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β_1-adrenoceptor selectivity of nebivolol and bisoprolol. A comparison of [^{3}H]CGP 12.177 and [^{125}I]iodocyanopindolol binding studies

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Abstract

There is an ongoing discussion on whether or not high β_1-adrenoceptor selectivity of β-adrenoceptor antagonists may be favorable in the treatment of patients with heart failure. The present study compared the β_1-adrenoceptor selectivity of nebivolol and bisoprolol with that of carvedilol in the human myocardium, using a binding assay in conjunction with either the hydrophilic ligand (±)-[^{3}H]4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-on HCl ([^{3}H]CGP 12.177) or the lipophilic ligand [^{125}I]iodocyanopindolol as radiolabeled compound. Measurements were made using membrane preparations obtained from identical nonfailing donor hearts. β-adrenoceptor density was found to be slightly higher when [^{125}I]iodocyanopindolol was used compared to [^{3}H]CGP 12.177 (256 ± 15 and 213 ± 18 fmol/mg protein, respectively). When the highly β_1-adrenoceptor-selective compound 2-hydroxy-5-(2-(hydroxy-3-(4((1-methyl-4-trifluoromethyl)-1-*H*-imidazol-2-yl)-phenoxy)-propyl)-aminoethoxyl)-benzamide (CGP 20.712A) and the highly β_2-adrenoceptor-selective compound erythro-(±)-1-(7-methylindan-4-yloyl)-3-isopropylaminobutan-2-ol HCl (ICI 118.551) were used in competition experiments, a similar proportion of β_1-adrenoceptors was seen for [^{3}H]CGP 12.177 (69.3 ± 1.6%) and for [^{125}I]iodocyanopindolol (67.0 ± 2.1%). $K_i(\beta_1)$ and $K_i(\beta_2)$ were obtained in the presence of 50 nM ICI 118.551 and 300 nM CGP 20.712A. The rank order of β_1-adrenoceptor selectivity ($K_i(\beta_2)/K_i(\beta_1)$ ratio) was nebivolol (for [^{3}H]CGP 12.177 46.1 and for [^{125}I]iodocyanopindolol 22.5)>bisoprolol (13.1 and 6.4)>carvedilol (0.65 and 0.41). To investigate whether in vivo metabolized nebivolol retains high β_1-adrenoceptor selectivity, serum specimens were collected before and 2 h after oral administration of 5 mg nebivolol. The samples were used for [^{125}I]iodocyanopindolol binding studies with the myocardial membrane preparations. In these samples, the binding of [^{125}I]iodocyanopindolol to β_1-adrenoceptors was inhibited by 46.4 ± 5.3%, whereas the binding to β_2-adrenoceptors was inhibited by 20.5 ± 1.1% compared to that of control samples. It is concluded that nebivolol is approximately 3.5 times more β_1-adrenoceptor-selective than bisoprolol in the human myocardium. Furthermore, in vivo metabolized nebivolol retains β_1-adrenoceptor selectivity.

Keywords: β_1-adrenoceptor selectivity; Nebivolol; Bisoprolol; Carvedilol; Myocardium, human

1. Introduction

β-adrenoceptor antagonist are undoubtedly one of the most effective drugs in the treatment of cardiovascular diseases. This holds true in patients with coronary heart disease and myocardial infarction, for patients with heart failure due to ischemic or dilatative cardiomyopathy, as well as for patients being treated for cardiac arrhythmias. Several noncardiovascular diseases can effectively be treated by β-adrenoceptor blockade as well, e.g. migraine (Limmroth and Michel, 2001), essential tremor (Louis, 2002), glaucoma (Stamper et al., 2002) or portal hypertension (Boyer, 2001). In certain cases, the therapeutic benefit of β-adrenoceptor antagonist therapy depends in part on the blockade of the β_2-adrenoceptor, as suggested by the treatment of portal hypertension (Boyer, 2001) and essential tremor (Louis, 2002).

However, several problems may arise when β_2-adrenoceptors are pharmacologically blocked. First, pulmonary diseases with bronchial hyperreactivity can be worsened by β_2-adrenoceptor blockade (Tafreshi and Weinacker, 1999). Secondly, patients suffering from diabetes mellitus are at special risk for hypoglycemia because β_2-adrenoceptor-

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mediated counterregulation might be affected (Verschoor et al., 1986). Thirdly, β_2-adrenoceptor-mediated vasodilatation is important for patients with peripheral vascular disease (Heintzen and Strauer, 1994) and has been suggested to be necessary to maintain a normal blood pressure under stress conditions (Dishy et al., 2001). Fourthly, some data suggest an unfavorable role of β_2-adrenoceptor antagonism on the lipid profile (Fogari et al., 1990). Fifthly, when β-adrenoceptor antagonist treatment is initiated in heart failure patients, it may be favorable when β_2-adrenoceptor-mediated effects on contractility and vascular resistance remain present (Bristow, 2000). Thus, the use of a β_1-adrenoceptor-selective antagonist might be advantageous for patients at risk of adverse effects mediated by β_2-adrenoceptor blockade.

