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THE β_1- AND β_2-ADRENOCEPTOR AFFINITY OF ATENOLOL AND METOPROLOL

A RECEPTOR-BINDING STUDY PERFORMED WITH DIFFERENT RADIOLIGANDS IN TISSUES FROM THE RAT, THE GUINEA PIG AND MAN

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Abstract—The radioligand binding technique was used to perform a systematic investigation of the β_1- and β_2-adrenoceptor affinity of atenolol and metoprolol in tissues from the rat, the guinea pig and man. Radioligands, [^{125}I](±)hydroxybenzylpindolol, [^{125}I](−)pindolol, [^{3}H](−)dihydroalprenolol and [^{3}H](−)CGP12177, with different degrees of lipophilicity were used in the binding experiments. In membrane preparations of rat ventricular myocardium and uterus, the number of specific binding sites was similar when comparing experiments performed with the different radioligands. The percentage of the β_1- and β_2-adrenoceptor subpopulations in the tissues studied was not dependent on the radioligand or displacing compound used. Furthermore, the affinity of metoprolol and atenolol for β_1- and β_2-adrenoceptors was independent of the radioligand used or the tissue studied. The β_1-adrenoceptor affinity of metoprolol was about 6–7 times higher than that of atenolol, while the β_1-adrenoceptor selectivity was similar (about 30-fold) for the two β-blockers. It is concluded that the physical–chemical properties of the radioactive ligands and β-blockers studied do not affect the results obtained from β-adrenoceptor-binding experiments in cellular membrane fractions. The β_1- and β_2-adrenoceptor affinities did not change in any experiments performed in tissues from the rat, the guinea pig and man for either atenolol or metoprolol.

The binding of an antagonist to the β-adrenoceptor has been described as a passive hydrophobic interaction, largely driven by an increase in entropy [1, 2]. In addition, electrostatic interaction appears to be of importance in antagonist binding to the β-adrenoceptor [2]. The non-specific interaction of a β-blocker has been suggested to be related, at least in part, to the lipophilicity of the compound [3]. The radioligand binding technique has been of great value for determination of β-adrenoceptor affinity of various compounds. A number of radioactive ligands have been used in these binding studies. These ligands differ regarding their affinity to β-adrenoceptors, specific radioactivity and physical–chemical properties, such as lipophilicity [4, 5].

Several authors have reported on the β-adrenoceptor affinity of various β-blockers, using the radioligand binding technique, but the results often vary considerably. For example, for the β_1-selective blockers atenolol and metoprolol, affinity constants to the β_1-adrenoceptor ranging from 0.1 μM [6] to 5.5 μM [7] (atenolol) and from 0.005 μM [6] to 0.5 μM [8] (metoprolol) have been presented. These two β-blockers were selected as examples, since the spectrum of their pharmacological properties is similar but their physical–chemical properties (e.g. degree of lipophilicity) are different [9]. With this background in mind, we considered it of importance to perform a systematic investigation of the β-adrenoceptor affinity of atenolol and metoprolol using binding assay with radioactive ligands with different degrees of lipophilicity. The β_1- and β_2-adrenoceptor affinity of the two β-blockers was determined in tissues from the rat, the guinea pig and man.

MATERIALS AND METHODS

Sprague–Dawley rats (250–300 g) of either sex from Møllegaard, Denmark, and male Duncin Hartley guinea pigs (350–500 g) from Sahlins, Sweden, were used. Uteri were obtained from rats pretreated with progesterone (10 mg/kg/day i.m.) for six consecutive days [10]. The free wall of the left cardiac ventricle and the right atrium were obtained from male rats. In the case of the guinea pigs, the free wall of the left ventricle and the soleus muscle were excised. All the tissues obtained were used for preparation of membranes (see below). The animals were killed by a blow on the neck.

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The human tissues were obtained from Akademisch Ziekenhuis, VUB, Brussels (Belgium). The free wall of the left ventricle was dissected (post mortem, <1 hr after death) from the heart of a 50-year-old man who had died of cerebral haemorrhage. Biopsy specimens from the uterus were obtained under routine anaesthesia from women undergoing hysterectomy on various medical or surgical indications. All women were taking medroxyprogesterone acetate as a contraceptive treatment [11].

