The β_1 - and β_2 -adrenoceptor affinity and β_1 -blocking potency of S- and R-metoprolol

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1 The β -adrenoceptor affinity and blocking potency of the two enantiomers and the racemate of metoprolol were investigated *in vitro*, by use of a receptor-binding technique, and *in vivo* in the anaesthetized cat.

2 The enantiomeric purity of the S- and R-form was: >99.2% and >99.9%, respectively.

3 The β_1 - and β_2 -adrenoceptor affinity ($-\log$ equilibrium dissociation constant) of the enantiomers was determined from competition binding experiments (radioligand: [125 I]-(S)-pindolol) performed in membranes prepared from the guinea-pig left ventricular free wall (predominantly β_1) and soleus muscle (β_2). The β_1 -adrenoceptor affinity was (means $\pm$ s.d.): 7.73 ± 0.10 and 5.00 ± 0.06 for the S- and R-form of metoprolol, respectively. The corresponding values for β_2 -adrenoceptors were 6.28 ± 0.06 (S) and 4.52 ± 0.09 (R). Thus, the difference in affinity for the two enantiomers was greater on β_1 - (about 500) than on β_2 -adrenoceptors (about 50). The β_1 -adrenoceptor selectivity of the S-form (about 30) was similar to that of the racemic metoprolol, while the R-form was almost non-selective (3 fold β_1 -selective).

4 In the anaesthetized cat, the ($-\log$) intravenous doses ($\mu\text{mol kg}^{-1}$) of S- and R-metoprolol causing a 50% reduction (ED_{50}) in the heart rate response to sympathetic nerve stimulation were determined. The doses inducing a 25% depression (DD_{25}) of the basal myocardial contractility were also estimated. For the two enantiomers, the β_1 -blocking potency ($-\log \text{ED}_{50}$) was 7.04 ± 0.16 (S) and 4.65 ± 0.16 (R). A significant cardiodepressive effect was observed at high doses ($-\log \text{DD}_{25}$): 4.18 ± 0.20 (S) and 4.08 ± 0.10 (R).

5 It is concluded that the binding of metoprolol to β_1 -adrenoceptors has a stricter steric requirement than that for the binding of this β -blocker to β_2 -adrenoceptors. Furthermore, the non-specific cardiodepressive effect of metoprolol was observed at equally high doses for the two enantiomers.

Introduction

Metoprolol is a β_1 -adrenoceptor selective antagonist used in the treatment of various cardiovascular disorders, including hypertension and ischaemic heart disease (Åblad *et al.*, 1980; Cruickshank & Prichard, 1987). Like most β -blockers, metoprolol is a racemic compound with two enantiomeric forms, S and R. For many β -blockers, the S-enantiomer has been shown to possess greater β -blocking potency than the corresponding R-form (Patil *et al.*, 1975; Toda *et al.*, 1978; Morris & Kaumann, 1984; Kaumann, 1987; El Tayar *et al.*, 1988; Nathanson, 1988), while other properties such as the membrane stabilizing effect is similar for the two enantiomers (Barrett & Cullum, 1968; Harry *et al.*, 1974; Badia *et al.*, 1984). In the majority of these earlier studies the enantiomeric purities of the two forms of the β -blocker were not stated. Thus, due to the possible contamination of the R-enantiomer with significant quantities of the S-form, conclusions regarding the actual β -adrenoceptor affinity of the R-form could not be drawn from these studies. One exception is a study by Kaumann and coworkers (Wächter *et al.*, 1980), demonstrating that the R-form of bupranolol (enantiomeric purity >99.9%) is about 100 times less potent as a β -blocker compared to the corresponding S-form.

Results obtained in a number of studies suggest that the steric requirements of the β_1 -adrenoceptor is more stringent than that of the β_2 -adrenoceptor (Lemoine & Kaumann 1983; Morris & Kaumann 1984; Walter *et al.*, 1984; Nathanson, 1988). Indeed, Nathanson (1988) demonstrated a marked potency of R-timolol (enantiometric purity >99.9%) to antagonize the β_2 -adrenoceptor stimulant effect of isoprenaline in the rabbit ciliary process. In contrast to the findings on β_1 -adrenoceptors in the heart, where the S-form was more potent than the R-enantiomer, the β -blocking potency of R-

timolol in the iris/ciliary body of the eye was equal to that of the S-form.

