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Effects of (±)-, (+)- and (−)-Celiprolol on the Rat Left Atria and Portal Vein

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Abstract—Differing effects of (±)-celiprolol at β-adrenoceptors have been reported. The effects of (±)-, (+)- and (−)-celiprolol on the contractile responses of the rat left atria and portal vein have therefore been studied. (±)-Celiprolol did not augment the responses of the atria to electrical stimulation and is therefore not an agonist at β_1-adrenoceptors. Prolonged treatment with (±)-celiprolol attenuated the contractile activity of the vein and this effect was blocked by ICI 118,551 and is therefore mediated by agonism at β_2-adrenoceptors. The pA_2 values for (±)-, (+)- and (−)-celiprolol at the β_1-adrenoceptors of the atria were 8·0, 6·0 and 8·6, respectively. On the portal vein, (±)- and (−)-celiprolol produced non-parallel rightward shifts of log isoprenaline attenuation curves with a reduction in isoprenaline maximum responses. This effect of (±)-celiprolol on the vein was not due to slowly reversible antagonism, as the inhibitory effect of (±)-celiprolol was readily reversible.

Racemic β-adrenoceptor antagonists (β-blockers) are commonly used in the treatment of hypertension, angina and cardiac arrhythmias. These drugs act as antagonists at β_1- and β_2-adrenoceptors and may also have ancillary properties (e.g. membrane stabilizing activity, intrinsic sympathomimetic activity). The properties of racemic drugs are not always shared equally between the stereoisomers.

(±)-Celiprolol is a new β-blocker that is reported to be β_1-selective, to have intrinsic sympathomimetic activity but to lack membrane stabilizing activity. The original studies of the effects of (±)-celiprolol on isolated preparations showed that it caused parallel rightward shifts of concentration response curves, and it was assumed that it was a readily reversible β-adrenoceptor antagonist. Its pA_2 values were determined and have demonstrated that it is a β_1-adrenoceptor selective antagonist, having a pA_2 value of 8·1 and 7·6 at the β_1-adrenoceptors of the left and right guinea-pig atria, respectively, and pA_2 values of 6·0 and 5·0 at the β_2-adrenoceptors of the calf tracheal muscle and rat uterus (reviewed by Smith & Wolf 1984). A more recent study of the effects of (±)-celiprolol at β-adrenoceptors has failed to confirm that (±)-celiprolol is a readily reversible β-adrenoceptor antagonist; thus (±)-celiprolol caused non-parallel rightward shifts of log concentration response curves at the β_1-adrenoceptors of the rat right ventricle and at the β_2-adrenoceptors of the rat aorta and I have suggested that (±)-celiprolol is a slowly dissociating, rather than readily reversible, β-adrenoceptor antagonist (Doggrell 1990a).

(±)-Celiprolol did not have membrane stabilizing activity on the electrically-driven guinea-pig left atria or on the cat papillary muscle (Smith & Wolf 1984). The intrinsic sympathomimetic actions reported with (±)-celiprolol are a small increase in the spontaneous rate of the isolated atria from guinea-pig and cat, a vasodilation in dogs (Smith & Wolf 1984) and relaxation of human abdominal arteries and veins (Thulesius et al 1982). More recent studies have reported a lack of intrinsic sympathomimetic actions with (±)-celiprolol on the human saphenous vein and mammary arteries (Hughes et al 1987), canine coronary artery, rat mesenteric artery (O'Rourke & Vanhoutte 1990) and the rat electrically driven right ventricle and rat aorta (Doggrell 1990a).

The aims of the present study were to determine whether celiprolol alone did have effects on the cardiovascular system and to further characterize the effects of celiprolol on β-adrenoceptor mediated responses of this system. We report the effects of (±)-, (+)- and (−)-celiprolol alone on the electrically-driven rat left atria and portal vein and on the β_1-adrenoceptor-mediated contractions of the electrically-driven rat left atria and the β_2-adrenoceptor mediated attenuations of the rat portal vein spontaneous contractions to isoprenaline.

