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Different intrinsic activities of bucindolol, carvedilol and metoprolol in human failing myocardium

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1 Clinical studies have shown different effects of β-blockers on the β-adrenergic system, tolerability and outcome in patients with heart failure.

2 The study examines β-adrenoceptor-G-protein coupling and intrinsic activity of bucindolol, carvedilol and metoprolol in human ventricular myocardium.

3 Radioligand binding studies ([^{125}I]-Iodocyanopindolol) were performed in membrane preparations of human failing and nonfailing myocardium. Functional experiments were carried out in isolated muscle preparations of human left ventricular myocardium from failing hearts.

4 Bucindolol and carvedilol bound non-selectively to β_1- and β_2-adrenoceptors and exerted guanine nucleotide modulatable binding. Metoprolol was 35-fold β_1-selective and lacked guanine nucleotide modulatable binding.

5 All β-blockers antagonized isoprenaline-induced enhancement of contractility.

6 In preparations in which the coupling of the stimulatory G-protein to adenylate cyclase was facilitated by forskolin, bucindolol increased force of contraction in three and decreased it in five experiments. Carvedilol increased force in one and decreased it in six experiments. Metoprolol decreased force in all experiments by $89.4 \pm 2.2\%$ ($P < 0.01$ metoprolol vs carvedilol and bucindolol). The negative inotropic effect of metoprolol was antagonized by bucindolol.

7 It is concluded that differences in intrinsic activity can be detected in human myocardium and have an impact on cardiac contractility. In human ventricular myocardium, bucindolol displays substantially higher intrinsic activity than metoprolol and carvedilol. Bucindolol can behave as partial agonist or partial inverse agonist depending on the examined tissue.

8 Differences in intrinsic activity may contribute to differences in β-adrenoceptor regulation and possibly to differences in tolerability and outcomes of patients with heart failure.
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Keywords: β-adrenoceptor antagonists; intrinsic activity; inverse agonism; human myocardium; heart failure

Abbreviations: β-AR, β-adrenergic receptor; B_{max}, maximum receptor density; °C, degrees centigrade; EC_{50}, concentration achieving 50% of maximum effect; FOC, force of contraction; Gpp(NH)p, guanylylimidodiphosphate; G_S, stimulatory G-protein; ICYP, [^{125}I]-Iodocyanopindolol; ISA, intrinsic sympathomimetic activity; K_d, K_i, dissociation constant; NF, nonfailing; n_H, slope factor; NYHA, New York Heart Association; R_H, receptors in a high-affinity state; s.e.mean, standard error of the mean

Introduction

Clinical studies have demonstrated beneficial effects of β-adrenoceptor antagonist treatment of chronic heart failure (Hash & Prisant, 1997 for review). However, effects of β-adrenoceptor antagonists on survival are different. Carvedilol (Packer *et al*., 1996), bisoprolol (CIBIS II, 1999) and metoprolol (MERIT-HF Study Group, 1999) were shown to reduce mortality in patients with chronic heart failure. In contrast, xamoterol had adverse effects on survival in patients with heart failure (Nicholas *et al*., 1990), and bucindolol failed to produce a beneficial effect compared to placebo (BEST, 1995; Bristow, 2000). Thus, differences among β-adrenoceptor antagonists are likely to occur.

Previous data indicate that these agents exert different effects on the β-adrenergic system. Gilbert *et al*. (1993) reported a restoration of down-regulated right ventricular β-adrenoceptor density after treatment with metoprolol, but not with carvedilol. These *in vivo* observations were confirmed by *in vitro* experiments in chick heart cells, where metoprolol, but neither carvedilol nor bucindolol, reversed agonist-induced down-regulation of β_1-adrenoceptors (Yoshikawa *et al*., 1996).

Transgenic mice overexpressing the human β_2-adrenoceptor (Bond *et al*., 1995) or a constitutively active mutant (CAM) adrenoceptor (Samana *et al*., 1993) have led to the expansion of the classical ternary complex model of receptor action (De Lean *et al*., 1980). The β-adrenoceptor exists in an equilibrium between an active (R*) and an inactive (R) conformation. When unoccupied, the β-adrenoceptor already exerts basal intrinsic activity. Agonists induce a conformational change towards the R*-state, whereas neutral antagonists cause no change. Some antagonists are able to stabilize the inactive conformation (R), thus decreasing the basal activity of the receptor. These antagonists are termed inverse agonists, as they exert a 'negative' intrinsic activity, driving the equilibrium to the opposite direction than the agonist (Bond *et al*., 1995).

The activation state of the β-adrenoceptor is of importance for receptor regulation. The intrinsic activity of several partial and full agonists was shown to correlate with the degree of receptor phosphorylation and in turn desensitization and downregulation (Benovic *et al*., 1988). In this light of receptor action of antagonists, many therapeutically used agents may

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need reclassification (Bond *et al*., 1995). In particular, in failing human tissue, direct evaluation of intrinsic activity of relevant compounds has not been performed.

The present study assessed the intrinsic activity of the β-adrenoceptor antagonists bucindolol, carvedilol and metoprolol directly in human left ventricular myocardium from patients with heart failure. By radioligand binding experiments, receptor-G-protein interaction was examined. For evaluating the functional relevance of the biochemical findings, contraction experiments were performed in the presence of forskolin, a diterpene that facilitates the coupling of the α-subunit of the stimulatory G-protein ($G_{S\alpha}$) with the catalytic unit of adenylate cyclase (Jasper *et al*., 1988).