The techniques for stereoselective synthesis of compounds have markedly improved in recent years. The use of optically pure precursors makes it possible to synthesize compounds with a high degree of enantiomeric purity (Lamm *et al.*, 1987). With this technique, the S- and R-forms of metoprolol with an enantiomeric purity of >99.2% (S) and >99.9% (R) were recently synthesized by the Department of Organic Chemistry, AB Hässle. The aim of this study was to investigate the β_1 - and β_2 -adrenoceptor affinity of these enantiomers and, furthermore, to compare the β_1 -blocking potency of S- and R-metoprolol with the doses producing significant cardiodepressive effects.

Methods

β -Adrenoceptor binding experiments

Male Dunkin-Hartley guinea-pigs (400–600 g) were used in the experiments. The animals were killed by a blow on the neck, and the left ventricular free wall (LV) and the soleus muscle were excised from each animal for preparation of membranes. Both this procedure and the binding assay were essentially the same as described previously (Abrahamsson & Nerme, 1987; Abrahamsson *et al.*, 1988). The membrane suspensions from LV and soleus muscle had a protein concentration of $0.29\text{--}0.50 \text{ mg ml}^{-1}$ and $0.30\text{--}0.73 \text{ mg ml}^{-1}$, respectively. The incubation with [125 I]-(S)-pindolol, at a final concentration of 100 pM, was performed at 30°C (18 min) and always in the presence of guanosine 5'-triphosphate (Nerme *et al.*, 1985). The specific binding of [125 I]-(S)-pindolol, determined in the presence of 50 μM R-isoprenaline, constituted 85–90% of the total binding.

The negative logarithm of the equilibrium dissociation constant (pK_D) for [125 I]-(S)-pindolol was derived from saturation binding experiments. In each tissue studied, saturation

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binding experiments were performed at 30°C (18 min incubation) with 9 concentrations of the radioligand (20–440 pM) and the pK_D values obtained were (means $\pm$ s.d., $n = 5$): 9.9 ± 0.11 (LV) and 10.3 ± 0.09 (soleus muscle). These constants are in good agreement with those obtained from association/dissociation kinetic experiments (means $\pm$ s.d., $n = 2$): 10.2 ± 0.18 (LV) and 10.1 ± 0.07 (soleus muscle). Moreover, the kinetic analysis of [125 I]-(S)-pindolol binding confirmed that an incubation period of 18 min was sufficient when binding experiments were carried out at 30°C (means $\pm$ s.d., $n = 2$): $k_{+1} = 2.3 \pm 0.57 \text{ nm}^{-1} \text{ min}^{-1}$, $k_{-1} = 0.16 \pm 0.008 \text{ min}^{-1}$ (LV) and $k_{+1} = 1.6 \pm 0.28 \text{ nm}^{-1} \text{ min}^{-1}$, $k_{-1} = 0.12 \pm 0.044 \text{ min}^{-1}$ (soleus muscle).

In order to determine the pK_D values for the various forms of metoprolol, competition binding experiments were performed with 14 concentrations for each drug. For S and racemic metoprolol the concentration ranges studied were 1 nM–20 μ M (LV) and 10 nM–0.2 mM (soleus muscle). The corresponding ranges for R-metoprolol were 10 nM–0.2 mM (LV) and 20 nM–0.5 mM (soleus muscle). The competition binding curves were analysed by fitting the experimental data to a non-linear function, based on the method of least squares, by means of a computer assisted program. Each curve was tested according to a mathematical equation describing a one-site or a two-site receptor interaction. The two-site model was accepted when the fitting gave an improvement in the goodness of fit value that was statistically significant ($P < 0.05$) compared to the fitting to the one-site model.

