$ s.e.mean.

* $P < 0.05$ vs control.

Comparison between the in vivo and in vitro studies

We have compared the *in vitro* and *in vivo* affinities of labetalol and the RR-SR combination of α_1 - and β_1 -adrenoceptors on the basis of their K_i (Table 4) and K_B values. In Table 5 are shown the dissociation constants (K_B) at α - and β -adrenoceptors for labetalol and the RR-SR (50:50) isomers calculated from the *in vivo* studies and their affinity constants (K_i) obtained from the *in vitro* studies. The dissociation constants for labetalol and the RR-SR combination at α_1 -adrenoceptors were similar *in vivo* ($K_B = 1.9 \times 10^{-7}$ and 7.6×10^{-8} respectively) and *in vitro* ($K_i = 1.2 \times 10^{-7}$ and 5.9×10^{-8} respectively), thus, giving estimates of the relative α_1/β_1 potency ratio of approximately 15 *in vivo* and 10 *in vitro*.

Table 4 Inhibition of radioligand binding (K_i) at adrenoceptors in the rat cerebral cortex (α_1 - and α_2 -adrenoceptors) and heart (β_1 -adrenoceptor)

Adrenoceptors Antagonists	Cerebral cortex		Heart
	α_1 - [^3H]-prazosin§	α_2 - [^3H]-clonidine§	β_1 - [^3H]-dihydroalprenolol§
Prazosin	$0.29 \pm 0.02 \times 10^{-9}$	$> 5 \times 10^{-5}$	—
Propranolol	—	—	$1.3 \pm 0.4 \times 10^{-9}$
Labetalol	$1.20 \pm 0.01 \times 10^{-7}$	$2.6 \pm 0.7 \times 10^{-5}$	$1.3 \pm 0.2 \times 10^{-8}$
RR-SR	$5.90 \pm 0.03 \times 10^{-8}^*$	$1.7 \pm 0.2 \times 10^{-5}$	$0.5 \pm 0.1 \times 10^{-8}^*$

Values are mean $\pm$ s.d. of parameter.

* $P < 0.05$ from labetalol with Student's *t* test on considered parameter.

§ Concentration and affinity constant for [^3H]-prazosin were 0.35 nM and 0.5 nM respectively, for [^3H]-clonidine were 0.4 nM and 2.5 nM respectively, and for [^3H]-dihydroalprenolol were 1.0 nM and 0.5 nM respectively. Inhibition curves were obtained by using 9–12 drug concentrations in triplicate.

Table 5 α - and β -Adrenoceptor blocking activities of labetalol and RR-SR expressed as dissociation constant (K_D) of the complex receptor-antagonist *in vivo* and as affinity constant (K_i) *in vitro*

	α_1 -	β_1 -	α_1/β_1 ratio
In vivo dissociation constant (K_D)			
Labetalol	1.9×10^{-7}	1.29×10^{-8}	14.7
RR-SR	7.6×10^{-8}	0.53×10^{-8}	14.3
Labetalol/RR-SR			
Ratio	2.5	2.4	
In vitro affinity constant (K_i)			
Labetalol	1.2×10^{-7}	1.3×10^{-8}	9.2
RR-SR	5.9×10^{-8}	0.5×10^{-8}	11.8
Labetalol/RR-SR			
Ratio	2.0	2.6	

Calculations based on data from Brittain & Levy ($n = 3$ rats for i.v. and $n = 5$ rats for p.o. kinetics).

The higher potency of the RR-SR (50:50) isomers compared with labetalol (approximately double) is also similar both *in vivo* and *in vitro*.

Discussion

Labetalol is a mixture of four optical isomers (RR, SS, SR, RS) the relative potency of which on adrenoceptors differs considerably. The RR stereoisomer was found to be a potent, non-selective antagonist at β -adrenoceptors and the SR stereoisomer a potent antagonist at α_1 -adrenoceptors as shown previously (Brittain *et al.*, 1982). Negligible α - and β -blocking activity was possessed by the SS and SR isomers, as shown previously (Brittain *et al.*, 1982).

In this study we compared the adrenoceptor blocking activities of labetalol with those of a 50:50 combination of the two active enantiomers, RR and SR both *in vivo* and *in vitro*.

In the pithed rat, labetalol and the RR-SR combination possess α - and β -blocking properties at adrenoceptors and the RR-SR combination was 2–3 times more potent than labetalol both at α - and β -adrenoceptors. The results obtained from i.v. and p.o. dosing studies were similar, this indicates that the kinetics of labetalol and the RR stereoisomer after the two routes of administration are not markedly different.

Prazosin, a selective α_1 -adrenoceptor antagonist, possesses a higher affinity at the α -adrenoceptor than either labetalol or the RR-SR combination, both in the pithed rat and in the rat cerebral cortex homogenate.

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Propranolol possessed a potency intermediate between labetalol and the RR-SR combination at β_1 -adrenoceptors. In the guinea-pig isolated atrium, previous studies have shown that propranolol was 4–5 times more potent than labetalol at β -adrenoceptors (Brittain & Levy, 1976). In the rat, very little is known about labetalol kinetics and differences between labetalol and propranolol in terms of absorption, protein binding, tissue/plasma partition or other pharmacokinetic factors, and their contributions to differences in potency between isolated atrium and *in vivo* studies. It was of interest therefore, to compare the activities at α_1 - and β_1 -adrenoceptors of labetalol and RR-SR (50:50) in the pithed rat with affinities for α_1 - and β -adrenoceptors obtained *in vitro* from the cerebral cortex and rat heart. There was a good agreement between labetalol/RR-SR isomer potency ratio and affinities for α_1 - and β_1 -adrenoceptors between *in vivo* and *in vitro* studies. The relative α_1/β_1 potency ratio for labetalol and the RR-SR isomer *in vivo* and *in vitro* studies was 15 and 10, respectively.

The RR-SR isomer/labetalol potency ratio at α - and β -adrenoceptors was approximately 2. This is expected since labetalol contains four optical stereoisomers, two of which are devoid of adrenoceptor activity.

It must be acknowledged that the possibility exists that the pharmacodynamics and/or pharmacokinetics of labetalol differ from that of its stereoisomers, as previously reported for several β -adrenoceptors blocking drugs (Walle & Walle, 1979; Lennard *et al.*, 1983; Williams & Lee, 1985). Also, we are aware that some of our assumptions are necessarily limited by their having being based on the only published paper on labetalol pharmacokinetics in the rat (Martin *et al.*, 1976).

Rather than defining the potential therapeutic implications of the tested compounds, the aim of our study was to compare the pharmacological characteristics of labetalol and the RR-SR combination *in vivo* and *in vitro*.

In conclusion, the active isomers of labetalol, RR and SR, can be expected to be a good substitute for labetalol in the clinical setting in the treatment of systemic arterial hypertension, since 50% of labetalol comprises SS and RS isomers which although devoid of α - and β -adrenoceptor blocking properties might contribute to the overall toxicity of the drug.

However, the recent withdrawal of the RR isomer preparation (dilevalol) from the market, due to liver toxicity, suggests that further work to explore the potential therapeutic advantages of the RR and SR combination is required.

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