Methods

Myocardial tissue

Isolated, electrically stimulated human left ventricular papillary muscle strips or cell membrane preparations from human left ventricular myocardium were used. Tissue was obtained during heart transplantations. Failing hearts were taken from seven patients with end-stage heart failure (New York Heart Association (NYHA) class IV), resulting from either idiopathic ($n=5$) or ischaemic ($n=2$) dilated cardiomyopathy (five male, two female; age, 64 ± 2 years; ejection fraction, $32\pm1\%$). Nonfailing hearts were taken from four organ donors whose hearts could not be used for transplantation (three male, one female; age 37 ± 3 years). For the latter group, echocardiography revealed normal left ventricular contractility. All patients gave written informed consent before surgery. Medical therapy of patients with heart failure consisted of diuretics, nitrates, ACE inhibitors and cardiac glycosides. Patients (nonfailing and failing) receiving catecholamines or β-adrenoceptor antagonists were withdrawn from the study. Drugs used for general anaesthesia were flunitrazepam, fentanyl and pancuronium bromide with isoflurane. The tissue was placed immediately in ice-cold cardioplegic solution (containing (in mmol l^{-1}) NaCl 15, KCl 10, $MgCl_2$ 4, histidine HCl 180, tryptophane 2, mannitol 30, and potassium dihydrogen oxoglutarate 1), and was delivered to the laboratory within 15 min. For functional experiments, ventricular samples were used immediately. For radioligand experiments, cardiac tissue was frozen in liquid nitrogen immediately after explantation and stored at $-70^{\circ}C$. Membrane preparation for binding experiments has been described elsewhere (Böhm *et al*., 1990b).

Isolated cardiac muscle strip preparation and measurement of force of contraction

Isometric force of contraction was determined on isolated, electrically driven muscle preparations as described previously (Böhm *et al*., 1990b). Bathing solution was maintained at $37^{\circ}C$, pH 7.4, and aerated with 95% O_2 and 5% CO_2. Muscles were stretched to the length at which force of contraction was maximal. In experiments with inotropic prestimulation, muscle strips were preexposed to isoprenaline (0.1 μmol l^{-1}, $\cong EC_{50}$) for 30 min and to forskolin (0.3 μmol l^{-1}, $\cong EC_{50}$) for at least 45 min.

The β-adrenoceptor antagonists bucindolol (0.1–1000 nmol l^{-1}), carvedilol (0.1–1000 nmol l^{-1}) and metoprolol (1–100,000 nmol l^{-1}) were applied cumulatively to the organ bath for 30 min at each concentration. Nevertheless, when concentrations of β-blockers are indicated as K_i or $100\times K_i$ (tables or bar graphs), the results are also derived from these cumulative dose-response curves.

β-Adrenoceptor binding studies

β-Adrenoceptors in cardiac tissue were investigated using [^{125}I]-iodocyanopindolol (ICYP) as the radiolabelled ligand (specific activity of 2000 Ci $mmol^{-1}$). For estimation of total β-adrenoceptor density (B_{max}) and dissociation constant (K_d), ICYP saturation curves with eight increasing concentrations of ICYP between 3 and 300 pmol l^{-1} and 3 μmol l^{-1} of propranolol for determination of nonspecific binding were used. Cold ligand binding affinity was measured by ligand-ICYP competition curves using 25 pmol l^{-1} of ICYP to maintain the radioligand concentration at approximate K_d. The assay was performed in a total volume of 250 μl. The total amount of protein used per assay was 20–30 μg. Protein concentrations were determined according to the method of Lowry *et al*. (1951). The incubation at $25^{\circ}C$ for 60 min allowed complete equilibration of the β-adrenoceptors with the radioligand. The reaction was terminated by rapid vacuum filtration through Whatman GF/C filters (Whatman Inc., Clifton, NJ, U.S.A.). The filters were washed immediately three times with 6 ml of ice-cold incubation buffer. All experiments were performed in triplicate.

Statistical analysis

Regression analysis was performed with the computer program GraphPadPrism (GraphPad Software, San Diego, Cal., U.S.A.). For determination of B_{max} and K_d of radioligand saturation experiments, linear regression according to the method of Scatchard *et al*. (1949) was performed. Competition curve slope (pseudo-Hill factor, n_H), the concentration at which 50% of the effect was achieved (EC_{50}) (for both binding studies and contraction experiments) and the percentage of receptors in a high affinity ($\%R_H$) or low affinity ($\%R_L$) state were determined by nonlinear regression analysis, comparing the fitting of the curve to either one or two receptor states by *F*-test analysis. Cold ligand dissociation constants for a high affinity (K_H) or a low affinity receptor state (K_L) were calculated according to the method of Cheng and Prussoff (1973). K_i values were calculated from EC_{50} values that were determined by fitting the results of competition experiments with a nonlinear regression analysis assuming only one receptor state, regardless if they actually reflect one or two affinity states.

Unless indicated, the data shown are mean ± s.e.mean. For multiple comparisons, ANOVA analysis was performed. Otherwise, statistical significance was analysed with the Mann–Whitney or Wilcoxon test. A value of $P<0.05$ was considered significant.

Materials

Chemicals were from Sigma Chemical Co. ICYP was produced by Amersham-Buchler (Freiburg i.Br., Germany). All other chemicals were of analytic grade or the best commercially available.

Results

Radioligand binding studies

[^{125}I]-Iodocyanopindolol (ICYP) saturation experiments revealed a 4.7-fold lower β-adrenoceptor density in failing

(NYHA IV) compared to nonfailing (NF) hearts (NYHA IV, 18.0 ± 3.4 fmol mg protein^{-1}; NF, 84.3 ± 22.3 fmol mg protein^{-1}; $P<0.01$) with no significant change in K_d (NF, 56.2 (95% confidence Interval: 29.0–83.33) pmol l^{-1}; NYHA IV, $37.6 \pm 23.1 - 52.2$) pmol l^{-1}).