Membrane preparation. The membranes were prepared in essentially the same way as described previously [12]. The membrane pellets obtained after the last centrifugation were diluted with ice-cold Tris buffer (20 mM Tris–HCl in 154 mM NaCl and 2 mM $MgCl_2$, pH 7.5) to give protein concentrations of 0.2–0.6 mg/ml and 1–4 mg/ml for suspensions used in experiments with iodinated and tritiated radioligands, respectively. Due to the low specific activity of [^{3}H](−)CGP12177, CGP*, experiments with this radioligand in rat uterus were performed on membranes prepared from 3–4 organs. The human tissues were homogenized in a Virtis 45 homogenizer at 45,000 rpm for 5 min and centrifuged. The final pellets were resuspended in Tris–HCl buffer (20 mM, pH 7.4) and stored in the presence of glycerol (10% v/v) at −80°. Protein was measured by the method of Lowry [13] using bovine serum albumin as the standard.

Radioligand binding assay. The binding assays were essentially the same as described previously [12]. Aliquots of membrane suspension (100 μl) were incubated with radioligand in a final volume of 0.25 ml and always in the presence of 100 μM GTP. The experiments with [^{125}I](±) hydroxybenzylpindolol (IHYP) and [^{125}I](−)pindolol (IPIN) were performed at 37° and incubation progressed for 30 and 20 min, respectively. In the case of [^{3}H](−)dihydroalprenolol (DHA) and CGP incubation was performed for 10 min, and the temperature was set at 30°. The specific binding for each ligand was defined with 50 μM (−) isoprenaline and constituted 80–90% of the total binding, which in itself was always less than 10% of the total amount of radioligand added. In the experiments with DHA, 10 μM phentolamine was added to all tubes to decrease nonspecific binding. In the initial experiments with DHA in membranes from rat uterus, the nonspecific binding was found to be unacceptably high (40–45%). Consequently, DHA was not used as radioligand in the subsequent experiments in rat uterus membranes.

To determine the number of β-adrenoceptors (B_{max}) in the tissue and the negative logarithm of the equilibrium dissociation constant (pK_d) for each radioligand, saturation binding experiments were performed at 9 concentrations of each ligand (6 concentrations of CGP in uterus). The concentration ranges used for iodinated and tritiated ligands were 20–400 pM and 0.2–8 nM, respectively.

In order to determine the pK_d for atenolol and metoprolol and, furthermore, to define the subpopulations of β-adrenoceptors in each tissue, competition binding experiments were performed at 14 concentrations for each drug. For atenolol, the concentration ranges studied were 5 nM–0.1 mM and 20 nM–0.5 mM for tissues dominated by β_1- or β_2-adrenoceptors, respectively. The correspondent ranges for metoprolol were 2 nM–0.05 mM and 10 nM–0.2 mM. In competition binding experiments iodinated ligands were used at about 100 pM and tritiated at about 1 nM.

Chemicals. IHYP, IPIN and DHA were purchased from New England Nuclear (MA, U.S.A.) and CGP from Amersham (U.K.).

The lipophilicity of the different drugs was investigated by determining their distribution (log D) between octanol and phosphate buffer at pH 7.4 (25°). Log D = log[amine (water phase)/amine (octanol phase) + ionized amine (octanol phase)]. The distribution ratio of the radioactive drugs was determined by counting aliquots from each phase in a γ- or β-counter. The distribution ratio for atenolol and metoprolol was determined by HPLC- and UV-detection.

Calculations and statistics. The saturation binding data were analysed according to the method of Scatchard [14] to obtain B_{max}- and pK_d-values. The competition 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. The analyses were constrained with regard to the level of nonspecific binding. 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 that 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. The relative size of the two subpopulations of β-adrenoceptors was calculated from the competition curves. The affinity constants for the two competing drugs (atenolol and metoprolol) were calculated from the equation described by Cheng and Prusoff [15]. The results are expressed as mean values ± SD. Statistical analyses were performed by using unbalanced two-way analysis of variance and Student's t-test.