Previously, the affinity of β-adrenoceptor antagonist for β_1- and β_2-adrenoceptors and, therefore, their β_1-adrenoceptor selectivity has been assayed under various conditions. Therefore, different data on β_1-adrenoceptor selectivity exist depending on the radioligand, tissue and species used. Bisoprolol is widely accepted to be a highly β_1-adrenoceptor-selective antagonist exceeding the selectivity of metoprolol and atenolol (Schnabel et al., 2000; Smith and Teitler, 1999). The newly developed nebivolol, a third-generation β-adrenoceptor antagonist which mediates vasodilatation via nitric oxide liberation (Cockcroft et al., 1995), has been shown to be uniquely β_1-adrenoceptor-selective in animal models. This unique β_1-adrenoceptor selectivity has also been confirmed recently in human myocardium in (±)-[^{3}H]4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-on HCl ([^{3}H]CGP 12.177) binding studies (Brixius et al., 2001). However, conflicting results for nebivolol were obtained using [^{125}I]iodocyanopindolol (Maack et al., 2001) and indicated that nebivolol is more or less nonadrenoceptor-selective in the human heart. These different data on the β_1-adrenoceptor selectivity of nebivolol led to confusion among physicians searching for the most selective β-adrenoceptor antagonist to avoid potential β_2-adrenoceptor-mediated adverse effects.

Thus, the present study aims to investigate the β_1-adrenoceptor selectivity of bisoprolol, nebivolol and carvedilol, using a binding assay with either the hydrophilic [^{3}H]CGP 12.177 or the lipophilic [^{125}I]iodocyanopindolol as the radiolabeled compound. In order to obtain comparable data, measurements were made using membrane preparations obtained from identical nonfailing donor hearts. Thus, disease-mediated effects, e.g. ischemic or dilatative cardiomyopathy, as well as the influence of treatment on the assay could be excluded.

2. Materials and methods

2.1. Myocardial tissue

Nonfailing human myocardium was obtained from five donors with brain death caused by traumatic injury (n = 4; 3 males, 1 female; age: 42 ± 6 years). The nonfailing hearts could not be transplanted for technical reasons. None of the patients had received β-adrenoceptor antagonist treatment before injury. Immediately after explantation, the nonfailing hearts were placed in ice-cold pretreated Bretschneider solution and delivered to the laboratory within 15 min. The investigation on the human heart complies with the principles outlined in the Declaration of Helsinki and was reviewed and approved by the local ethics committee.

2.2. Membrane preparation

Myocardial tissue was taken from the interventricular septum. The myocardial tissue was mechanically reduced to small pieces after freezing in liquid nitrogen. Afterwards, it was chilled in 30 ml of ice-cold homogenization buffer that contained (in mM) Tris 20, EDTA 1 and dithiothreitol 1, pH 8.0, and was homogenized after thawing at 4 °C with a motor-driven glass–teflon potter for approximately 10 min. The homogenate was centrifuged at 480g for 15 min (Beckmann JA 20). The supernatant was filtered through two layers of gauze and was used in the experiments. The pellet was discarded. This homogenate was diluted with an equal volume of ice-cold 1 M KCl and was stored on ice for 10 min. The supernatant was centrifuged at 100,000g for 45 min. The pellet was resuspended in incubation buffer that contained (in mM) Tris 50 and $MgCl_2$ 10, pH 7.4 (50 volumes), and was centrifuged again at 100,000g for 45 min. The pellet was finally resuspended in incubation buffer and was stored at −80 °C. The preparation has been described previously by Schwinger et al. (1990, 1991).

2.3. Radioligand binding assay

β-adrenoceptors in cardiac tissue were investigated using [^{3}H]CGP 12.177 ((±)-[^{3}H]4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-on HCl) (specific activity 55 Ci/mmol) or [^{125}I]iodocyanopindolol (specific activity 2000 Ci/mmol) as the radiolabeled ligand. The assay was performed in a total volume of 500 μl of incubation buffer. Serum experiments were performed with 450 μl of serum instead of incubation buffer. The volume of incubation buffer was reduced to 50 μl in this particular experiment. A total of 100–200 μg of protein was used for each sample. The incubation was carried out at 37 °C for 60 min. These conditions allowed complete equilibration of the receptor with the ligands. The reaction was terminated by filtration through Whatman GF/C filters by use of a 48-well cell harvester. All experiments were performed in triplicate. Filters were dried at 60 °C and placed in 5 ml of scintillation cocktail (ACS II, Amersham, Braunschweig, Germany) and radioactivity was determined in a liquid scintillation counter (Packard, 1600 TR, Ratingen, Germany). Specific binding was determined as the difference of binding in the absence and presence of DL-propranolol (10 μM). Protein was determined according to Bradford (1976).