Figure 1 shows representative results from competition experiments of the β-adrenoceptor ligands isoprenaline, metoprolol, carvedilol and bucindolol in human myocardium. Figure 1A represents the binding properties of the agonist isoprenaline. It is characterized by a biphasic binding curve

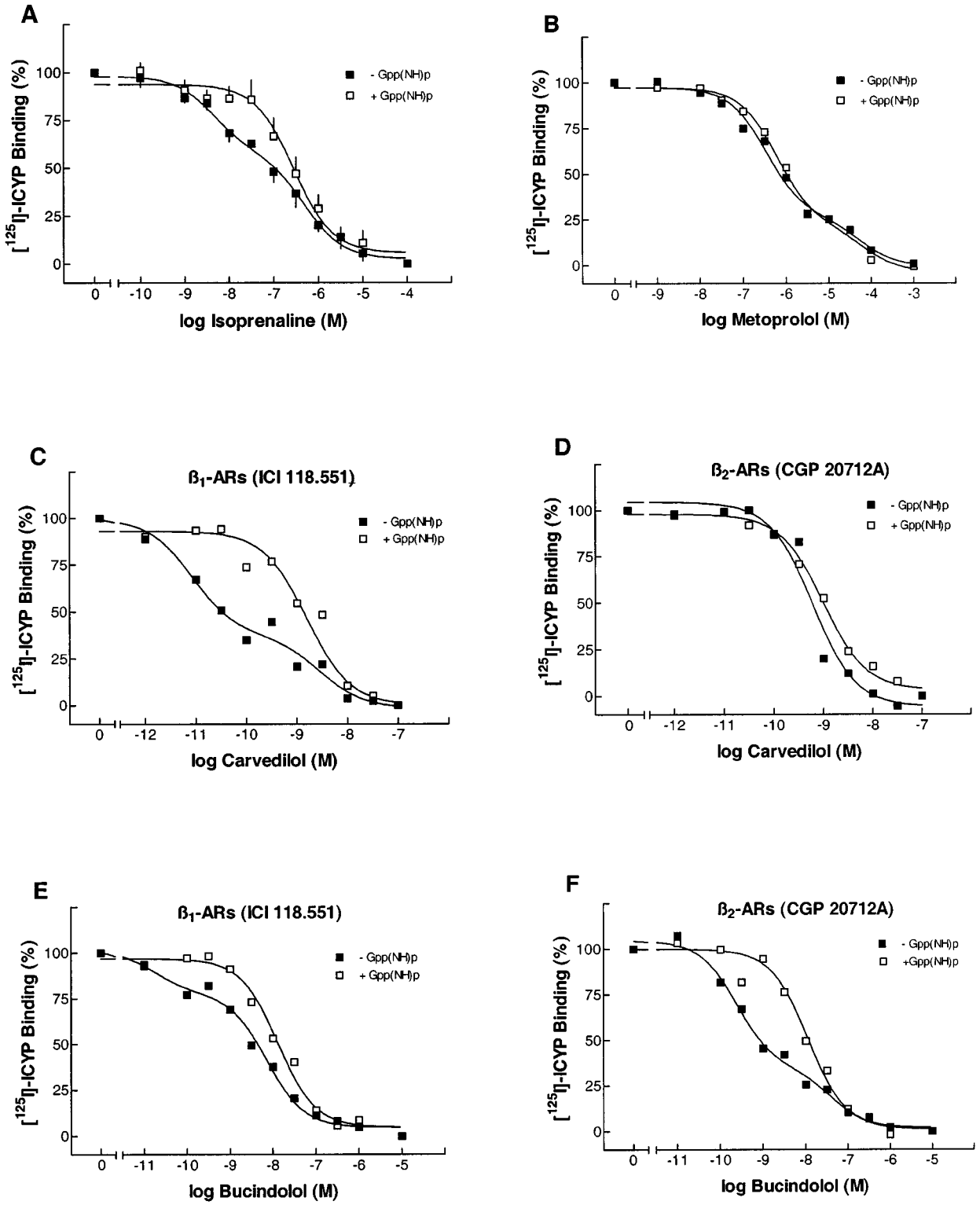

Figure 1 [^{125}I]-ICYP competition curves in human left ventricular myocardium from nonfailing hearts. (A) ICYP-isoprenaline competition in the absence and presence of 100 μmol l^{-1} of Gpp(NH)p. (B) ICYP-metoprolol competition in the absence and presence of Gpp(NH)p. (C) ICYP-carvedilol competition, both curves in the presence of ICI 118,551 (50 nmol l^{-1}), in the absence and presence of Gpp(NH)p. (D) ICYP-carvedilol competition, both curves in the presence of CGP 20712A (300 nmol l^{-1}), in the absence and presence of Gpp(NH)p. (E, F) ICYP-bucindolol competition, identical conditions like C, D. A, data are the means ± s.e.mean from $n=4$ experiments. B–F, data are from one representative experiment. β-ARs, β-adrenoceptors.

with the identification of a high- and a low-affinity binding site. In the presence of guanylylimidodiphosphate (Gpp(NH)p), a non-hydrolyzable guanine nucleotide, binding becomes monophasic with the detection of only one (low-affinity) binding site. As a consequence, the slope factor (n_H) steepens and approaches unity (Tables 1 and 2).

Binding of metoprolol to β-adrenoceptors in human myocardium also revealed two distinct affinity sites (Figure 1B). This biphasic binding is not converted into monophasic binding by the presence of Gpp(NH)p. When adding ICI 118,551 (50 nmol l^{-1}), a highly selective β_2-adrenoceptor antagonist, or CGP 20712A (300 nmol l^{-1}), highly selective for β_1-adrenoceptors, ligand binding to a homogenous population of β_1- or β_2-adrenoceptors could be observed, respectively (Tables 1 and 2). The K_L values in the presence of ICI 118,551 and CGP 20712A were similar to K_H and K_L in the absence of these compounds (Tables 1 and 2, Figure 1B). The β_1-selectivity of metoprolol was 35-fold.