RESULTS

Characterization of ligands and tissues

The lipophilicity, determined as the distribution between organic solvent and water, for the com-

Table 1. Distribution (log D) in octanol: phosphate buffer at pH 7.4 (25°). The pK_a-values are about 9.5 for all drugs

Ligand	Log D
Atenolol	−2.14
Isoprenaline	−1.88*
CGP	−0.55
Metoprolol	−0.28
IPIN	1.05
DHA	1.32
IHYP	2.50

* See Ref. [26].

* Abbreviations used: CGP, [^{3}H](−)CGP12177; IHYP, [^{125}I](±)hydroxybenzylpindolol; IPIN, [^{125}I](−)pindolol; DHA, [^{3}H](−)dihydroalprenolol; B_{max}, total number of specific binding sites; pK_d, negative logarithm of the equilibrium dissociation constant.

Table 2. B_{max} and pK_d-values in the left ventricular free wall (LV) and uterus from the rat, determined with different radioligands. Values are means ± SD

	LV			Uterus		
Ligand	N	B_{max}	pK_d	N	B_{max}	pK_d
CGP	6	35 ± 20	9.4 ± 0.19	4	114 ± 20.7	9.2 ± 0.10
IPIN	31	21 ± 8.5	10.0 ± 0.15	4	109 ± 23.2	10.0 ± 0.13
DHA	5	41 ± 13.2	9.1 ± 0.08		—	—
IHYP	12	27 ± 7.4	10.3 ± 0.11	4	138 ± 35.0	10.5 ± 0.08

N = number of animals.

Table 3. The β_1-adrenoceptor population (%) in the left ventricular free wall (LV) and right atrium (RA) from different species, determined by displacement of different radioligands with atenolol and metoprolol. Values are means ± SD

		% β_1-adrenoceptors	
Species/tissue	Ligand	Atenolol	Metoprolol
Rat, LV	CGP	67 ± 16.0 (6)	61 ± 15.8 (7)
	IPIN	57 ± 8.0 (6)	54 ± 14.2 (6)
	DHA	69 ± 7.0 (6)	63 ± 8.2 (7)
	IHYP	60 ± 16.9 (6)	66 ± 6.6 (7)
Guinea-pig, LV	IHYP	80 ± 10.9 (12)	81 ± 8.4 (11)
Human, LV	CGP	80 (1)	57 (1)
	IPIN	58 (1)	66 (1)
Rat, RA	IPIN	60 ± 8.4 (7)	58 ± 14.3 (7)

Number of individuals within brackets.

Table 4. The β_2-adrenoceptor population (%) in the uterus and the soleus muscle from different species, determined by displacement of different radioligands with atenolol and metoprolol. Values are means ± SD

		% β_2-adrenoceptors	
Species/tissue	Ligand	Atenolol	Metoprolol
Rat, uterus	CGP	86 ± 15.0 (7)	99 ± 3.0 (7)
	IPIN	95 ± 9.5 (11)	98 ± 5.7 (11)
	IHYP	97 ± 6.8 (9)	98 ± 4.4 (8)
Human, uterus	CGP	100 ± 0 (3)	100 ± 0 (3)
	IPIN	100 ± 0 (5)	100 ± 0 (4)
Guinea-pig, soleus	IHYP	92 ± 7.0 (12)	87 ± 15.0 (11)

Number of individuals within brackets.

pounds used in this study are summarized in Table 1. For a range of compounds, an increase in the log *D*-value indicates an increased lipophilicity.

In left ventricular free wall and uterus of the rat, the B_{max} obtained from Scatchard analyses was independent of the radioligand used. B_{max} and pK_d-values for the ligands in the two tissues are summarized in Table 2. The relative size of the two subpopulations of β-adrenoceptors in the different tissues, estimated from displacement curves with either metoprolol or atenolol, are presented in Tables 3 and 4. The percentage of the β_1- and β_2-adrenoceptor populations obtained in the tissues studied was not dependent on the radioligand or the displacing compound used in the experiments.