To obtain a homogeneous population of β_1- or β_2-adrenoceptors, competition experiments were performed in the presence of the highly β_1-adrenoceptor-selective antagonist CGP 20.712A (2-hydroxy-5-(2-(hydroxy-3-(4((1-methyl-4-trifluoromethyl)-1-*H*-imidazol-2-yl)-phenoxy)-propyl)-aminoethoxyl)-benzamide) (300 nM) or the highly β_2-adrenoceptor-selective antagonist ICI 118.551 (erythro-(±)-1-(7-methylindan-4-yloyl)-3-isopropylaminobutan-2-ol HCl) (50 nM), respectively.

2.4. Materials

[^{3}H]CGP 12.177 ((±)-[^{3}H]4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-on HCl) and [^{125}I]iodocyanopindolol were commercially obtained from Amersham Pharmacia (Braunschweig, Germany). Nebivolol was provided by Berlin Chemie (Berlin, Germany), bisoprolol was from Merck (Darmstadt, Germany) and carvedilol was provided by Boehringer (Mannheim, Germany). All other chemicals were of analytical grade or the best grade commercially available.

2.5. Statistics

All values are means ± S.E.M. Statistical significance was analyzed with Student's *t*-test for paired observations. Significance was imparted at a P value of <0.05. For calculating B_{max} and K_d of radioligand saturation experiments, as well as competition binding isotherms, nonlinear regression analyses were performed.

Selectivity ratios for nebivolol, bisoprolol and carvedilol were obtained according to the method of Cheng and Prussoff (1973). K_i values were calculated from IC_{50} values determined by fitting the competition curve with a nonlinear regression analysis, assuming only one receptor state (one site fit), regardless if they actually reflect one or two affinity states. The selectivity ratios for the highly selective antagonists ICI 118.551 and CGP 20.712A, respectively, were calculated by determination of the dissociation constants of the high- and the low-affinity site (K_H and K_L, respectively) in two-site fitting binding curves.

Regression analyses were performed using the computer software GraphPadPrism (GraphPad Software, San Diego, USA).

3. Results

3.1. Identification of β_1- and β_2-adrenoceptors in [^{3}H]CGP 12.177 and [^{125}I]iodocyanopindolol binding assays

Binding of [^{3}H]CGP 12.177 (0.06–10 nM) and [^{125}I]iodocyanopindolol (0.003–0.5 nM) was monophasic and saturable. β-adrenoceptor density was found to be slightly higher when the lipophilic ligand [^{125}I]iodocyanopindolol was used as compared to the hydrophilic ligand [^{3}H]CGP 12.177 (256 ± 15 and 213 ± 18 fmol/mg protein, respectively) (Table 1). The nonspecific binding was higher for [^{125}I]iodocyanopindolol binding, amounting to 25.4% at K_d as compared to [^{3}H]CGP 12.177 binding (10.9% at K_d). Thus, [^{3}H]CGP 12.177 showed lower nonspecific binding, possibly due to the hydrophilic character of the compound.

Determination of β_1- and β_2-adrenoceptor subpopulations was performed using the highly β_1-adrenoceptor-selective CGP 20.712A and the highly β_2-adrenoceptor-selective ICI 118.551 (0.0003–10 μM). The means of three individual experiments, each performed in triplicate for [^{3}H]CGP 12.177 and [^{125}I]iodocyanopindolol competition are presented in Fig. 1. The competition curves were biphasic, indicating two classes of binding sites. The proportion of β_1- and β_2-adrenoceptors was obtained by dividing the two-site fitted competition curves of the highly selective ligands into one fraction of high affinity and one fraction of low affinity, as indicated in Fig. 1.