In two out of seven experiments, carvedilol exerted biphasic binding properties. In both cases, in the presence of Gpp(NH)p the competition curve fitted to a single receptor state (Tables 1 and 2). When looking at β-adrenoceptor subtypes (in the presence of ICI 118,551 or CGP 20712A, respectively), biphasic binding of carvedilol was present in four of seven cases at the β_1-adrenoceptor (Figure 1C), but only one of seven cases at the β_2-adrenoceptor (1D). In all cases, biphasic binding curves converted into monophasic ones in the presence of Gpp(NH)p. In human myocardium, carvedilol was rather non-selective.

Bucindolol exerted biphasic binding in the absence of Gpp(NH)p, with slope factors substantially less than unity (Tables 1 and 2). In only 50% of the experiments, the computer modeled two-affinity state fit could be converted into a one-affinity state fit by the presence of Gpp(NH)p. Bucindolol identified a rather high percentage of high-affinity binding states (Tables 1 and 2). In the presence of ICI 118,551 and CGP 20712A (Figure 1E,F, Table 1), respectively, these agonist-like binding properties could be observed at β_1- as well as at β_2-adrenoceptors. The slope factors in the presence of Gpp(NH)p (Tables 1 and 2) indicate that the guanine

Table 1 Radioligand competition experiments in human nonfailing myocardium

Agent	*Gpp NHp*	*2-s-f*	R_H (%)	K_H (nmol l^{-1})	K_L (nmol l^{-1})	*Slope* (n_H)
Isoprenaline	–	4/4	39.2±2.7	4.0±2.0	255.9±121.2	0.44±0.09
	+	0/4	–	–	275.3±71.8	0.90±0.14*
Carvedilol	–	1/3	35.9	0.001	0.5±0.2	0.76±0.21
+ICI	+	0/3	–	–	1.2±0.3	0.83±0.08
	–	3/3	49.4±7.0	0.02±0.02	1.8±0.3	0.32±0.09
+CGP	+	0/3	–	–	1.0±0.1	0.84±0.08*
	–	0/1	–	–	0.5	2.81
	+	0/1	–	–	0.7	0.82
Metoprolol	–	1/1	70.0	227.5	33080	0.50
	+	1/1	69.8	410.2	20040	0.62
+ICI	–	0/1	–	–	209.4	0.98
+CGP	–	0/1	–	–	9806	1.22
Bucindolol	–	4/4	69.1±2.2	0.6±0.1	99.7±38.1	0.52±0.04
	+	3/4	69.6±2.8	0.5±0.1	221.3±20.6	0.56±0.12
+ICI	–	3/3	48.6±12.8	1.2±0.9	91.3±78.3	0.58±0.02
	+	1/3	49.6	1.4	41.8±34.6	0.70±0.12
+CGP	–	3/3	52.6±7.3	0.1±0.02	35.9±17.9	0.36±0.13
	+	1/3	47.2	0.4	31.7±27.0	0.62±0.13

*$P<0.05$ vs −Gpp(NH)p; +ICI, in the presence of ICI 118.551 (50 nmol l^{-1}); +CGP, in the presence of CGP 20712A (300 nmol l^{-1}); GppNHp, in the presence (+) or absence (−) of Gpp(NH)p (100 μmol l^{-1}); 2-s-f, number of experiments that modelled for a two-site competition fit; R_H, fraction of receptors in a high affinity state; K_H, K_i-values of the receptors in a high affinity state; K_L, K_i-values of the receptors in a low-affinity state; n_H, slope factor.

Table 2 Radioligand competition experiments in human failing myocardium

Agent	*Gpp NHp*	*2-s-f*	R_H (%)	K_H (nmol l^{-1})	K_L (nmol l^{-1})	*Slope* (n_H)
Isoprenaline	–	4/4	27.9±3.7	0.7±0.4	90.4±29.4	0.58±0.03
	+	0/4	–	–	104.3±16.3	0.76±0.04*
Carvedilol	–	1/4	24.6	0.05	0.9±0.2	0.92±0.05
	+	0/4	–	–	0.5±0.1	1.06±0.08
+ICI	–	1/4	27.3	0.02	1.2±0.5	0.90±0.13
	+	0/4	–	–	0.8±0.2	0.87±0.09
+CGP	–	1/4	48.8	0.02	1.1±0.7	1.07±0.26
	+	0/4	–	–	1.2±0.8	1.24±0.25
Metoprolol	–	2/3	55.5±14.8	117.8±31.0	6879±5153	0.61±0.03
	+	1/3	53.8	12.7	651.9±308.1	0.76±0.17
+ICI	–	0/3	–	–	170.9±43.4	0.72±0.03
	+	0/2	–	–	180.6±8.3	0.97±0.18
+CGP	–	0/3	–	–	5157±1876	1.33±0.35
	+	0/2	–	–	3836±2463	0.79±0.05
Bucindolol	–	2/3	64.0±16.4	0.3±0.1	28.6±15.7	0.59±0.17
	+	0/3	–	–	20.7±18.1	0.62±0.04

*$P<0.05$ vs −Gpp(NH)p; +ICI, in the presence of ICI 118.551 (50 nmol l^{-1}); +CGP, in the presence of CGP 20712A (300 nmol l^{-1}; GppNHp, in the presence (+) or absence (−) of Gpp(NH)p (100 μmol l^{-1}); 2-s-f, number of experiments that modelled for a two-site competition fit; R_H, fraction of receptors in a high affinity state; K_H, K_i-values of the receptors in a high affinity state; K_L, K_i-values of the receptors in a low-affinity state; n_H, slope factor.

nucleotides do not sufficiently resolve agonist-like binding properties of bucindolol. In human myocardium, bucindolol was non-selective.

Functional studies

In order to estimate the implications of different β-adrenoceptor binding properties of β-adrenoceptor antagonists on myocardial contractile function, experiments in left ventricular papillary muscle strips from patients with terminal heart failure due to idiopathic or ischaemic dilated cardiomyopathy were performed.