A β_2 -adrenoceptor selectivity of about 3 has been obtained for [125 I]-(S)-pindolol (McGonigle *et al.*, 1986; Neve *et al.*, 1986; Abrahamsson & Nerme, 1987). Such a selectivity may give rise to potential problems when competition binding experiments are used to determine the β_1 - and β_2 -adrenoceptor affinity of a drug (McGonigle *et al.*, 1986; Neve *et al.*, 1986). Thus, in this study the β_1 -adrenoceptor affinity of metoprolol was determined from the β_1 -adrenoceptor population in the LV (about 80% β_1 -adrenoceptors, Abrahamsson *et al.*, 1988), while the corresponding β_2 -adrenoceptor affinity was determined in the soleus muscle, a tissue with an almost homogeneous β_2 -adrenoceptor population (Abrahamsson *et al.*, 1988). The β_1 -selectivity of the compounds was then calculated from the β_1 - and β_2 -adrenoceptor affinities obtained from the two tissues from each animal.

As described above, the β -adrenoceptor affinity of the radioligand was determined in each tissue preparation studied (both LV and soleus muscle). Since the guinea-pig LV contains both β_1 - and β_2 -adrenoceptors, the affinity of the radioligand obtained in this tissue was regarded as an approximate value of the β_1 -adrenoceptor affinity of [125 I]-(S)-pindolol. However, we do not think that this approximation will significantly affect the calculated β_1 -adrenoceptor affinity of metoprolol. In fact, recalculation of the pK_D values for metoprolol, with the 'true' β_1 -adrenoceptor affinity of [125 I]-(S)-pindolol (mean value $pK_D = 9.7$; Neve *et al.*, 1986), gave similar values to those obtained when the affinity values of the radioligand obtained in the guinea-pig LV were used (see above). In this study, a comparison between the receptor affinities for the radioligand obtained from saturation binding experiments in the LV and soleus muscle revealed a β_2 -adrenoceptor selectivity of 2.9 ± 1.10 (mean $\pm$ s.d., $n = 5$).

In vivo experiments

Male cats (body weight 3.2–5.2 kg) were used in the experiments. Anaesthesia was induced by sodium pentobarbitone (30 mg kg $^{-1}$ i.v.) and maintained by i.v. infusion of 0.5 mg kg $^{-1}$ h $^{-1}$. A tracheotomy was performed and the animals were ventilated mechanically with room air. The respiratory rate and tidal volume were adjusted and bicarbonate was administered to keep blood gases and blood pH within the normal range. The body temperature was kept between 37.5°C and 38.5°C by an automatic thermoregulator con-

nected to a water-heated operating table. Catheters were inserted into the right carotid artery (for blood pressure measurement), the left jugular vein (for drug administration) and the left femoral vein for administration of pentobarbitone. After sternectomy, a tip-transducer (Millard) was passed through the apex of the heart into the left ventricular cavity. The transducer was used to measure left ventricular pressure, and the $dP/dt \text{ max}$ was derived from these signals. Pace electrodes were recorded on the right atrial appendage. Bilateral vagotomy was performed to prevent reflex vagal effects secondary to sympathetic stimulation. The stellate ganglionic rami going to the heart were identified by test stimulation, the rami were then cut and a stimulating electrode placed on the distal part. Throughout the experiment the haemodynamic signals were recorded on a Grass Polygraph and the signals (heart rate, $dP/dt \text{ max}$, mean arterial blood pressure) were analysed by a computerized system (Axenborg, 1988).

The cardiac sympathetic nerves were stimulated (supramaximal current, duration 1 ms) for 60 s and the chronotropic response was recorded. The stimulating frequency required to obtain 80% of the maximal chronotropic response was determined in each cat (mean value $\pm$ s.d.: $1.6 \pm 0.78 \text{ Hz}$). In three groups of five cats, increasing doses of S, R and racemic metoprolol were administered i.v. over a period of 15 min. At each dose level, the nerves were stimulated at the end of the perfusion period (13–15 min) for determination of the β -blocking effect. In each cat the non-specific cardio-depressive effect of the compounds was studied. Starting at 10 μ mol kg $^{-1}$, doses in increments of two were given until the left ventricular $dP/dt \text{ max}$ (determined at constant heart rate) was decreased by more than 50%.