Materials and Methods

General

Male Wistar rats, 250–350 g, were stunned and exsanguinated. The heart or portal vein was rapidly removed and placed in Krebs solution saturated with 5% CO_2 in oxygen. All experiments were performed in the presence of a modified Krebs solution (composition (mM): NaCl 116, KCl 5·4, $CaCl_2$ 2·5, $MgCl_2$ 1·2, NaH_2PO_4 1·2, $NaHCO_3$ 22·0, D-glucose 11·2, Na_2EDTA 0·04) at 37°C which was bubbled with 5% CO_2 in oxygen. Contractile responses were measured isometrically with force displacement transducers (Grass model FT03C) and displayed on a polygraph (Grass model 79B). In each series of experiments, the individual values (percentages, slopes, pD_2 values, concentration-ratios and pA_2 values) obtained were subject to Student's *t*-test. Differences were considered significant for $P < 0.05$. Mean values ± s.e.m. were also obtained.

Contractile responses of the rat electrically-driven left atria (method described by Doggrell 1988)

Left atria were removed from the heart and halved. Each atria half was mounted longitudinally between two platinum electrodes under 1 g tension in 5 mL organ baths containing Krebs solution (with 10^{-5} M guanethidine and atropine at 10^{-6} M) and allowed to equilibrate with washing for 60 min. Tissues were electrically stimulated at 4 Hz (5 ms, 10 V).

After 9 min of stimulation, a cumulative challenge with isoprenaline was made. One of the tissues was then treated with (±)-, (+)- or (−)-celiprolol for 75 min while the other tissue of the pair remained untreated throughout. During this time about 1 L of drug-free or drug-containing Krebs overflowed the tissue. The tissues were electrically stimulated and cumulatively challenged with isoprenaline until an isoprenaline maximal response was obtained. This procedure was repeated with one tissue remaining untreated throughout and the paired tissue being treated with a higher concentration of the same drug.

The contractile responses to electrical stimulation just before the second and third challenges with isoprenaline were calculated as a percentage of the response to stimulation before the first challenge with isoprenaline. For each challenge with isoprenaline, the response to electrical stimulation just before challenge with isoprenaline was subtracted from the combined response to electrical stimulation and isoprenaline. The maximal combined responses to electrical stimulation and isoprenaline were calculated as a percentage of the maximum of the first challenge to isoprenaline. If the maximum response to electrical stimulation and isoprenaline between treated and untreated tissues was not significantly different, response curves were calculated as a percentage of the maximum of the individual curves, i.e. normalized.

Attenuation responses of the rat portal vein (method described by Doggrell 1990b)

Each portal vein was cleared of surrounding tissue and mounted under 1 g tension in a 5 mL organ bath containing Krebs solution and allowed to equilibrate with washing for 30 min. Tissues were treated with phenoxybenzamine at 10^{-4} M for 45 min and then rapidly washed for 20 min. The wash was then stopped, and the tissues were allowed to stabilize for 20 min. During this period, the amplitude of the spontaneous contractions became constant and then three series of experiments were performed.

(i) The effects of (±)-, (+)- and (−)-celiprolol on responses to isoprenaline. A cumulative challenge to isoprenaline was made and then tissues were rapidly washed in the presence of (±), (+)- or (−)-celiprolol for 45 min. During this time about 450 mL of drug-containing Krebs solution overflowed the tissue. Washing was stopped for 20 min before a second challenge to isoprenaline. Tissues were then treated with a higher concentration of the same drug in a rapid wash. After a 45 min/500 mL wash and a 20 min stabilization, a third challenge to isoprenaline was initiated.

In some experiments, the effects of washing on the inhibitory effects of (±)-celiprolol and labetalol were determined. In these experiments after the cumulative challenge to isoprenaline in the presence of drug, tissues were transferred to drug-free Krebs solution and 450 mL of this solution was allowed to flow over the tissues in 45 min. After a 20 min stabilization, a third challenge to isoprenaline was initiated.

(ii) Cumulative challenge to (±)-celiprolol. A cumulative challenge to (±)-celiprolol at 10^{-8}, 10^{-7}, 10^{-6} and then 10^{-5} M was made.

(iii) The effect of ICI 118,551 on the attenuation responses to (±)-celiprolol. Tissues were treated with either 450 mL of drug-free or 450 mL of (±)-celiprolol-containing Krebs solution over 45 min. Washing was stopped for 20 min and then ICI 118,551 was added to each tissue.

On the portal vein the measurement of the contractile response was taken as the average of the amplitude of the final 3 contractions in a 5 min period. The attenuation in the presence of a drug or drugs was calculated as a percentage of the contractile response in the first stabilization period.