The application of 0.1 μmol l^{-1} of isoprenaline enhanced basal force of contraction by 101±24% ($n=18$). After equilibration, cumulative concentrations of the β-adrenoceptor antagonists bucindolol, carvedilol and metoprolol were added to the organ bath. There was no significant difference among the three groups in basal as well as in isoprenaline-enhanced force of contraction (Table 3). All β-adrenoceptor antagonists significantly antagonized isoprenaline enhanced force of contraction (Figure 2, Table 3). The extent to which force of contraction was reduced significantly differed among the groups. Whereas bucindolol and carvedilol at $100 \times K_i$ (reflecting approximately 100% receptor occupation) reduced force of contraction close to basal values (Figure 2B,C), the negative inotropic effect of metoprolol was substantially more pronounced ($P<0.01$ vs carvedilol and bucindolol, respectively; Figure 2A).

As both carvedilol and bucindolol exert agonist-like binding properties, the coupling of β-adrenoceptors to adenylate cyclase was facilitated by forskolin at a concentration of 0.3 μmol l^{-1}. By this method, intrinsic sympathomimetic activity (ISA) of ligands in human myocardium can be detected (Böhm *et al*., 1990b). Forskolin increased force of contraction by 54±17% ($n=21$). Metoprolol decreased cardiac contractility by 53.4±12.9% and 89.4±2.2% at 50% (K_i) and 100% ($100 \times K_i$) receptor occupation, respectively (Figure 3A $P<0.01$ vs forskolin). Carvedilol had a negative inotropic effect in six out of seven experiments and a slight positive inotropic effect in only one experiment. The total negative inotropic effect was 6.6±2.5% and 27.1±8.1% at K_i and $100 \times K_i$, respectively (Figure 3C, $P<0.05$ vs forskolin, $P<0.01$ vs metoprolol). At concentrations of K_i and $100 \times K_i$, bucindolol decreased force of contraction slightly, but insignificantly (Figure 3E). Bucindolol caused a significant positive inotropic effect in 38% of the muscles (three out of eight), increasing force of contraction by 17.1±8.4% at 100 nmol l^{-1} (Figure 3F). In the other 62%, bucindolol caused a negative inotropic effect of 36.0±7.0% at the highest concentration. In the presence of bucindolol

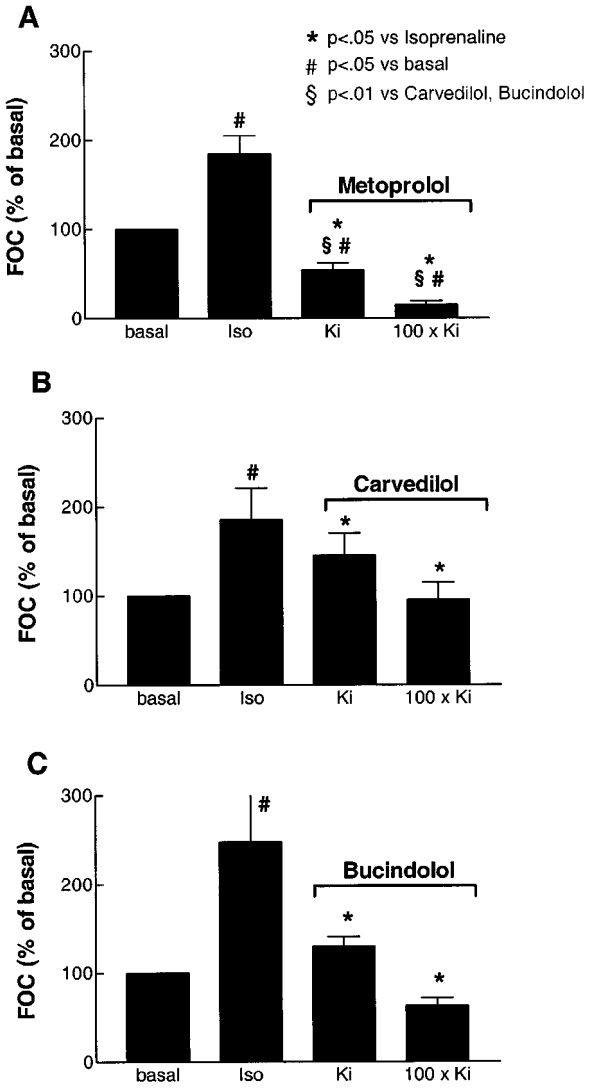

Figure 2 The effects of metoprolol ($n=6$ experiments from $n=5$ hearts, A), carvedilol ($n=6/5$, B) and bucindolol ($n=6/5$, C) at K_i and $100 \times K_i$ on isoprenaline (Iso, 0.1 μmol l^{-1}) enhanced force of contraction (FOC) in human left ventricular myocardium from patients with heart failure. Results are from cumulative dose-response experiments, but only K_i- and $100 \times K_i$-values are indicated as bar graphs. K_i-values are derived from radioligand binding experiments. The concentrations used in the experiment are 0.01 (K_i) and 1 μmol l^{-1} ($100 \times K_i$) for bucindolol, 0.001 (K_i) and 0.1 μmol l^{-1} ($100 \times K_i$) for carvedilol and 1 (K_i) and 100 μmol l^{-1} ($100 \times K_i$) for metoprolol. These concentrations are used to achieve approximately 50% (K_i) and 100% ($100 \times K_i$) β-adrenoceptor occupation, respectively.