The percentage blockade of the chronotropic response to nerve stimulation was plotted against the cumulative dose of the test compound. The negative logarithm of the dose causing 50% blockade of the chronotropic response ($-\log \text{ED}_{50}$) was calculated as the geometrical mean of ED_{50} values from the individual experiments. Similarly, the $-\log$ dose reducing the $dP/dt \text{ max}$ by 25% ($-\log \text{DD}_{25}$) was obtained by plotting the negative inotropic effect against the cumulative dose of the compound. A dose-ratio comparing the $-\log \text{ED}_{50}$ and the $-\log \text{DD}_{25}$ was calculated for each cat.

Drugs

S, R and racemic metoprolol were obtained from the department of Organic Chemistry, AB Hässle. The method for synthesis of the S-enantiomer of metoprolol has recently been described (Lamm *et al.*, 1987). The R-antipode used in the present work was prepared from D-mannitol via (R)-1-O-methanesulphonyl-2,3-O-isopropylidene-glycerol, following a previously published method (Danilevicz & Kemp, 1973) with one modification, the cyclic sulphite was used instead of the primary tosylate of the 1-aryloxy-2,3-propanediol. The preparation of racemic metoprolol via cyclic sulphite has been described in a recent patent (Jpn. Kokai Tokyo Koho, 1985). The enantiomeric purity of the R- and S-form of metoprolol was determined by h.p.l.c. analysis of the diastereomeric (R)-O-methyl-mandelic acid amides (Helmchen & Strubert, 1974) and was shown to be at least 99.9 and 99.2%, respectively.

[125 I]-(S)-pindolol was purchased from New England Nuclear. R-isoprenaline bitartrate was obtained from Sigma.

Results

β_1 - and β_2 -adrenoceptor affinity (in vitro)

Competition binding curves for S, R and racemic metoprolol in LV and soleus muscle are shown in Figure 1. These curves clearly show that considerably higher concentrations were needed for R-metoprolol to displace the radioligand when compared to the other compounds studied. The β_1 - and β_2 -adrenoceptor affinity of S, R and racemic metoprolol are summarized in Table 1. The β_1 -adrenoceptor affinity of the

Figure 1 Inhibition of binding of [125 I]-(S)-pindolol ([125 I]-Pin) by increasing concentrations of S (○), R (□) and racemic (▲) metoprolol in membrane preparations of the guinea-pig left ventricular free wall (LV) (a) and soleus muscle (b). Shown are curves (binding was performed in duplicate) from the two tissues in one animal. In this experiment the affinity (pK_D) of S-metoprolol was 7.69 (β_1 LV), 6.16 (β_2 LV) and 6.23 (soleus). The corresponding values for racemic metoprolol were 7.49 (β_1 LV), 5.97 (β_2 LV) and 5.97 (soleus). For R-metoprolol the β -adrenoceptor affinity was 5.00 (LV) and 4.50 (soleus).

S-form was about 500 times greater than that of the R-form, while the corresponding difference for the β_2 -adrenoceptors was about 50. As expected, the β -adrenoceptor affinity of the S-form was about twice as great as that of racemic metoprolol. The β_1 -adrenoceptor selectivity ratio of the compounds was calculated by comparison of the β_1 - and β_2 -adrenoceptor affinity obtained from LV and soleus muscle in each animal. The β_1 -adrenoceptor selectivity was similar for the racemic and the S-enantiomer of metoprolol, while the R-enantiomer was only 3 fold β_1 -selective.

From the displacement curves with racemic and S-metoprolol in the LV (Figure 1), we found evidence of two subpopulations of β -adrenoceptors (mean $\pm$ s.d., $n = 5$): $74 \pm 2.2\%$ (β_1) and $26 \pm 2.2\%$ (β_2). This confirms the work on guinea-pig LV previously described (Lemoine *et al.*, 1985; Abrahamsson *et al.*, 1988). In contrast to the other compounds studied, it was not possible to identify the two subpopulations in the LV with R-metoprolol. The β_2 -adrenoceptor affinity (pK_D) of racemic and S-metoprolol

Table 1 The β_1 - and β_2 -adrenoceptor affinity for S, R and racemic metoprolol obtained from receptor binding studies *in vitro*