Assessment of data

Slopes were determined for all isoprenaline response curves. In addition, when responses to isoprenaline were normalized, pD_2, concentration-ratio and pA_2 values were determined. The slope (difference in percentage maximum of the response/unit of logarithm of molar concentration of isoprenaline) and pD_2 value were computed by regression line analysis. The regression line analysis was performed on the steepest part of the log concentration response curve, which was usually over the range 20–80% of the maximum response. For each tissue, the concentration ratios were determined between each challenge with isoprenaline. Previous studies have shown that the sensitivity to isoprenaline decreased with successive challenges in the rat left atria (Doggrell 1988) but not the rat portal vein (Doggrell 1990b). Consequently concentration ratios obtained in the presence of drugs had to be corrected for changes occurring in untreated atria but not portal veins. Thus the difference in the mean pD_2 values from untreated atria were subtracted from the difference in the individual pD_2 values from treated atria. pA_2 values were determined for concentrations of drugs that had no effect on the slope of the isoprenaline response curves by use of the formula:

$$pA_2 = pA_x + \log (x - 1)$$

where pA_x is the negative logarithm of the molar concentration of drug and x is the concentration-ratio.

Drugs

The drugs used were labetalol hydrochloride* (Allen & Hanbury's Research Ltd), (±)- (+)-, and (−)-celiprolol hydrochloride* (Chemie Linz AG), guanethidine hydrochloride* (Ciba-Geigy), atropine sulphate (Serva), (−)-isoprenaline bitartrate (Sigma Chemical Co.) and phenoxybenzamine hydrochloride (Smith Kline and French). Compounds marked with an asterisk were donated.

Results

Left atria

(±)-, (+)- and (−)- Celiprolol had no effect on the resting tone or on the force of contraction of the rat electrically-driven left atria. The responses to isoprenaline alone were not altered by (+)-celiprolol at 10^{-7} M (data not shown). (±)-Celiprolol at 10^{-7}–10^{-6} M, (+)-celiprolol at 10^{-6} M and (−)-celiprolol at 10^{-7}–10^{-6} M caused parallel rightward shifts of the log isoprenaline concentration response curves (i.e. the slopes of the log isoprenaline concentration response curves from treated and untreated tissues were not significantly different, Fig. 1), had no effect on isoprenaline maximum responses and reduced the isoprenaline pD_2

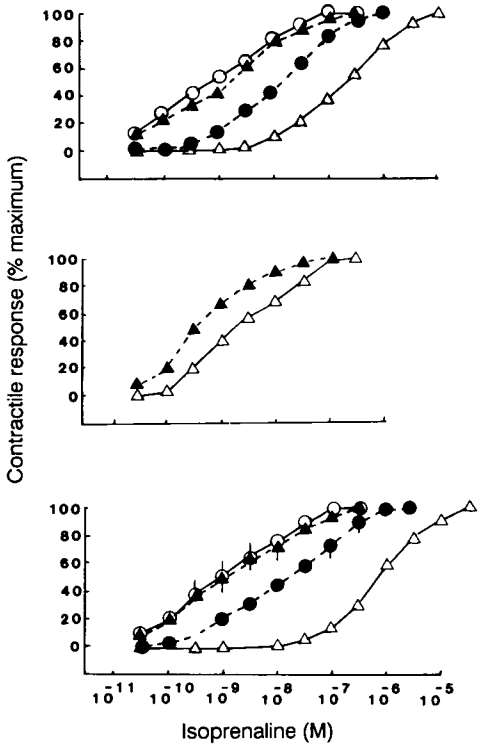

FIG. 1. Effects of (±)-, (+)- and (−)-celiprolol on the contractile responses of the rat electrically-driven left atria to isoprenaline. Top: responses in the absence (○) and presence (●) of (±)-celiprolol at 10^{-7} M and in the absence (▲) and presence (△) of (±)-celiprolol at 10^{-6} M. Middle: responses in the absence (▲) and presence (△) of (+)-celiprolol at 10^{-6} M. Bottom: responses in the absence (○) and presence (●) of (−)-celiprolol at 10^{-7} M and in the absence (▲) and presence (△) of (−)-celiprolol at 10^{-6} M. Responses are calculated as a percentage of the maximum and plotted against the log of the molar concentration of isoprenaline. Each value is the mean from 5 to 8 tissues; vertical lines show s.e.m.

