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Nebivolol, bucindolol, metoprolol and carvedilol are devoid of intrinsic sympathomimetic activity in human myocardium

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1 The present study investigated whether or not there may be differences in the direct cardiac actions of the novel, highly β_1-selective adrenoceptor antagonist nebivolol (NEB) in comparison to metoprolol (MET), bisoprolol (BIS), carvedilol (CAR) and bucindolol (BUC) in human myocardium ($n=9$).

2 The rank order of β_1-selectivity as judged by competition experiments to ^{3}H-CGP 12.1777 in the presence of CGP 207.12 A (300 nmol l^{-1}, $K_i\beta_2$) or ICI 118.551 (50 nmol l^{-1}, $K_i\beta_1$) were NEB($K_i\beta_2$/$K_i\beta_1$: 40.7) > BIS(15.6) > MET(4.23) > CAR(0.73) > BUC(0.49).

3 The rank order of the negative inotropic potency of the β-adrenoceptor antagonists measured in left ventricular trabeculae (dilated cardiomyopathy, DCM) as judged by the concentration needed to induce a 50% decrease in isoprenaline (1 μmol l^{-1})-stimulated force (IC_{50}) was: MET (0.6 μmol l^{-1}) > CAR (4.1 μmol l^{-1}) > NEB (7.0 μmol l^{-1}).

4 NEB, BUC, MET and CAR did not not exert an intrinsic sympathomimetic activity (ISA) as determined by measurements of force development in forskolin (0.3 μmol l^{-1}) pre-treated left ventricular trabeculae, nor by measuring adenylate cyclase activity in forskolin (0.3 μmol l^{-1})-stimulated assays (crude membranes). This also holds true for radioligand binding assays with or without guanine nucleotide guanyl-5′-yl imidodiphosphate (Gpp(NH)p).

5 Although all studied β-adrenoceptor antagonists lack intrinsic sympathomimetic activity (ISA), they differ in the β_1-selectivity as well as in their direct negative inotropic action. These differences as well as the mode of extracardiac action may have an impact on outcome of patients treated with β-adrenoceptor antagonists.

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Keywords: Nebivolol; bucindolol; carvedilol; metoprolol; bisoprolol; heart failure; human myocardium; intrinsic sympathomimetic activity; β_1-selectivity

Abbreviations: βAA, β-adrenoceptor antagonists; BIS, bisoprolol; BUC, bucindolol; CAR, carvedilol; Gpp(NH)p, guanine nucleotide guanyl-5′yl-imidodiphosphate; ISA, intrinsic sympathomimetic activity; MET, metoprolol; NEB, nebivolol

Introduction

It has been shown by clinical trials that β-adrenoceptor antagonists (βAAs) improve survival of patients suffering from heart failure (MERIT-HF Study Group, 1999; CIBIS II Investigators and Committees, 1999; Packer *et al*., 1996). Whether the beneficial effects hold true for all βAAs in an ongoing matter of debate, since the βAA bucindolol improved symptoms but did not improve outcome of heart failure patients (BEST Steering Committee, 1995; Bristow, 2000). In addition, it is unclear, whether or not differences in the direct cardiac action have an impact on prognostic benefit of βAAs or not. On one hand, this has been due to their negative inotropic and bronchiconstructive effects *via* inhibition of β_2-adrenoceptors. On the other hand, it has been shown previously that βAAs with intrinsic sympathomimetic activity (ISA, e.g. xamoterol (Schwinger *et al*., 1990b)) are contraindicated in human heart failure, because of the detrimental increase in heart rate (The Xamoterol in Severe Heart Failure Study Group, 1990). In contrast, βAAs with high inverse agonistic efficacy may cause receptor up-regulation (Milligan *et al*., 1995), and may therefore contribute to the restoration of the blunted β-adrenoceptor adenylate cyclase system in human heart failure. This holds true for metoprolol, whereas carvedilol does not lead to receptor up-regulation (Gilbert *et al*., 1996). Thus, when treating patients with already compromised left ventricular function, it may be wise to use a βAA with minor cardiodepressant effects, high β_1-selectivity and no ISA.