Table 3 Effects of β-blockers on cardiac contractility after prestimulation

	n	*Basal*	*Prestimulation (Iso/Forskolin)*	K_i	$100 \times K_i$
Isoprenaline					
Bucindolol	6/5	3.08±1.06	6.47±2.54*	3.83±1.32#	1.83±0.50#
Carvedilol	6/5	3.17±0.98	5.13±1.37*	4.13±1.05	2.83±0.76#
Metoprolol	6/5	3.49±1.00	6.07±1.55*	2.02±0.66*#	0.43±0.10**§§
Forskolin					
Bucindolol	8/5	4.15±1.39	5.21±1.40*	4.97±1.52	4.79±1.66
Carvedilol	7/4	4.98±1.48	7.31±1.48	6.84±1.38	5.42±1.35
Metoprolol	6/6	6.34±1.94	7.38±1.70	3.68±1.40*#	0.85±0.32**§§

Force of contraction (mN) was determined at basal levels, after isoprenaline (Iso, 0.1 μmol l^{-1})- or forskolin (0.3 μmol l^{-1})-prestimulation, respectively, and after the addition of the mentioned β-blockers at concentrations occupying 50% (K_i) or 100% ($100 \times K_i$) of β-adrenoceptors, respectively. K_i- and $100 \times K_i$-concentrations are given in legend of Figure 2. *$P<0.05$ vs basal; **$P<0.01$ vs basal; #$P<0.05$ vs isoprenaline- or forskolin-prestimulation, respectively; §$P<0.01$ vs isoprenaline- or forskolin-prestimulation, respectively; §$P<0.05$ vs carvedilol, bucindolol.

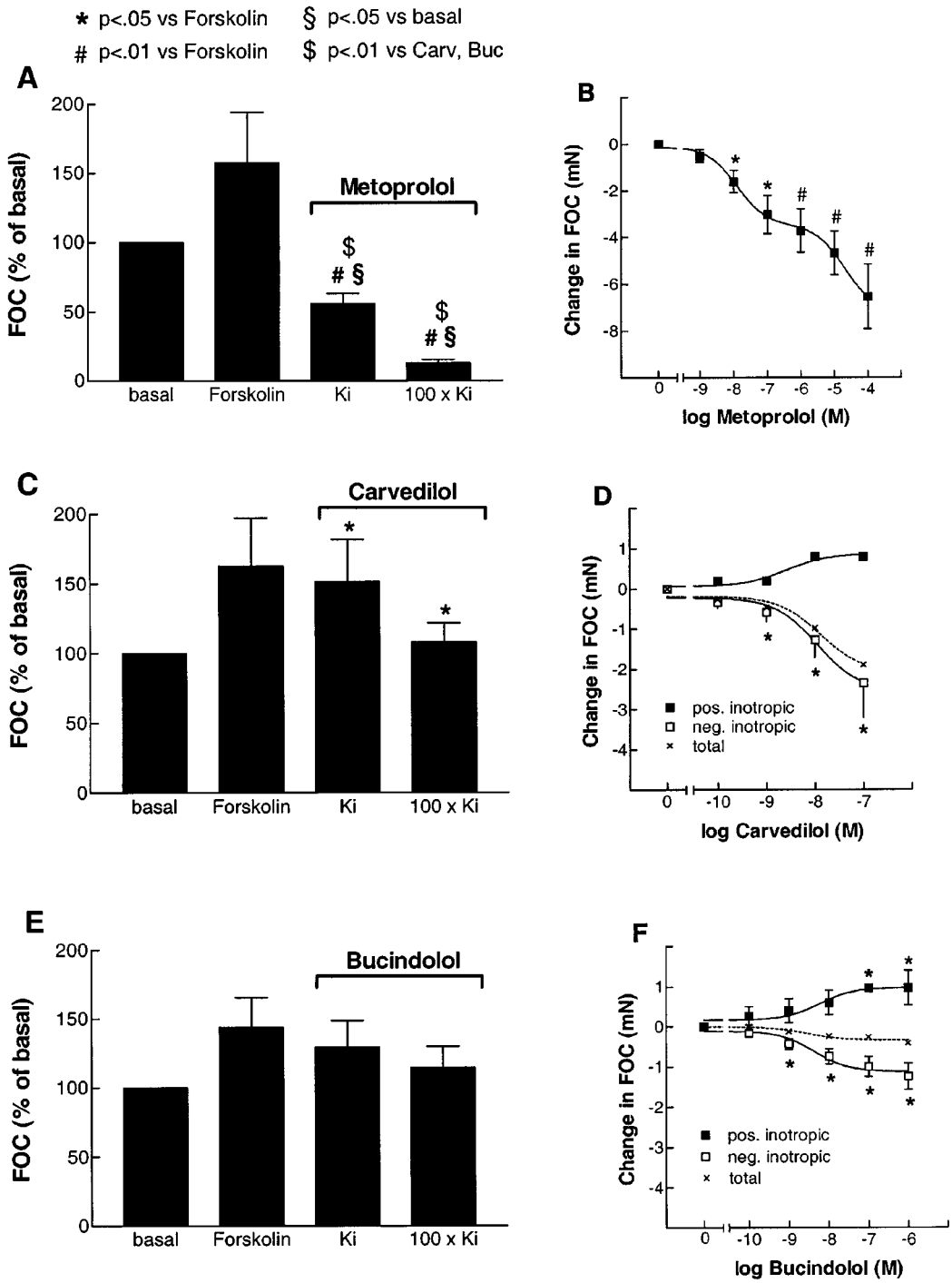

Figure 3 The effects of metoprolol ($n=6$ experiments from six hearts, A, B), carvedilol (Carv, $n=7/4$, C, D) and bucindolol (Buc, $n=8/5$, E, F) at K_i and $100 \times K_i$ on forskolin ($0.3\ \mu mol\ l^{-1}$) enhanced force of contraction (FOC) in human left ventricular myocardium from patients with heart failure. K_i-values are the same as in Figure 2. Bar graphs (A,C,E) give the means of all experiments in per cent of basal FOC at the indicated concentrations. In B,D,F, cumulative concentrations of the respective β-adrenoceptor antagonists are plotted against the change in FOC (mN). In D, F, the total group (dashed line) is divided into one group of muscles with a positive inotropic response to β-blocker ($n=1$ for carvedilol, $n=3$ for bucindolol), and the other group with a negative inotropic response.