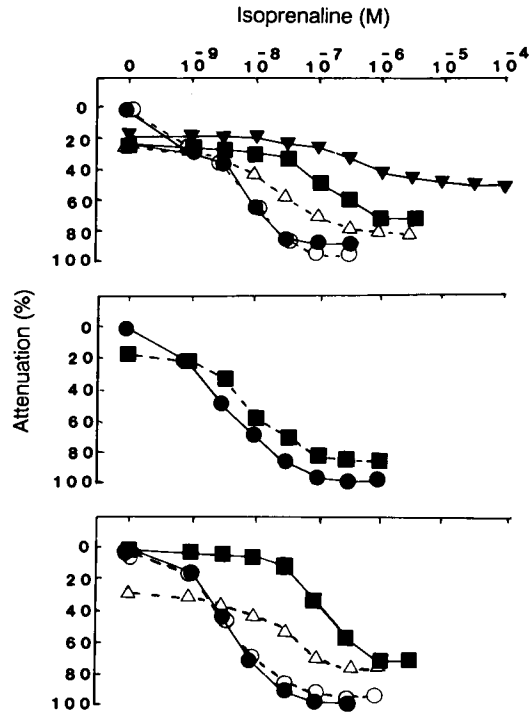

FIG. 2. Effects of (±)-, (+)- and (−)-celiprolol alone and on the attenuation responses of the rat portal vein to isoprenaline. Top: responses in the absence (○) and presence of (±)-celiprolol at 10^{-6} (△) and 10^{-5} M (■) and, from other tissues, responses in the absence (●) and presence (▼) of (±)-celiprolol at 10^{-4} M. Middle: responses in the absence (●) and presence (■) of (+)-celiprolol at 10^{-5} M. Bottom: responses in the absence (●) and presence (△) of (+)-celiprolol at 10^{-6} M and, from other tissues, in the absence (○) and presence (■) of (+)-celiprolol at 10^{-5} M.

values. The mean pA_2 values for (±)-celiprolol at 10^{-7} and 10^{-6} M, (+)-celiprolol at 10^{-6} M and (−)-celiprolol at 10^{-7} and 10^{-6} M were 8·03±0·10 (n=8), 7·99±0·13 (8), 6·04±0·20 (8), 8·55±0·10 (7) and 8·59 and 0·17 (6), respectively.

Portal vein

The spontaneous contractile activity of the phenoxybenzamine-treated portal vein remains unchanged for 6 h (Doggrell 1990b). Following a rapid 45 min wash in Krebs containing (±)- and (−)-celiprolol, but not (+)-celiprolol, the spontaneous contractile activity of the rat portal vein was attenuated. Thus the responses were reduced by 24, 23 and 18% by (±)-celiprolol at 10^{-6}, 10^{-5} and 10^{-4} M and by 27% by (−)-celiprolol at 10^{-6} M (Fig. 2), respectively. (−)-Celiprolol at 10^{-5} M did not attenuate the contractile activity (Fig. 2).

Three successive challenges of the rat portal vein to isoprenaline produced identical attenuation curves (Doggrell 1990b). (±)-Celiprolol at 10^{-6}–10^{-4} M and (−)-celiprolol at 10^{-6}–10^{-5} M caused non-parallel rightward shifts of the log isoprenaline concentration response curves (Table 1) and reduced the maximum attenuation to isoprenaline (Fig. 2). This data with (±)- and (−)-celiprolol was not suitable for use to determine pA_2 values. (+)-Celiprolol at 10^{-6} M had no effect and at 10^{-5} M caused parallel rightward shifts of isoprenaline response curves (Table 1) and a small reduction of the maximum attenuation to isoprenaline (Fig. 2). The pA_2 value for (+)-celiprolol was 5·36±0·10 (8).

The effects of (±)-celiprolol and labetalol on the rat portal vein were compared. Labetalol, like (±)-celiprolol, is considered to be a readily reversible β-adrenoceptor antagonist. Labetalol at 3×10^{-7} M and (±)-celiprolol at 10^{-5} M caused similar attenuations of the contractile activity of the portal vein (24 and 18%, respectively) and a similar magnitude of rightward shift of the isoprenaline response curve (Fig. 3).

Table 1. Effects of (±)-, (+)- and (−)- celiprolol on the slopes of log isoprenaline concentration response curves on rat portal vein.