The novel βAA nebivolol increases survival in cardiomyopathic hamsters with congestive heart failure (Ver Donck *et al*., 1991). Due to the altered β-adrenoceptor-adenylate cyclase coupling in failing human myocardium (e.g. downregulation of the β-adrenoceptors (Bristow *et al*.,

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1982; 1986; Schwinger *et al*., 1990a; 1991); upregulation of G_i (Feldman *et al*., 1988); up-regulation of the β-adrenoceptor kinase (Ungerer *et al*., 1993), the inotropic responsiveness of βAAs in failing myocardium might be different from that in animal models. Therefore, the present study investigates the direct cardiac effects on force of contraction, β_1-selectivity and ISA of the βAA nebivolol in comparison to metoprolol and carvedilol. The non-selective βAA bucindolol (Hershberger *et al*., 1990) and the β_1-selective βAA bisoprolol were also studied. The chemical structures of the different β-adrenoceptor antagonists are shown in Figure 1.

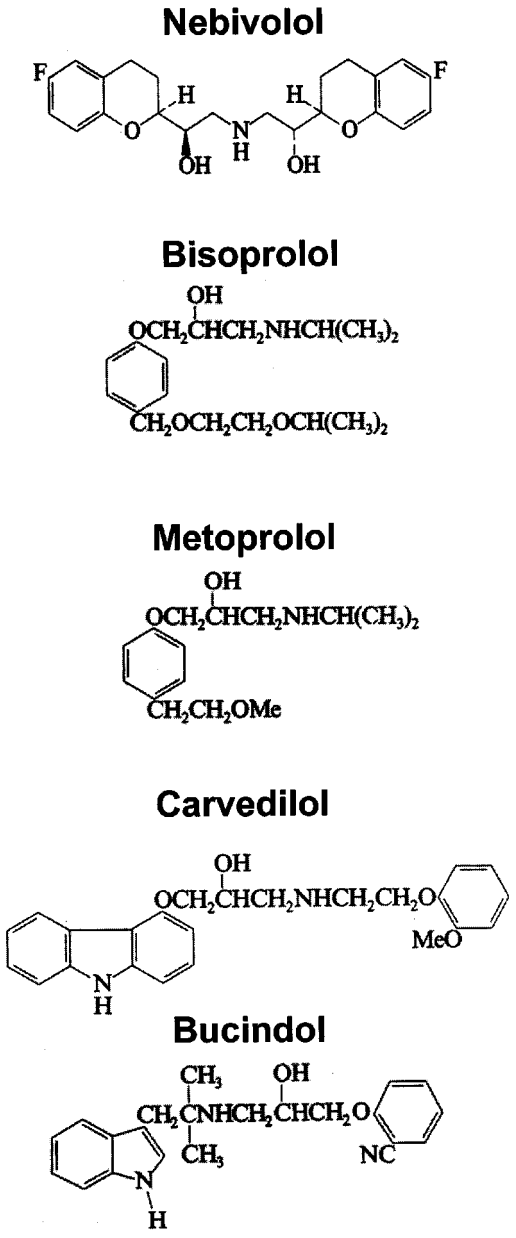

Figure 1 Chemical structures of the different β-adrenoceptor antagonists.

Methods

Myocardial tissue

Human left ventricular trabeculae were obtained from seven non-failing donor hearts (mean age: 54±4 years, one woman, six men) that could not be transplanted for technical reasons and from five hearts with end-stage heart failure due to dilated cardiomyopathy at the time of heart transplantation (mean age: 52±6 years, one woman, four men). None of the donors had a history of heart disease and all had normal left ventricular function as measured by the attending cardiologist. Mean ejection fraction of the heart failure group was 27.3±3.3%, mean cardiac index 2.1±0.1 l min m^{-2}, mean left ventricular end-diastolic pressure 16.2± 2.4 mmHg. Medication consisted of nitrates, diuretics, angiotensin-converting enzyme inhibitors, and digoxin. None of the patients have received Ca^{2+} channel antagonists or Ca^{2+} channel agonists within 7 days of surgery, or β-adrenoceptor agonists 48 h before surgery. Drugs used for general anaesthesia were flunitrazepam, fentanyl, and pancuronium bromide with isoflurane. The investi- gation conforms with the principles outlined in the Declaration of Helsinki and was approved by the local ethics committee.

Contraction experiments

The experiments were performed on isolated, electrically driven, isometrically contracting muscle preparations. The experiments were performed as previously described (Schwinger *et al*., 1990b).