($100\ nmol\ l^{-1}$), the negative inotropic effect of metoprolol could be competitively inhibited (Figure 4).

In Figure 5, the effects of 100% receptor occupation by bucindolol on forskolin enhanced FOC is compared to the effects of receptor occupation by carvedilol (Figure 5A) and metoprolol (Figure 5B) in the hearts from the same patients, respectively. There was a close correlation between the effects of bucindolol with the effects of carvedilol (Figure 5A) and metoprolol (Figure 5B).

Discussion

Recent studies in different cell systems have demonstrated that the effects of β-blocking agents on adenylate cyclase stimulation, chronotropy or inotropy are strongly dependent on the examined system. For a number of β-blockers, no definite classification as partial agonist or inverse agonist can be made, since their effects were variable in different systems. In the present study we examined the intrinsic activity of the

compounds bucindolol, carvedilol and metoprolol directly in human failing myocardium, since these agents are frequently used in patients with heart failure.

The examination of intrinsic activity in intact human myocardium is complicated by several mechanisms. In myocardium from patients with heart failure, the total amount of β-adrenoceptors is reduced (Bristow *et al*., 1982), and the remaining receptors are desensitized to agonist stimulation (Hausdorff *et al*., 1990). In addition, increased membrane concentrations of inhibitory G-protein α-subunits (Böhm *et al*., 1990a) might also reduce partial agonist or inverse agonist responses. Therefore, in contraction experiments forskolin was added to the organ bath. This diterpene facilitates the coupling of $G_{S\alpha}$ to the catalytic unit of adenylate cyclase, and responses of both partial agonists (Böhm *et al*., 1990b) and inverse agonists (Mewes *et al*., 1993) become amplified and detectable.

Using this approach, it could be demonstrated that bucindolol behaved as a partial agonist in 38% of the experiments and as an inverse agonist in 62% of the experiments. Also carvedilol displayed slight partial agonist activity in one experiment. This might be due to different initial activation states of the receptors in the different tissue samples. In β_2-adrenoceptor-expressing Sf9 cells, dichloroisoproterenol was shown to act as either a partial agonist or inverse agonist, depending on the degree of isoprenaline-induced desensitization of the system (Chidiac *et al*., 1996). In Figure 5, the effects of 100% receptor occupation by bucindolol on forskolin enhanced FOC is compared to the effects of receptor occupation by carvedilol (Figure 5A) and metoprolol (Figure 5B) in the hearts from the same patients, respectively. Assuming that the initial state of receptor activation is similar in samples that come from the same tissue, these plots indicate that the activation state of the β-adrenoceptors has an influence on the functional response to the respective ligands. In a study on the pithed rat, bucindolol but not carvedilol behaved as a partial agonist by producing a dose-related increase in heart rate (Willette *et al*., 1998). This positive chronotropy of bucindolol was also detected in several other *in vivo* studies (Deitchman *et al*., 1980; Marwood *et al*., 1986), whereas in a study on human myocardium, no increase in adenylate cyclase activity or force of contraction in response to bucindolol or carvedilol could be detected (Hershberger *et al*., 1990). In another study on human atrial myocardium, small amounts of partial agonism of bucindolol, but not carvedilol could be detected after muscle preparations had been depleted of catecholamines (Trochu *et al*., 1999). In a very recent study on constitutively active mutants of the β_1-adrenoceptor, metoprolol displayed inverse agonist activity, whereas carvedilol had partial agonist activity (Lattion *et al*., 1999). Bucindolol had not been investigated. These variable results, but also the fact that in our study even under identical experimental conditions the intrinsic activity of bucindolol and carvedilol was variable, may be related to different basal activation states of the β-adrenoceptors in the respective tissues.

The effects of metoprolol on cardiac contractility are substantially different to those of bucindolol and carvedilol. In contrast to the latter two agents, metoprolol not only inhibited isoprenaline stimulation, but it further reduced force of contraction to 15% of basal levels. Moreover, when forskolin stimulated cardiac contractility independent from β-adrenoceptor activation, 100% receptor occupation by metoprolol led to nearly complete depression of contractility. Hence, metoprolol is an inverse agonist that primarily stabilizes the inactive conformation of the β-adrenoceptor (R). Consistently, in Sf9 cells transfected with a baculovirus expression system, metoprolol was shown to exert a relatively high amount of inverse agonism at the β_2-adrenoceptor compared to carvedilol and bucindolol (Yoshikawa *et al*., 1996).

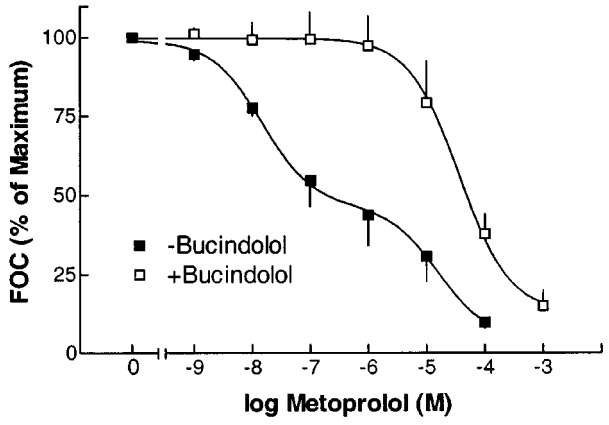

Figure 4 Negative inotropic effect of metoprolol in the presence (+) and absence (−) of bucindolol (0.1 μmol l^{-1}), and in the presence of forskolin (0.3 μmol l^{-1}), respectively. Ordinate: per cent of maximum forskolin-enhanced force of contraction (FOC).