	Slopes[a]
Control	55±6 (8)
(±)-Celiprolol, 10^{-6} M	36±7 (8)*
Control	54±4 (7)
(±)-Celiprolol, 10^{-5} M	39±4 (7)*
(±)-Celiprolol, 10^{-4} M	28±1 (7)*
Control	60±5 (8)
(+)-Celiprolol, 10^{-5} M	49±7 (8)
Control	53±4 (8)
(−)-Celiprolol, 10^{-6} M	29±5 (8)*
Control	62±3 (8)
(−)-Celiprolol, 10^{-5} M	49±4 (6)*

[a] Mean±s.e.m. (n)=number of animals. * $P<0·05$ t-test with own control.

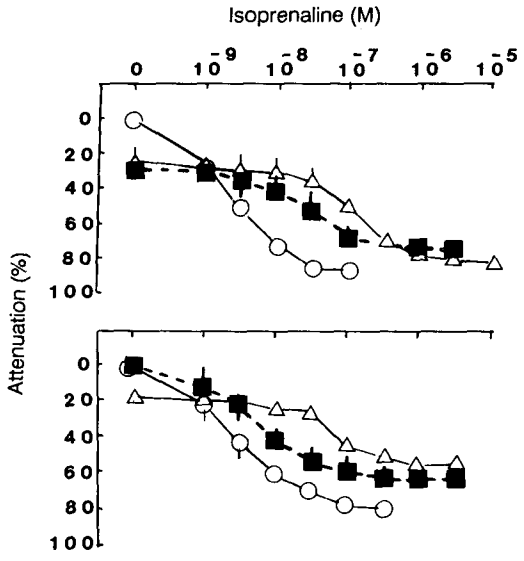

FIG. 3. Effect of washing in drug-free Krebs solution on the effects of labetalol and (±)-celiprolol and on the isoprenaline responses of the portal vein. Top: responses before treatment (○) and during treatment with labetalol at 3×10^{-7} M (△), and labetalol-treated tissues after washing with 450 mL of drug-free Krebs over 45 min (■). Bottom: responses before treatment (○) and during treatment with (±)-celiprolol at 10^{-5} M (△) and (±)-celiprolol-treated tissues after washing (■). Responses are calculated as a percentage attenuation and plotted against the log of the molar concentration of isoprenaline. Each value is the mean from 6–7 tissues; vertical lines show s.e.m.

The rightward shift was parallel in the presence of labetalol at 3×10^{-7} M (slope of 53 ± 7 in the absence and 52 ± 14 in the presence of labetalol) but not parallel in the presence of (±)-celiprolol at 10^{-5} M (48 ± 7 in the absence and 29 ± 5 in the presence of (±)-celiprolol). Labetalol had no effect whereas (±)-celiprolol reduced the maximum attenuation to isoprenaline (Fig. 3). The attenuating effect of labetalol alone at 3×10^{-7} M was not altered whereas that of (±)-celiprolol alone at 10^{-5} M was completely reversed by washing the tissue in 450 mL of drug-free Krebs solution for 45 min (Fig. 3). The antagonism observed with labetalol and (±)-celiprolol was reversed to a similar incomplete extent by the 45 min wash of the tissues with 450 mL of drug-free Krebs (Fig. 3).

Attenuating responses to (±)-celiprolol: effect of ICI 118,551

Further experiments were performed in order to examine the effects of ICI 118,551 on the attenuating responses to (±)-celiprolol. In the first attempt at this, a cumulative challenge of the rat portal vein to (±)-celiprolol was made by adding a small sample (≤ 5 μL) of a concentrated solution of the drug to the 5 mL organ bath on a 10 min cycle to produce final concentrations of 10^{-8}, 10^{-7}, 10^{-6} and then 10^{-5} M. (±)-Celiprolol added in this manner did not attenuate the contractile activity of the rat portal vein (n = 4, data not shown). In order to produce attenuating responses to (±)-celiprolol the protocol used in the previous study had to be repeated. Following a 45 min wash with drug-free Krebs solution the contractile activity of the portal vein was unchanged and remained unaltered on the addition of ICI 118,551 at 10^{-6} M being 100 ± 4 and 97 ± 5% (n = 4) before and after the addition of ICI 118,551, respectively. Following a 45 min wash with 450 mL of Krebs solution containing (±)-celiprolol at 10^{-6} M, the contractile activity of the rat portal vein was attenuated by 12%, to 88 ± 4% (n = 4, $P < 0.05$) and this attenuation was reversed by ICI 118,551 to 105 ± 7% (n = 4, $P < 0.05$).