β_1- and β_2-adrenoceptor binding studies

Membrane preparation For the investigation of the β_1-adrenoceptor selectivity, human non-failing left ventricular myocardium was used. Due to the described β_1-adrenoceptor downregulation in human failing myocardium (Bristow *et al*., 1982; 1986; Schwinger *et al*., 1990a; 1991), failing left ventricular cardiac tissue may not be suitable for the determination of β_1-adrenoceptor selectivity. The tissue of the left ventricle from human non-failing mycardium were chilled in 15 ml ice-cold homogenization buffer (in mmol l^{-1}: Tris/HCl 40, EDTA 1, dithiotreitol 1, pH 8.0). Connective tissue was trimmed away and myocardial tissue was minced with scissors and homogenized with a motor-driven glass-teflon potter for 1 min. Afterwards, the crude membrane preparation was homogenized by hand for 1 min with a glass-glass potter. The homogenate was centrifuged at 480 *g* for 15 min. The supernatant was diluted with an equal volume of ice-cold 1 M KCl, stored on ice for 10 min, and then centrifuged at 100,000×*g* for 45 min. The pellet was re-suspended in incubation buffer (in mmol l^{-1}: Tris-HCl 50, $MgCl_2$ 10, pH 7.4), and homogenized for 1 min with a glass-glass potter. This suspension was centrifuged at 100,000×*g* for 45 min. The pellet was finally re-suspended in incubation buffer and stored at −80°C.

Radioligand binding assay β-adrenoceptors in cardiac tissue homogenates were investigated using ^{3}H-CGP

12.177 [(−)-4-(3-t-butylamino-2-hydroxy-propoxy)-(5,7-^{3}H) benzimidazol-2-one)] as the radiolabelled ligand (specific activity 50 Ci mmol l^{-1}). Specific binding was determined as the difference of binding in the absence and presence of 10 μmol l^{-1} DL-propranolol β-adrenoceptor subtypes were determined by competition experiments using the β_1-selective antagonist CGP 207.12 A (0.3 μmol l^{-1}) and the β_2-selective antagonist ICI 118.551 (0.05 μ mol l^{-1}). β_2 and β_1 ratio was calculated as described previously (De Lean *et al*., 1982). Experiments were performed as described previously (Schwinger *et al*., 1991).