Metoprolol	n	pK_D		β_1 -selectivity
		β_1	β_2	
Racemate	5	7.32 ± 0.13	5.94 ± 0.09	25 ± 9
S-form	5	7.73 ± 0.10	6.28 ± 0.06	29 ± 6
R-form	5	5.00 ± 0.06	4.52 ± 0.09	3 ± 0.1

Mean values $\pm$ s.d. are shown. n = number of experiments. pK_D = $-\log$ equilibrium dissociation constant.

obtained from the small β_2 -adrenoceptor population in the LV were ($n = 5$): 5.61 ± 0.35 (racemic) and 6.09 ± 0.39 (S). Although the variability was greater, these values from the LV are in agreement with those obtained from the soleus muscle (Table 1).

β_1 -adrenoceptor blocking potency and non-specific cardiodepressive effects (in vivo)

Before the start of the drug administration, the haemodynamic parameters monitored were similar for the three groups of animals (Table 2). In addition, the chronotropic responses to nerve stimulation were the same in the three groups (increase in beats min^{-1}): 77 ± 8 ('racemate'), 72 ± 11 ('S') and 74 ± 7 ('R'). The heart rate response to nerve stimulation was antagonized following administration of increasing doses of S, R or racemic metoprolol (Figure 2). The β -blocking potency was markedly lower for the R-form than for S and racemic metoprolol. Calculations of $-\log \text{ED}_{50}$ values showed that the S-form was about 250 times more potent than the R-enantiomer (Table 3).

Table 2 Basal haemodynamics in the anaesthetized cats before drug administration

Metoprolol	n	Heart rate (beats min^{-1})	MABP (mmHg)	Left ventricular peak dP/dt max
Racemate	5	165 ± 4	110 ± 5	2873 ± 150
S-form	5	158 ± 3	120 ± 5	3486 ± 84
R-form	5	158 ± 3	105 ± 8	3305 ± 233

Mean values $\pm$ s.d. are shown. n = number of experiments. MABP = mean arterial blood pressure. The different groups were subsequently given S, R or racemic metoprolol.

Figure 2 Blockade of the heart rate response to sympathetic nerve stimulation after intravenous administration of increasing doses of S (○, $n = 5$), R (□, $n = 5$) and racemic (▲, $n = 5$) metoprolol. Each dose of the drug was administered over a 15 min period and the cardiac nerves were stimulated at the end of the perfusion period (min 13–15) for determination of the β -blocking effect. The β -blocking effect is presented for all the doses administered to each individual cat.

Table 3 The β_1 -adrenoceptor blocking potency and non-specific cardiodepressive effects in anaesthetized cats of S, R and racemic metoprolol

Metoprolol	n	β_1 -blockade ($-\log \text{ED}_{50}$)	Cardiac depression ($-\log \text{DD}_{25}$)	Dose-ratio ($\text{DD}_{25}/\text{ED}_{50}$)
Racemate	5	6.74 ± 0.13	4.30 ± 0.13	293 ± 129
S-form	5	7.04 ± 0.16	4.18 ± 0.20	779 ± 302
R-form	5	4.65 ± 0.16	4.08 ± 0.10	4 ± 2

Mean values $\pm$ s.d. are shown. n = number of experiments. ED_{50} : dose ($\mu\text{mol kg}^{-1}$) causing 50% blockade of the increased heart rate. DD_{25} : dose ($\mu\text{mol kg}^{-1}$) causing 25% reduction of cardiac contractility (at paced heart rate).

The non-specific cardiodepressive effects of the compounds were studied after administration of high doses. A significant decrease in basal cardiac contractility (dP/dt_{max}) was obtained at similar concentrations for S, R and racemic metoprolol (Table 3). The ratio between the dose causing a β -blocking effect (ED_{50}) and the dose producing a 25% reduction in basal contractility (DD_{25}) was about twice as high for S-metoprolol compared to the racemic form, while the corresponding ratio for the R-form was markedly lower (Table 3).

Discussion

The enantiomeric purity of the two forms of metoprolol studied was very high. This is particularly essential when investigating the β -adrenoceptor affinity and β -blocking potency of the less active R-enantiomer. The enantiomeric purity of the R-form used in this study was more than 99.9%. Thus, the contamination of the R-form with the more active S-enantiomer of metoprolol was less than 0.1%.