As judged from K_H and K_L values, CGP 20.712A has a β_1-adrenoceptor selectivity of 812 in [^{3}H]CGP 12.177 binding assays, and of 430 in [^{125}I]iodocyanopindolol binding assays. The β_2-adrenoceptor selectivity of ICI 118.551 was 212 in [^{3}H]CGP 12.177 binding assays, and 507 in [^{125}I]iodocyanopindolol binding assays. A similar ratio of β_1- and β_2-adrenoceptors was obtained using the different radioligands, as presented in Table 1. The overall proportion

Table 1
Identification of β_1- and β_2-adrenoceptor subtypes in nonfailing human hearts ($n=3$, each experiment performed in triplicate) determined by competition of CGP 20.712A and ICI 118.551 in [^{3}H]CGP 12.177 and [^{125}I]iodocyanopindolol binding assays

	B_{max} (fmol/mg protein)	K_d (nM)	CGP 20.712A					ICI 118.551				
			β_1 (%)	β_2 (%)	K_H (nM)	K_L (nM)	β_1-sel. (K_L/K_H)	β_1 (%)	β_2 (%)	K_H (nM)	K_L (nM)	β_2-sel. (K_L/K_H)
[^{3}H]CGP	213.9 ± 17.6	0.263 ± 0.01	67.8 ± 1.0	32.2 ± 1.0	3.43 ± 0.35	2785 ± 525	812	70.7 ± 3.1	29.3 ± 3.1	1.69 ± 0.50	357.6 ± 27.3	212
[^{125}I]ICYP	259.7 ± 14.6	0.238 ± 0.03	64.6 ± 2.3	35.4 ± 2.3	7.00 ± 2.74	3007 ± 385	430	69.5 ± 2.9	30.5 ± 2.9	0.80 ± 0.21	405.3 ± 81.3	507

[^{3}H]CGP: [^{3}H]CGP 12.177. [^{125}I]ICYP: [^{125}I]iodocyanopindolol. B_{max}: maximum specific radioligand bound. K_d: apparent affinity. K_H: K_i values of the high-affinity binding site. K_L: K_i values of the low-affinity binding site. β-sel.: β-adrenoceptor selectivity.