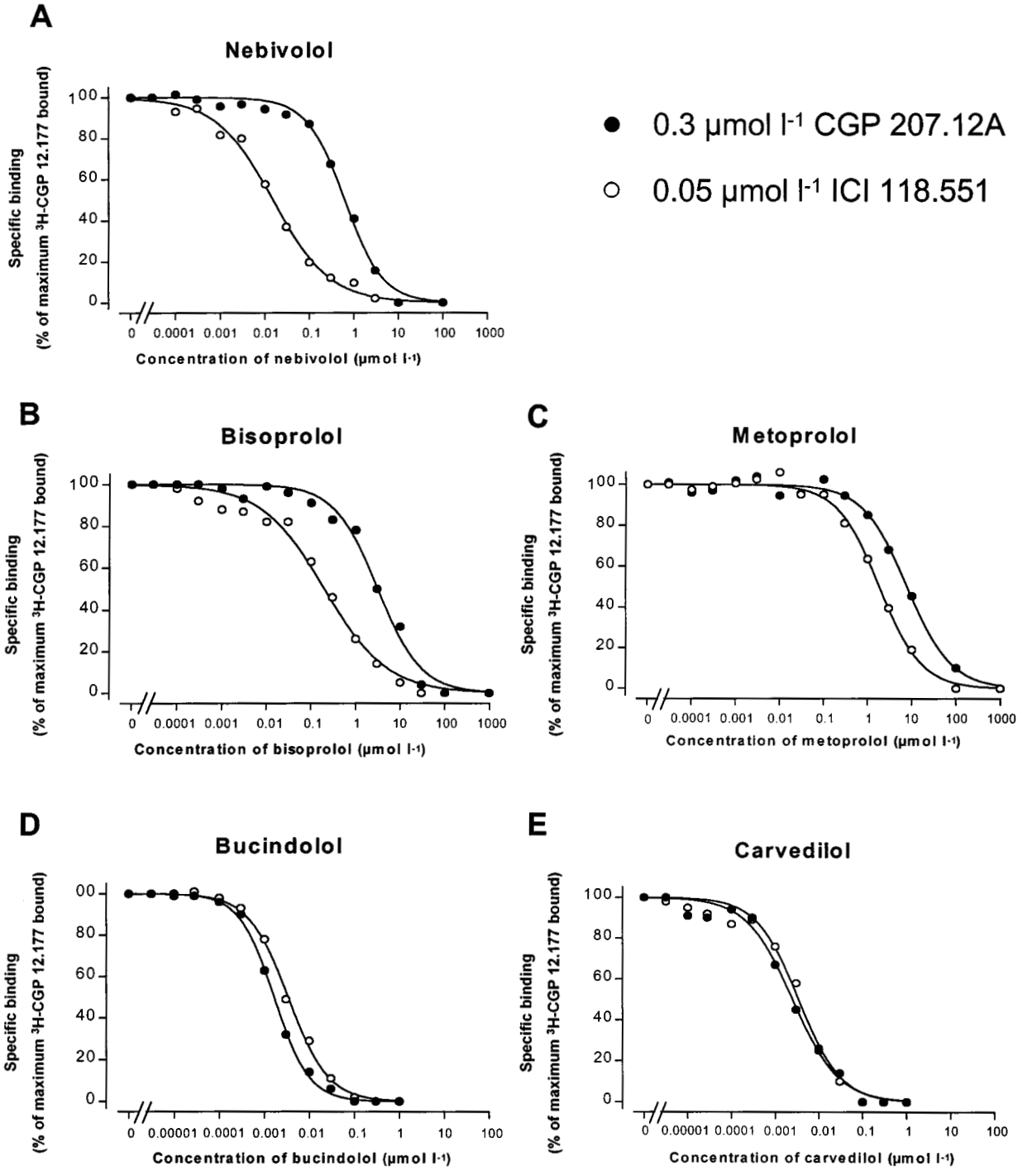

Figure 2 β-adrenoceptor selectivity. Experiments with nebivolol (A), bisoprolol (B), metoprolol (C), bucindolol (D) and carvedilol (E) were performed in the presence of 0.3 μmol l^{-1} CGP 207.12A or 0.05 μmol l^{-1} ICI 118.551 in order to obtain competition at a homogeneous population of β_2- or β_1-adrenoceptors on left ventricular myocardial membranes obtained from human myocardium.

Adenylate cyclase activity

Particulate membrane fractions from human non-failing hearts were obtained as described in the preparation for cardiac β_1- and β_2-receptors except that after the first centrifugation step at $100{,}000 \times g$, the pellet was resuspended in a hypotonic medium (in mmol l^{-1}: ATP 2.5, $MgCl_2$ 2.5, $KHCO_3$ 1, and Tris/HCl 2, pH 7.4) and that it was finally resuspended in $KHCO_3$ (1 mmol l^{-1}). Adenylate cyclase activity was determined as described previously (Schmidt *et al*., 1995).

Guanine-nucleotide modulated binding

Particulate membrane fractions from human non-failing hearts were obtained as described in the preparation for cardiac β_1- and β_2-adrenoceptors. A competition curve of the βAAs was performed as described above (radioligand binding assay) in the presence and in the absence of a high concentration of the non-hydrolysable guanine nucleotide guanyl-5′-yl imidodiphosphate (Gpp(NH)p, 30 μmol l^{-1}). Experiments were performed as described previously (Schmidt *et al*., 1995; Bristow *et al*., 1992).

Materials

Nebivolol was generously provided by Berlin-Chemie AG, Berlin, Germany; metoprolol by Astra GmbH, Wedel, Germany; carvedilol by Boehringer Mannheim, Mannheim, Germany; bisoprolol by Merck, Darmstadt, Germany. Bucindolol was kindly given by Knoll AG, Mannheim, Germany and CGP 20712 by Novartis, Basel, Switzerland. ^{3}H-CGP 12.177 was obtained from Amersham, Braunschweig, Germany, and ICI 118.551 from Tocris, Bristol, U.K.

Statistics

All values are mean ± s.e.mean. Statistical significance was analysed with the student's *t*-test for unpaired or paired observations as well as by using analysis of variance followed by a protected *t*-test (Bonferroni) or paired *t*-test.