Affinity of metoprolol and atenolol

The pK_d-values of metoprolol and atenolol to β_1-adrenoceptors, determined with the four different ligands in the β_1-adrenoceptor dominated organs, are presented in Table 5. The β_1-adrenoceptor affinity of metoprolol was about 6–7 times higher than that of atenolol. Analysis of variance of pK_d-values obtained from the experiments with different radioligands showed no statistically significant difference for the β_1-adrenoceptor affinity of metoprolol and atenolol, respectively. The affinities of metoprolol and atenolol to β_2-adrenoceptors, in rat uterus and guinea-pig soleus muscle, estimated with three different radioligands, were also found to be independent of the radioligand used (Table 6). Analysis

Table 5. β_1-Adrenoceptor affinity (pK_d) of atenolol and metoprolol, determined in LV and RA from different species by displacement of different radioligands. Values are means ± SD

Species/tissue	Ligand	pK_d β_1-adrenoceptors	
		Atenolol	Metoprolol
Rat, LV	CGP	6.3 ± 0.20 (6)	7.3 ± 0.33 (7)
	IPIN	6.4 ± 0.31 (6)	7.4 ± 0.34 (6)
	DHA	6.5 ± 0.08 (6)	7.3 ± 0.19 (7)
	IHYP	6.4 ± 0.21 (6)	7.1 ± 0.22 (7)
Guinea-pig, LV	IHYP	6.3 ± 0.27 (12)	7.1 ± 0.14 (11)
Human, LV	CGP	6.6 (1)	7.3 (1)
	IPIN	6.6 (1)	7.4 (1)
Rat, RA	IPIN	6.6 ± 0.18 (7)	7.5 ± 0.29 (7)

Number of individuals within brackets. Abbreviations: see Table 2.

Table 6. β_2-Adrenoceptor affinity (pK_d) of atenolol and metoprolol determined in the uterus and the soleus muscle from different species by displacement of different radioligands. Values are means ± SD

Species/tissue	Ligand	pK_d β_2-adrenoceptors	
		Atenolol	Metoprolol
Rat, uterus	CGP	4.9 ± 0.15 (7)	5.9 ± 0.29 (7)
	IPIN	5.0 ± 0.20 (11)	5.8 ± 0.23 (11)
	IHYP	5.0 ± 0.41 (9)	5.9 ± 0.43 (8)
Human, uterus	CGP	5.0 ± 0.06 (3)	5.5 ± 0.24 (3)
	IPIN	4.8 ± 0.13 (3)	5.5 ± 0.29 (4)
Guinea-pig, soleus	IHYP	5.1 ± 0.19 (12)	5.6 ± 0.16 (11)

Number of individuals within brackets.

Table 7. β_1-Selectivity ratio for atenolol and metoprolol. Comparison between different tissues, species and ligands

Species/tissue	Ligand	β_1-Selectivity ratio	
		Atenolol	Metoprolol
Rat, LV-uterus	CGP	23	20
	IPIN	36	39
	IHYP	20	22
Rat, RA-uterus	IPIN	36	42
Guinea-pig, LV-soleus	IHYP	15	26
Human, LV-uterus	CGP	23	69
	IPIN	27	40
Mean value ± SD	All ligands	26 ± 7.9	37 ± 16.5

Abbreviations: see Table 2.

of the variance of pK_d-values for β_2-adrenoceptors showed no statistically significant differences between the different experiments with atenolol and metoprolol.

Selectivity of atenolol and metoprolol

A β_1-selectivity ratio was calculated from the affinity of metoprolol and atenolol to β_1- and β_2-adrenoceptors. The mean β_1-selectivity ratio for metoprolol and atenolol was calculated to be 37 and 26, respectively (Table 7).

DISCUSSION

In this study, the receptor-binding technique was used to determine the β_1- and β_2-adrenoceptor affinity of the two β-blockers, atenolol and metoprolol. For both atenolol and metoprolol, the β-adrenoceptor affinity constants were found to be very similar in the various receptor-binding experiments using radioactive ligands with different physical–chemical properties. There is a markedly greater difference in lipophilicity between atenolol and the lipophilic IHYP (40,000-fold) than between atenolol and CGP (40-fold). However, despite this difference, a similar affinity constant of atenolol was obtained when calculated from the displacement curves of either radioligand.