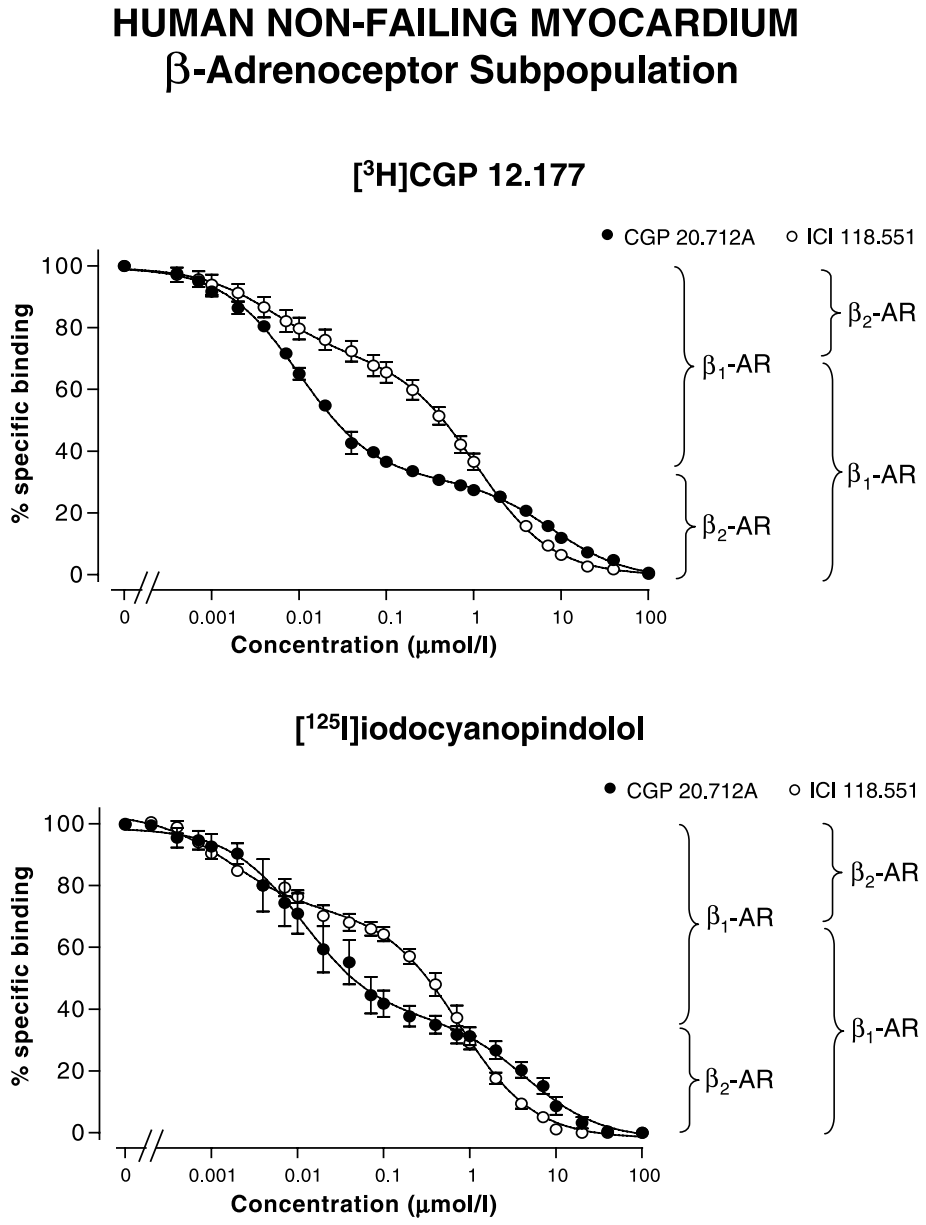

Fig. 1. Inhibition of specific radioligand binding (at K_d) to cardiac β_1- and β_2-adrenoceptors by the highly β_1-adrenoceptor-selective antagonist CGP 20.712A and the highly β_2-adrenoceptor-selective antagonist ICI 118.551, respectively. Note that a similar ratio of β_1- and β_2-adrenoceptors was detected with both selective antagonists and in both [^{3}H]CGP 12.177 and [^{125}I]iodocyanopindolol binding assays. Data are expressed as means of three individual experiments, each performed in triplicate.

of β_1-adrenoceptors was 69.3 ± 1.6% for [^{3}H]CGP 12.177 and 67.0 ± 2.1% for [^{125}I]iodocyanopindolol.

3.2. Determination of β_1-adrenoceptor selectivity of nebivolol, bisoprolol and carvedilol

To obtain a homogeneous population of β_1- or β_2-adrenoceptors, competition experiments were performed in the presence of the highly β_1-adrenoceptor-selective CGP 20.712A (300 nM) or the highly β_2-adrenoceptor-selective ICI 118.551 (50 nM), respectively. These concentrations were chosen according to the results of the previous experiments. Fig. 1 indicates that at a concentration of 50 nM ICI 118.551, the binding of [^{125}I]iodocyanopindolol to β_2-adrenoceptors was selectively inhibited. The concentration of 300 nM CGP 20.712A led to a selective inhibition of [^{125}I]iodocyanopindolol binding to β_1-adrenoceptors.

β_1- and β_2-adrenoceptors were labeled with [^{3}H]CGP 12.177 or [^{125}I]iodocyanopindolol at K_d. Competition curves for nebivolol (0.00003–100 μM), bisoprolol (0.0001–1000 μM) and carvedilol (0.003–1000 nM) were calculated assuming one binding site only (Fig. 2). The rank order of β_1-adrenoceptor selectivity ($K_i(\beta_2)/K_i(\beta_1)$ ratio) was nebivolol (for [^{3}H]CGP 12.177 46.1 and for [^{125}I]iodo-