Results

β_1-selectivity

To study the β_1-selectivity of nebivolol, carvedilol and metoprolol, competition experiments to ^{3}H-CGP 12.1777 binding were performed in the presence of ICI 118.551 (50 nmol l^{-1}) in order to obtain a homogeneous population of β_1-adrenoceptors, as well as in the presence of CGP 207.12 A (300 nmol l^{-1}) to determine competition of β_2-adrenoceptors. The non-selective βAA bucindolol and the β_1-selective βAA bisoprolol were studied for comparison.

Figure 2 shows competition curves obtained with nebivolol (upper part, A), bisoprolol (middle part, left side, B), metoprolol (middle part, right side, C), bucindolol (lower part, left side, D) and carvedilol (lower part, right side, E) in crude membrane preparations of human non-failing left ventricular myocardium. Figure 3 summarizes the results. Nebivolol exerts the highest β_1-selectivity in human myocardium, displaying a 40.7-fold selectivity ratio (Table 1). Metoprolol and bisoprolol also displayed β_1-selectivity but less than nebivolol. Bucindolol and carvedilol were not selective for β_1-adrenoceptors (Table 1).

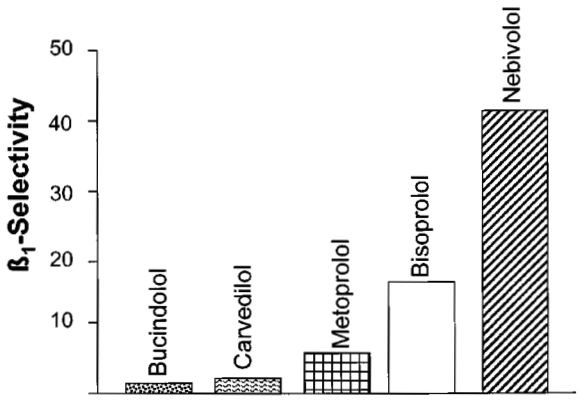

Figure 3 Rank order of β_1-adrenoceptor selectivity of nebivolol, bisoprolol, metoprolol, bucindolol and carvedilol.

Inhibition of β-agonist-stimulated muscle contraction

Figure 4 shows original tracings of the force of contraction under basal conditions, after application of isoprenaline (1 μmol l^{-1}), and after application of a low (0.3 μmol l^{-1}) and high (10 μmol l^{-1}) concentration of the βAAs nebivolol (upper panel), carvedilol (middle panel) and metoprolol (lower panel). Figure 5 gives the percentage changes of isoprenaline pre-stimulated force of contraction obtained in human left ventricular failing myocardium after application of the different βAAs. Basal as well as isoprenaline (1 μmol l^{-1})-stimulated force of contraction measured at a stimulation frequency of 1 Hz and an extracellular Ca^{2+}-concentration of 1.8 mM was similar in all groups (Table 2). The rank order of negative inotropic efficacy as judged from the negative inotropic effect on isoprenaline pre-stimulated force of contraction measured at 30 μM of the respective β-blockers was: metoprolol > carvedilol ⩾ nebivolol (Table 2). By analysing the concentration necessary to induce a 50% decrease on isoprenaline pre-stimulated force of contraction (IC_{50}) the following rank of negative inotropic potency order was obtained: metoprolol ⩾ carvedilol > nebivolol (Table 2).

Intrinsic sympathomimetic activity

To functionally study the ISA of metoprolol, carvedilol, bucindolol, and nebivolol in human myocardium, cumulative concentration-response curves were obtained measuring force of contraction in isolated trabeculae of human non-failing hearts in the presence of forskolin (Figure 6). Forskolin has been shown to be a tool for the detection of ISA mediated by βAAs (Jasper *et al*., 1988). In isolated left ventricular trabeculae of human non-failing left ventricular myocardium, application of forskolin (0.3 μmol l^{-1}) induced similar positive inotropic effects in all groups studied (nebivolol-

Table 1 Binding constants for displacement of CGP 207.12 A (300 $nmol^{-1}$, $K_i(\beta_2)$) and of ICI 118.551 (50 $nmol^{-1}$, $K_i(\beta_1)$), as well as the β_1-adrenoceptor selectivity

	n	+ *CGP 207.12 A* (300 nmol l^{-1}) (nmol l^{-1})	+ *ICI 118.551* (50 nmol l^{-1}) (nmol l^{-1})	$K_i(\beta_2)/K_i(\beta_1)$
Bucindolol	6	0.83 (0.76–0.91)	1.69 (1.51–1.91)	0.49
Carvedilol	6	1.25 (0.95–1.65)	1.72 (1.30–2.27)	0.73
Metroprolol	6	3856.5 (3121–4765)	912.5 (767.5–1084)	4.23
Bisoprolol	6	1557.0 (1163–2086)	99.6 (71.5–138.6)	15.6
Nebivolol	9	309.5 (259.0–370.0)	7.6 (6.2–9.3)	40.7