The hydrophilic β-adrenoceptor agonist isoprenaline was chosen to define the specific binding sites in

this study. Despite the wide range of lipophilicity among the different radioligands, the nonspecific binding was low and reproducible in all experiments. This may be due to the high specificity to β-adrenoceptors of both the radioligands and isoprenaline. A high specificity of isoprenaline for the β-adrenoceptors was indicated by the observation that specific binding of the radioligand (DHA) was similar when defined in concentrations of up to 1000 μM of isoprenaline [3]. The percentage of β-adrenoceptors found in a membrane preparation was the same comparing saturation binding experiments with different radioactive ligands. In addition, it was demonstrated that the size of the two subpopulations of β-adrenoceptors in a membrane preparation did not depend on the radioactive ligands used or on whether atenolol or metoprolol was used to displace the ligand. Altogether, these findings suggest that the physical–chemical properties of the radioactive ligands, as well as the β-blockers studied, do not influence the results obtained from β-adrenoceptor binding experiments in cellular membrane fractions.

In the human uterus a significant β_1-adrenoceptor population has only been detected when the tissue was obtained from pregnant women [16, 17] or nonpregnant women in the midfollicular phase [11]. In the present study, demonstrating a homogeneous β_2-adrenoceptor population, the myometrical tissue was obtained from individuals taking medroxyprogesterone acetate as a contraceptive treatment. This tissue has earlier been demonstrated, using the β_2-selective antagonist ICI 118,551, to contain a homogeneous β_2-adrenoceptor population [11].

The densities of β_1- and β_2-adrenoceptors in the ventricular free wall of the rat and human heart observed in this study are in agreement with earlier reports [18]. However, in contrast to earlier results obtained in the guinea-pig ventricular muscle [19], showing a homogeneous population of β_1-adrenoceptors, we observed a significant population (20%) of β_2-adrenoceptors in this tissue. This discrepancy may be due to differences in the computer-modelling methods used to determine the β-adrenoceptor subtypes. In the present study, the computer-modelling method was based on mass-action law principles [20], while in the earlier study [19], "pseudo-Scatchard" plots [21] were used to determine the size of the two β-adrenoceptor populations.

When the binding experiments performed on tissues from different species (rat, guinea-pig and man) were compared, it was found that the β-adrenoceptor affinity constants for both atenolol and metoprolol did not vary greatly. This is in accordance with the reported structural similarities of β-adrenoceptors from different mammalian species [22]. It also suggests that the β_1- and β_2-adrenoceptor affinity of compounds, determined in binding experiments on membrane fractions from rat and guinea-pig tissues, can be used to predict the affinity constant of a β-blocker to human β-adrenoceptors.

The β_1-adrenoceptor affinity constant (K_d) obtained for metoprolol in this study was within the range of 30 to 80 nM. It is interesting to relate this level to the plasma concentration of metoprolol required for therapeutic β_1-adrenoceptor blockade in man [23, 24]. It has been shown that the concentration of β-adrenoceptor antagonist present in plasma in most cases is representative of the concentration in the effect compartment [25]. Clinical studies with metoprolol have demonstrated significant β_1-blockade (about 20% decrease in exercise heart rate) at plasma concentrations of 100–200 nM [24]. Thus, an antagonist concentration occupying about 70–80% of the β_1-adrenoceptors is required in order to obtain β_1-adrenoceptor blockade in man. The β_1-adrenoceptor affinity constant for atenolol in this study was within the range of 250–500 nM. With atenolol, significant β_1-blockade in man (about 20% decrease in exercise heart rate) is obtained at plasma concentrations of 1000–1500 nM [24]. Thus, in similarity to metoprolol, a concentration occupying about 70–80% of the β_1-adrenoceptors is required to obtain significant β_1-adrenoceptor blockade in man. In this study, comparison of the β_1-adrenoceptor affinity constants of metoprolol and atenolol indicates a potency ratio of about 6–7. This is in agreement with the ratio in plasma concentrations required for the two β-blockers in order to produce an equal degree of β_1-blockade in man [24].

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