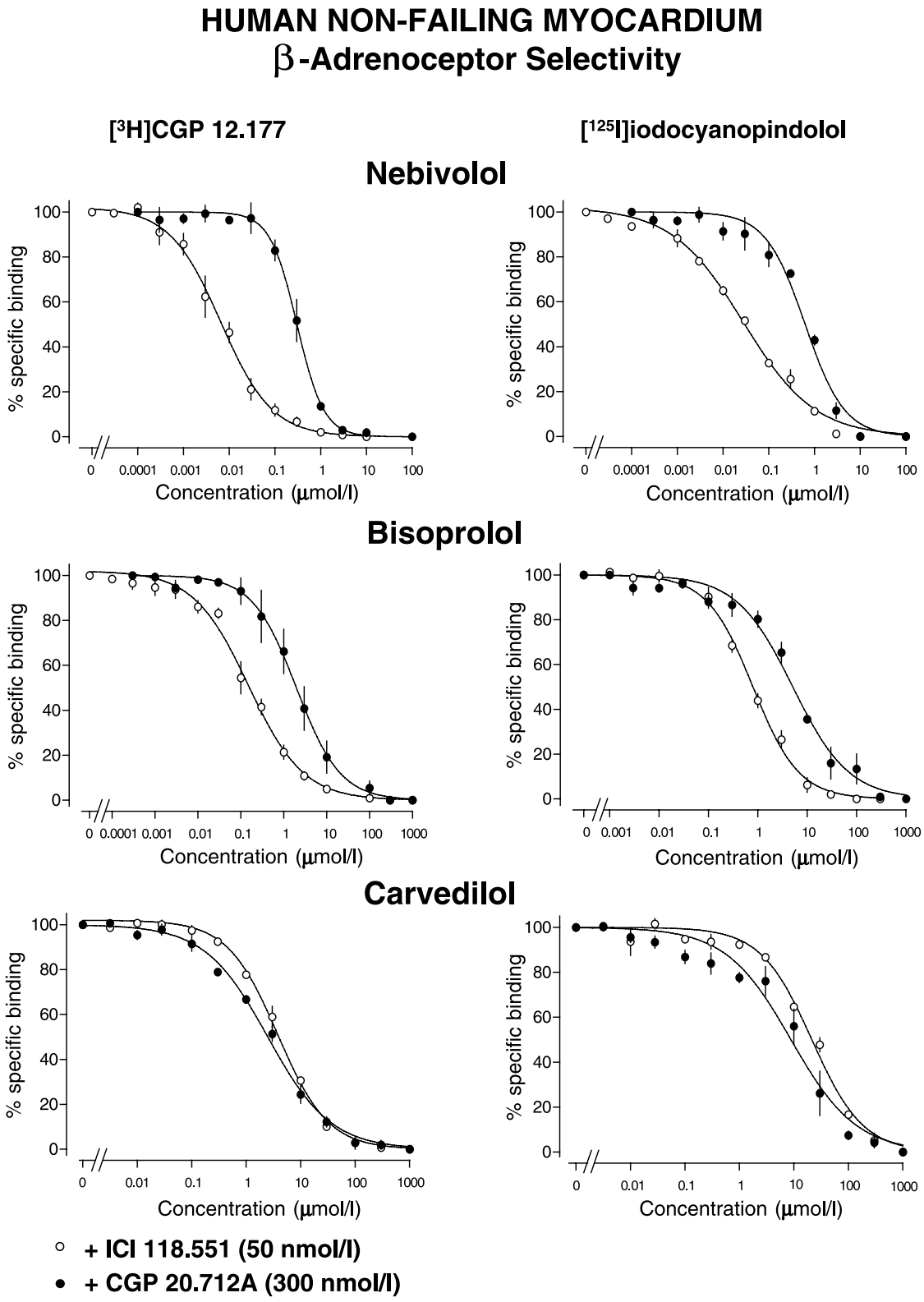

Fig. 2. Inhibition of specific radioligand binding (at K_d) to cardiac β_1- and β_2-adrenoceptors by the respective β-adrenoceptor antagonist. To obtain a homogeneous population of β_1- or β_2-adrenoceptors, competition experiments were performed in the presence of the highly β_1-adrenoceptor-selective antagonist CGP 20.712A (300 nM) or the highly β_2-adrenoceptor-selective antagonist ICI 118.551 (50 nM), respectively. Data are expressed as means of three individual experiments, each performed in triplicate.

Table 2
Identification of β_1-selectivity in nonfailing human hearts ($n = 3$, each experiment performed in triplicate) determined by competition in [^{3}H]CGP 12.177 and [^{125}I]iodocyanopindolol binding assays

	[^{3}H]CGP 12.177					[^{125}I]iodocyanopindolol				
	$K_i(\beta_1)$ (nM)	$N_H(\beta_1)$	$K_i(\beta_2)$ (nM)	$n_H(\beta_2)$	β_1-sel.	$K_i(\beta_1)$ (nM)	$n_H(\beta_1)$	$K_i(\beta_2)$ (nM)	$n_H(\beta_2)$	β_1-sel.
Nebivolol	3.30 (2.79–3.87)	0.77 (0.69–0.86)	152.2 (139.1–166.4)	1.48 (1.30–1.65)	46.1	13.9 (11.1–17.4)	0.56 (0.49–0.62)	313.1 (234.3–418.5)	1.00 (0.75–1.27)	22.5
Bisoprolol	75.8 (61.8–92.7)	0.68 (0.60–0.76)	991.0 (875.5–1122)	0.84 (0.76–0.93)	13.1	398.8 (352.7–450.9)	0.93 (0.84–1.02)	2552 (1925–3383)	0.76 (0.62–0.91)	6.4
Carvedilol	1.91 (1.69–2.14)	0.94 (0.85–1.02)	1.25 (1.06–1.87)	0.75 (0.67–0.83)	0.65	10.7 (8.69–13.17)	0.93 (0.77–1.08)	4.43 (2.96–6.62)	0.72 (0.54–0.91)	0.41

$K_i(\beta_1)$: K_i values of cold ligand in the presence of 50 nM ICI 118.551. $K_i(\beta_2)$: K_i values of cold ligand in the presence of 300 nM CGP 20.712A. β_1-sel.: β_1-adrenoceptor selectivity ($K_i(\beta_2)/K_i(\beta_1)$). n_H: slope factor. Data are given as means with 95% confidence limit.

cyanopindolol 22.5)>bisoprolol (13.1 and 6.4)>carvedilol (0.65 and 0.41). Inhibition constants are presented in Table 2.