The β_1-adrenoceptor selectivity was quantified by calculating the ratio of $K_i(\beta_2)$ over $K_i(\beta_1)$.

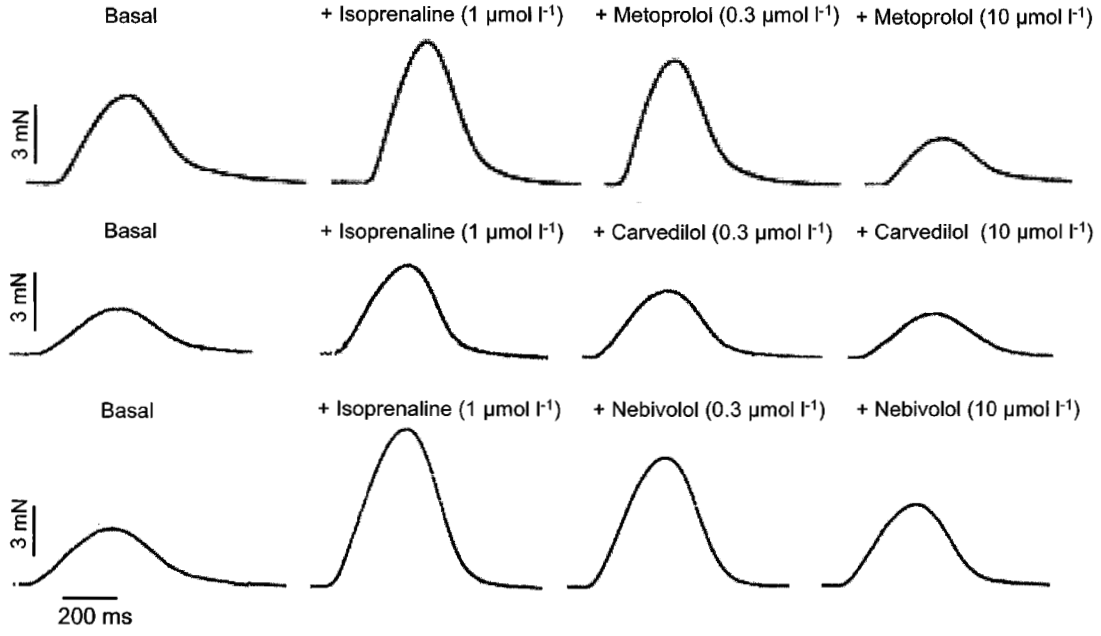

Figure 4 Original tracings illustrating the effect of a low (0.3 $\mu mol\ l^{-1}$) and high (10 $\mu mol\ l^{-1}$) concentration of metoprolol, carvedilol and nebivolol in isoprenaline (1 $\mu mol\ l^{-1}$) pre-stimulated isolated, electrically driven (1 Hz) trabeculae of human failing myocardium.

group: $+38\pm4\%$, $n=5$; metoprolol-group: $+40\pm5\%$, $n=5$; carvedilol-group: $+35\pm6\%$, $n=4$; bucindolol-group: $+39\pm8\%$, $n=5$). Neither metoprolol, nor carvedilol, bucindolol or nebivolol increased force of contraction after forskolin pretreatment, indicating that none of the βAAs studied possessed ISA.

The ISA of nebivolol in comparison to bucindolol was also studied in crude membrane preparations of human non-failing left ventricular myocardium by measuring the adenylate cyclase activity after pre-stimulation with forskolin (0.3 $\mu mol\ l^{-1}$). Bucindolol (10^{-10}–10^{-5} $mol\ l^{-1}$) as well as nebivolol (10^{-9}–10^{-4} $mol\ l^{-1}$) did not change adenylate cyclase activity under these conditions (Figure 7).