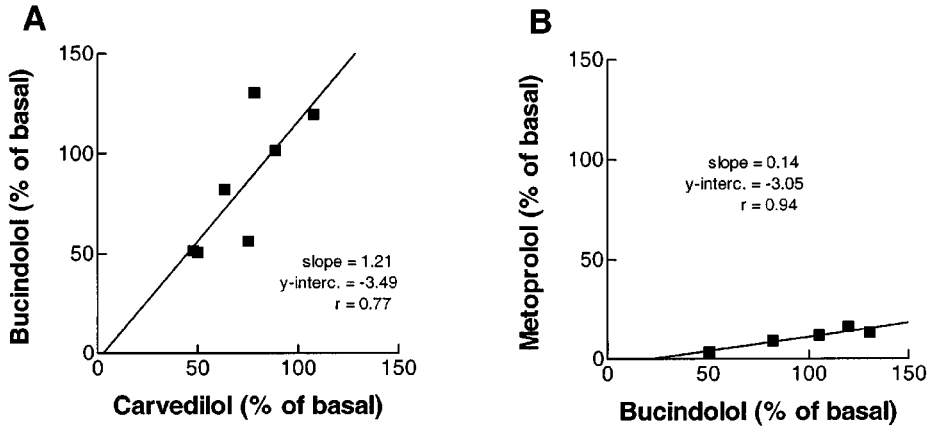

Figure 5 Comparison of intrinsic activity of bucindolol, carvedilol and metoprolol. (A) FOC in per cent of basal values after $100 \times K_i$ of carvedilol (abscissa) is plotted against FOC in per cent of basal values after $100 \times K_i$ of bucindolol (ordinate) in preparations *from the same hearts*, after forskolin (0.3 μmol l^{-1}) prestimulation of left ventricular myocardium from patients with heart failure. (B) bucindolol (abscissa) against metoprolol (ordinate), the same conditions as in A.

The examination of intrinsic activity can be complicated by the presence of contaminating catecholamines in the underlying tissues. The presence of catecholamines could lead to an enhancement of negative inotropic effects of β-adrenoceptor antagonists. However, in experiments with metoprolol in the presence of bucindolol, the combined application of two β-adrenoceptor antagonists would be expected to have additive effects on force of contraction. In contrast, bucindolol is able to inhibit the metoprolol-induced reduction of force of contraction. Thus, the presence of contaminating catecholamines can be ruled out.

The examined β-blockers were shown to affect cardiac β-adrenoceptor regulation in substantially different ways (Gilbert *et al*., 1993; Yoshikawa *et al*., 1996). In this study, carvedilol and bucindolol exerted guanine nucleotide modulatable binding at cardiac β-adrenoceptors. Similar results have been obtained by other studies (Yoshikawa *et al*., 1996; Hershberger *et al*., 1990; Bristow *et al*., 1992). In contrast, in our study β-adrenoceptor binding was examined at human ventricular β_1- and β_2-adrenoceptor subgroups separately by performing experiments in the presence of ICI 118,551 and CGP 20712A, respectively. While for carvedilol, guanine nucleotide modulatable binding had been observed predominantely to β_2-adrenoceptors (Bristow *et al*., 1992), the present study identifies agonist-like binding especially to β_1-adrenoceptors. For bucindolol, our study obtains comparable results to those of Hershberger *et al*. (1990), who also observed complex binding characteristics for bucindolol on both β_1- and β_2-adrenoceptors.

[^{125}I]-ICYP-metoprolol competition curves were not affected by the presence of Gpp(NH)p. In addition, metoprolol exerted inverse agonism in functional experiments. These results indicate that this compound rather stabilizes the inactive conformation (R) of the β-adrenoceptor. Thus, phosphorylation of the β-adrenoceptor by β-adrenergic receptor kinases and subsequent desensitization or even down-regulation might be effectively prevented. These observations may explain why *in vitro* models, and also clinical studies observed up-regulation of β-adrenoceptors with consecutive increased haemodynamic response to catecholamine stimulation following metoprolol treatment, but a lack of up-regulation or even further down-regulation of β-adrenoceptors upon treatment with carvedilol and bucindolol (Heilbrunn *et al*., 1989; Gilbert *et al*., 1993; Yoshikawa *et al*., 1996; Böhm *et al*., 1998).

One possible limitation of the present study could be that it examines effects of β-adrenoceptor antagonists on isolated myocardium. The *in vivo* effects of these agents could be slightly different. Nevertheless, the study evaluates intrinsic activity of therapeutically relevant β-blockers in intact human myocardium, and thus, might possess some advantage compared to studies in animal or cell models.

The data may be critical to understand differences in regulation of cardiac β-adrenoceptors in patients with heart failure that are treated with bucindolol, carvedilol or metoprolol. Furthermore, different intrinsic activity may provide one explanation for different tolerability or even outcomes due to β-blocker treatment in heart failure. In two recent meta-analyses of the effects of β-blockers on mortality in patients after myocardial infarction, β-blockers with intrinsic sympathomimetic activity (ISA) were associated with a lower risk-reduction compared to agents that lack ISA (Soriano *et al*., 1997; Freemantle *et al*., 1999). Xamoterol, a β-blocker with ISA in human myocardium (Böhm *et al*., 1990b), had detrimental effects on the survival of patients with heart failure (Nicholas *et al*., 1990). These data indicate that intrinsic activity of β-blockers is of clinical relevance. In general, β-blockers with high intrinsic activity appear to have worse effects on mortality than agents with low intrinsic activity. However, when classifying β-blockers with respect to their intrinsic activity, it should be considered that the effects of the respective agents are very much dependent on the examined system. Thus, it may be difficult to subdivide β-blockers into agents with or without ISA. For human ventricular myocardium, it can be concluded that bucindolol and carvedilol activate the receptor-G-protein complex and display substantial higher intrinsic activity compared to metoprolol. In a part of the experiments, bucindolol even stimulated cardiac contractility, displaying the ability of this agent to behave as a partial agonist in human failing myocardium. Whether this comparable high intrinsic activity may have contributed to the lack of benefit provided by this agent in the BEST-trial (Bristow, 2000) remains to be further elucidated.

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