3.3. Semiquantitative assessment of β_1-adrenoceptor selectivity of in vivo metabolized nebivolol

To investigate whether in vivo metabolized nebivolol retains high β_1-adrenoceptor selectivity, serum specimens from three healthy volunteers were collected before and 2 h after 5 mg nebivolol was administered orally (T_{max} of nebivolol after a single oral dose is 2.7 ± 1.2 h with a C_{max} of 159 ± 61 ng/ml and a terminal half-time of 22.0 ± 8.2 h; Van Rooy, 1994, unpublished data). The samples were used for [^{125}I]iodocyanopindolol binding studies with the myocardial membrane preparations. The experimental set-up did not differ from that of the former experiments, except the buffer volume, which was reduced in favor of the serum specimens which contained metabolized nebivolol. The use of serum instead of buffer led to a substantial reduction of specific and nonspecific binding of [^{125}I]iodocyanopindolol to the membrane preparation (only approximately 3–5% of specific and nonspecific binding sites were detected in the presence of 90% serum as compared to no serum at K_d).

In the test serum samples, the binding of [^{125}I]iodocyanopindolol to β_1-adrenoceptors was reduced by 46.4 ± 5.3% whereas binding to β_2-adrenoceptors was reduced by 20.5 ± 1.1% as compared to that of control serum samples (Fig. 3), providing evidence that the β_1-adrenoceptor selectivity of nebivolol is retained after in vivo metabolism.

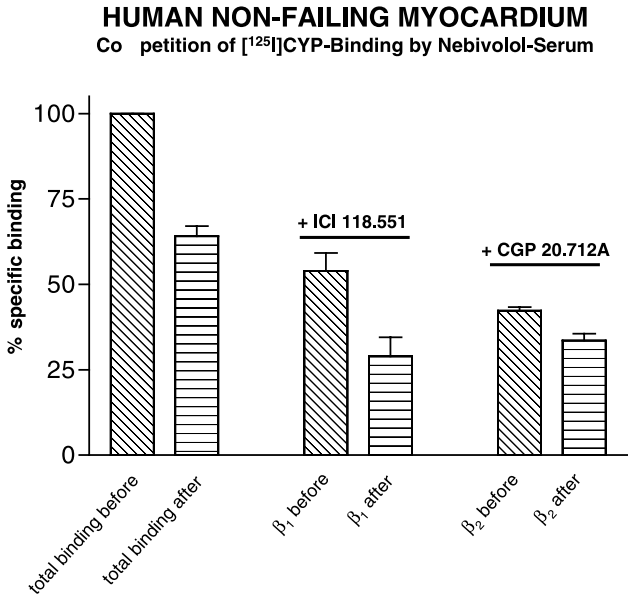

Fig. 3. Inhibition of specific [^{125}I]iodocyanopindolol binding (at K_d) to cardiac β_1- and β_2-adrenoceptors by serum samples ($n = 3$, each experiment performed in triplicate) obtained before and 2 h after administration of 5 mg nebivolol orally. Note that a dilution factor of 0.8 has to be considered. In this semiquantitative experiment, β_1-adrenoceptor selectivity of metabolized nebivolol was retained.

4. Discussion

In the investigated nonfailing human myocardium, a β_1/β_2 ratio of approximately 66:34 and a β-adrenoceptor density of 256 ± 15 ([^{125}I]iodocyanopindolol) and 213 ± 18 ([^{3}H]CGP 12.177) fmol/mg protein were found. This is in line with a former investigation in human nonfailing hearts using the same membrane preparation (Schwinger et al., 1991). However, a considerable difference in the β_1/β_2 ratio and the β-adrenoceptor density was found from values reported in the literature, which may be due to prior drug administration and different methodological set-ups.

In the present study, nebivolol and bisoprolol were found to be β_1-adrenoceptor-selective antagonists while carvedilol was nonselective and even had a modest selectivity for β_2-adrenoceptors. The bisoprolol and carvedilol selectivity ratios obtained in this study are consistent with those found in former investigations with cloned β_1- and β_2-adrenoceptors (Schnabel et al., 2000; Smith and Teitler, 1999). Thus, our experimental conditions, in which a homogeneous adrenoceptor population is obtained by using highly selective antagonists, gives reliable results.

The β_1-adrenoceptor selectivity of nebivolol was more pronounced than the β_1-adrenoceptor selectivity of bisopro-

lol (approximately 3.5 times higher), independently of which radioligand ([^{3}H]CGP 12.177 or [^{125}I]iodocyanopindolol) was used to label β_1- and β_2-adrenoceptors of the human myocardium. This finding contrasts with the results reported previously for [^{125}I]iodocyanopindolol binding studies in human myocardium, in which nebivolol was found to be a rather nonselective β-adrenoceptor antagonist (Maack et al., 2001). However, our results are in line with investigations of the β_1-adrenoceptor selectivity of nebivolol in animals (Van de Water et al., 1988) and at recombinant human adrenoceptors (Pauwels et al., 1991).