An interesting observation in this study was that the affinity difference for the S- and R-enantiomers of metoprolol was 10 times greater on β_1 - than on β_2 -adrenoceptors. Moreover, the β_1 -adrenoceptor selectivity of the S-form was similar to that of the racemate, while the R-form was only 3 fold β_1 -selective. Thus, it seems as if the binding of metoprolol to include β_1 -adrenoceptors has a stricter steric requirement than that for the binding of this drug to β_2 -adrenoceptors.

Several other stereoisomeric pairs of β -blockers have been shown to possess a different potency with regard to β_1 - and β_2 -adrenoceptor blockade. Using a receptor-binding technique, Morris & Kaumann (1984) demonstrated a two fold greater difference between the β_1 - and β_2 -adrenoceptor affinity for the S-enantiomers of ten different β -ligands compared to that of the corresponding R-forms. A similar result was also obtained for the enantiomers of the β -blocker bunolol (Quast & Vollmer, 1984). Taken together, these earlier observations and the findings in this study clearly show that the steric requirements for the binding of β -blockers are different for β_1 - and β_2 -adrenoceptors. Nathanson (1988) recently suggested that the β_2 -adrenoceptor, due to its different configuration, may provide an additional binding site for the R-enantiomer of a β -blocker.

In the anaesthetized cat, the S-enantiomer of metoprolol was demonstrated to be about 250 times more potent than the R-form in attenuating the chronotropic response to sympathetic nerve stimulation. Moreover, the S-enantiomer was about twice as potent as racemic metoprolol. The increase in

heart rate produced by sympathetic nerve stimulation in this model is predominantly mediated via stimulation of cardiac β_1 -adrenoceptors (Åblad *et al.*, 1974; Bryan *et al.*, 1981). Accordingly, the difference in potency for the enantiomers in the cat experiments was quite similar to the affinity difference for the S- and R-forms on β_1 -adrenoceptors, obtained from the binding studies *in vitro*.

A non-specific cardiodepressive effect of β -blockers has been demonstrated at high concentrations (Badia *et al.*, 1984; Hellenbrecht & Enenkel, 1984). This effect, due to the membrane stabilizing properties of the β -blocker, seems to be related to the lipophilicity of the compound (Hellenbrecht *et al.*, 1973; Hellenbrecht & Enenkel, 1984). In this study, a significant decrease in basal cardiac contractility was not observed until doses $> 50 \mu\text{mol kg}^{-1}$ for the three forms of metoprolol. The cardiodepressive effect of the S-form and racemate was observed at doses several hundred fold greater than those required for β_1 -adrenoceptor blockade, while these effects were observed at similar (high) doses for the R-enantiomer.

It has been claimed that racemic drugs are impure compounds with an 'isomeric ballast' (Ariëns, 1986). For the β_1 -selective blocker metoprolol it is clear from this study that the β_1 -adrenoceptor blocking effect of the racemic compound, at therapeutically effective plasma concentrations (50–300 nM) (Harron *et al.*, 1981; Rydén *et al.*, 1988) resides solely in the S-enantiomer ($K_D = 20 \text{ nM}$). The R-form is much less active as a β -adrenoceptor antagonist ($K_D = 10,000 \text{ nM}$) than the S-enantiomer. Although the difference in affinity for the two enantiomers was less pronounced on β_2 - than on β_1 -adrenoceptors, this difference does not mean that the β_1 -selectivity of racemic metoprolol is less than that of the S-enantiomer. It is also very unlikely that the R-form, by its membrane stabilizing properties, will cause any significant effects during therapy with clinically relevant doses of racemic metoprolol.

Today, racemic metoprolol is widely used in the treatment of various cardiovascular disorders. Like other racemic β -blockers in clinical use, metoprolol is well tolerated and the adverse effects are mild and relatively uncommon (Cruickshank & Prichard, 1987). Thus, since β -blockers constitute a group of drugs with a very high benefit/risk ratio, it may be concluded that it is not likely that an active S-enantiomer of a β -blocker will have any therapeutic advantages over the racemic form of the drug.

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