Discussion

Some cardiac preparations have a mixed population of β_1- and β_2-adrenoceptors whilst others have only functional β_1-adrenoceptors. Previous studies have demonstrated that (±)-celiprolol has intrinsic sympathomimetic activity on some but not all cardiac preparations. Thus (±)-celiprolol increased the rate of guinea-pig and cat atrial preparations to a small extent (Smith & Wolf 1984) but had no effect on the rat right ventricle (Doggrell 1990a) or, in the present study, on the rat left atria. The guinea-pig and cat atria have a functional subpopulation of β_2-adrenoceptors (O'Donnell & Wanstall 1985) whereas rat left atrium (Doggrell 1988) and right ventricle (Doggrell 1989) do not have functional β_2-adrenoceptors. As (±)-celiprolol only augments the responses of cardiac preparations containing β_2-adrenoceptors, it is likely that the intrinsic sympathomimetic activity of (±)-celiprolol on the heart is mediated by β_2-adrenoceptors.

There is disagreement on whether (±)-celiprolol causes β_2-adrenoceptor mediated vasodilation. Thus (±)-celiprolol has been shown to relax some, but not all, isolated blood vessels. This discrepancy between studies may relate to the method of (±)-celiprolol application. Thus, in the present study, the spontaneous contractile activity of the rat portal vein was attenuated by prolonged, but not by short, exposures to (±)-celiprolol and this relaxation was readily reversed by ICI 118,551, the selective β_2-adrenoceptor antagonist. Thus the vasodilatory effect of (±)-celiprolol is probably due to the slow association of (±)-celiprolol with β_2-adrenoceptors.

The original studies of the effects of (±)-celiprolol on isolated β_1- (guinea-pig left and right atria) and β_2-adrenoceptor-containing (calf tracheal muscle and rat uterus) tissues showed that it caused parallel rightward shifts of concentration response curves, and it was assumed that it was a readily reversible β-adrenoceptor antagonist (Smith & Wolf 1984). More recently I have shown that (±)-celiprolol causes non-parallel rightward shifts of β_1-adrenoceptor-mediated response curves of the rat electrically-driven right ventricle and of β_2-adrenoceptor-mediated response curves of the rat KCl-contracted aorta and have suggested that (±)-celiprolol is a slowly reversible β-adrenoceptor antagonist (Doggrell 1990a). In the present study (±)-celiprolol caused parallel rightward shifts of the isoprenaline β_1-adrenoceptor-mediated log concentration response curves of the rat left atria but non-parallel rightward shifts of the isoprenaline β_2-adrenoceptor-mediated response curves of the rat portal vein. On portal vein tissue, in which (±)-celiprolol caused non-parallel rightward shifts, further experiments were undertaken to ascertain whether (±)-celiprolol was slowly reversible. The results show that similar inhibitory effects of (±)-celiprolol and labetalol (an agent that is considered to be readily reversible and which causes parallel shifts of log isoprenaline concentration response curves) were reversed to a similar extent by washing in drug-free Krebs solution. Thus the ability of (±)-celiprolol to cause

non-parallel rightward shifts of β-adrenoceptor-mediated response curves in some tissues is not due to it being a slowly reversible antagonism and the reason for the non-parallel rightward shift remains unknown. However, it is possible that the type of antagonism observed with (±)-celiprolol is related to the receptor reserve of the tissue. Thus on tissues with large β-adrenoceptor reserves (e.g. guinea-pig atria (Ng & Malta 1989), rat atria (Doggrell 1990b)) (±)-celiprolol causes parallel shifts of β-adrenoceptor-mediated responses whereas on tissues with small β-adrenoceptor reserves (rat ventricle and aorta (Doggrell 1990b), portal vein (Doggrell 1990c)), (±)-celiprolol causes non-parallel shifts of β-adrenoceptor mediated responses.

In a study comparing the effects of (±)-, (+)- and (−)-celiprolol on the rat right ventricle and aorta, the majority of the β-adrenoceptor blocking activity of (±)-celiprolol was demonstrated to be due to the (−)-isomer (Doggrell 1990a). The present study using the rat left atria and portal vein confirms this and also demonstrates that the intrinsic sympathomimetic activity of (±)-celiprolol at β_2-adrenoceptors is due to the (−)-isomer.

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