Selectivity ratios varied depending on the radioligand used in our study. For all cold ligands investigated in this study, including the highly selective antagonists CGP 20.712A and ICI 118.551, the β_1-adrenoceptor selectivity ratio was approximately halved in [^{125}I]iodocyanopindolol compared to [^{3}H]CGP 12.177 binding assays. Another difference in radioligand binding properties was that the lipophilic [^{125}I]iodocyanopindolol was able to detect slightly more β-adrenoceptors. One possible explanation for this is that parts of the β-adrenoceptor protein or of its lipid environment might be inaccessible to hydrophilic compounds.

The β_1-adrenoceptor selectivity ratios of nebivolol were 46.1 and 22.5, respectively. The experimental antagonist CGP 20.712A has a much higher β_1-adrenoceptor selectivity than nebivolol (β_1-adrenoceptor selectivity ratios: 812 and 430, respectively). These data suggest that even the most selective β-adrenoceptor antagonist used in clinical practice has the potential to block at least some β_2-adrenoceptors. This is underlined by the serum experiments (Fig. 3), which showed that at physiological concentrations, nebivolol inhibited [^{125}I]iodocyanopindolol binding to β_2-adrenoceptors by approximately 20%. However, the β_1-adrenoceptor selectivity of nebivolol seems to be of clinical relevance. This has been demonstrated in trials investigating the influence of nebivolol on pulmonary function of normal subjects (Mohammed et al., 1991) and of patients with mild asthma (Cazzola et al., 2000). In addition, there is no evidence that β_1-adrenoceptor-selective antagonists may affect counterregulation of hypoglycemia in diabetic patients (UK Prospective Diabetes Study Group, 1998; Sawicki and Siebenhofen, 2001) Taken together, it is not justified to withhold patients with diabetes or patients with mild chronic obstructive pulmonary disease from β-adrenoceptor antagonist therapy when a β_1-adrenoceptor-selective antagonist like nebivolol or bisoprolol could be used. The beneficial effects of β_1-adrenoceptor antagonist treatment have been documented in patients with heart failure (NYHA II–IV) as well (CIBIS II Investigators and Committees, 1999; MERIT-HF Study Group, 1999).

The present study was performed under in vitro conditions. It cannot be excluded that in vivo effects may differ from those observed in vitro. It is a particular problem that adrenoceptor binding constants vary considerably depending on the experimental conditions, including incubation time and temperature (Convents et al., 1987), salt concentrations (McPherson et al., 1985) and the investigated tissue (Muntz et al., 1986). This might explain the different results for values of the β_1-adrenoceptor selectivity ratios presented in the literature for a given β-adrenoceptor antagonist. For this reason, the β_1-adrenoceptor selectivity ratios and K_i values presented in this study probably do not reflect the true in vivo values. Recently, we have published selectivity ratios of 40.7:15.6:0.73 for nebivolol, bisoprolol and carvedilol, respectively, obtained with a very similar assay using [^{3}H]CGP 12.177 (Brixius et al., 2001). The small differences between the former investigations and the independent present study may reflect differences in the investigated myocardial tissue.

Measurements in serum specimens have several limitations. First, we did not measure the metabolites of nebivolol in serum. Thus, the importance of metabolized or non-metabolized nebivolol for β_1-adrenoceptor selectivity after oral administration remains unclear. Given the complex metabolism of nebivolol (Van Peer, 1991) and the relatively small number of volunteers, the true selectivity of orally administered nebivolol and its metabolites might differ from that suggested by the results of the present serum experiments. Secondly, binding properties, at least the calculated B_{max} of [^{125}I]iodocyanopindolol, seem to be altered in the presence of 90% serum in the incubation assay. Thirdly, we cannot exclude a confounding influence due to the in vivo conditions, such as the liberation of endogenous catecholamines. However, the results of the semiquantitative serum experiments were consistent with the other results of the present study, and the method seems to be appropriate for further investigations of β_1-adrenoceptor selectivity. The characterization of the influence of serum in the binding assay has to be addressed in further studies.

We have demonstrated that nebivolol is approximately 3.5 times more β_1-adrenoceptor-selective than bisoprolol in human myocardium and thus might be the most β_1-adrenoceptor-selective antagonist available for clinical practice at the moment. Furthermore, in vivo metabolized nebivolol seems to retain β_1-adrenoceptor selectivity.

In addition, the results of the present study suggest that a rank order of β_1-adrenoceptor selectivity is unlikely to be changed by the use of a different radioligand; however, a direct comparison of absolute values for β_1-adrenoceptor selectivity, as is frequently done in clinical medicine and the pharmaceutical industry, is not correct.

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