To further investigate whether the βAAs were capable of binding to the agonist binding site and inducing ISA, radioligand binding experiments with the β_1-selective agonist ^{3}H-CGP 12.177 (0.6 $nmol\ l^{-1}$) were performed in the presence as well as in the absence of Gpp(NH)p (30 $\mu mol\ l^{-1}$) in crude membrane preparations of human non-failing myocardium. Gpp(NH)p is able to induce alterations in the β-adrenoceptors when the receptors are coupled to the adenylate cyclase by virtue of agonist binding resulting in a rightward shift of the binding displacement curve. Figure 8 presents the results obtained for the guanine nucleotide modulated binding studies for nebivolol (Figure 8B), carvedilol (Figure 8C), and bucindolol (Figure 8D). The β_1-adrenoceptor agonist isoprenaline was studied for compar-

ison (Figure 8A). Only the binding displacement curve of isoprenaline was shifted to the right in the presence of Gpp(NH)p (Figure 8A). Thus, all experiments investigating whether nebivolol, metoprolol, carvedilol, and bucindolol possess β-adrenoceptor agonistic moiety demonstrate that these compounds are devoid of intrinsic sympathomimetic properties.

Discussion

Some, but not all βAAs improve symptoms and prolong survival of heart failure patients (MERIT-HF Study Group, 1999; CIBIS II Investigators and Committees, 1999; Packer *et al*., 1996). Initiation of βAA therapy is often limited by worsening congestive heart failure, which may manifest as a decrease in haemodynamics (Kukin *et al*., 1999) or by β_2-mediated bronchoconstriction. Therefore, low doses of the βAA are a necessity, especially in the beginning of a β-adrenoceptor blocker treatment. Thus, it is important to know whether βAAs differ in their inotropic action. The present study investigated the direct cardiac effects of the βAAs nebivolol, metoprolol, carvedilol, bucindolol and bisoprolol in human myocardium.

Nebivolol was the βAA with the highest β_1-selectivity in human myocardium as compared with bisoprolol and metoprolol. A high β_1-selectivity may be of special benefit for the βAA-treatment of heart failure patients due to a reduction of undesired side-effects (e.g. bronchoconstriction). More importantly, recent studies have shown that blockade with β_1-adrenoceptor-selective antagonists improves survival in patients with congestive heart failure (MERIT-HF Study Group, 1999; CIBIS II Investigators and Committees, 1999). It is not yet clear whether this also holds true for non-selective βAAs. As shown in the BEST trial, bucindolol produced a non-significant reduction of 10% in total mortality (BEST Steering Committee, 1995; Bristow, 2000).

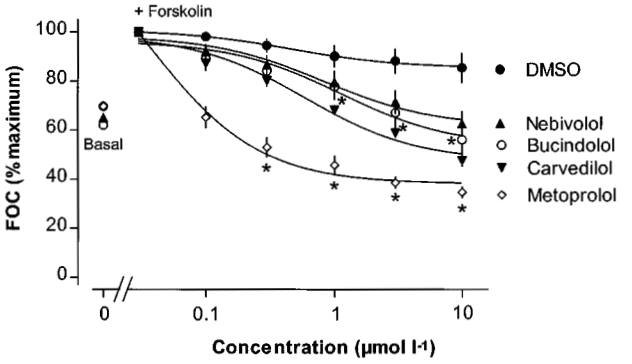

Figure 6 Concentration-response curves for the cardiac effects of the β-adrenoceptor antagonists metoprolol, carvedilol and nebivolol in human left ventricular non-failing myocardium after pre-stimulation with forskolin.

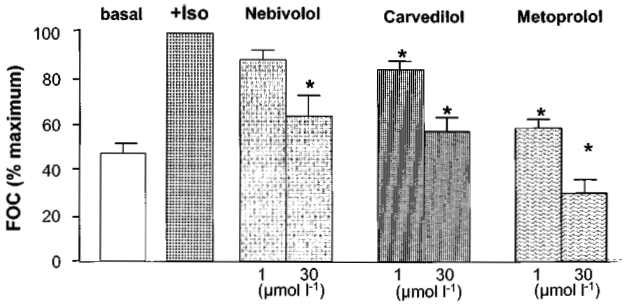

Figure 5 Percentage changes of isoprenaline pre-stimulated force of contraction obtained in human left ventricular failing myocardium after application of metoprolol, carvedilol and nebivolol.

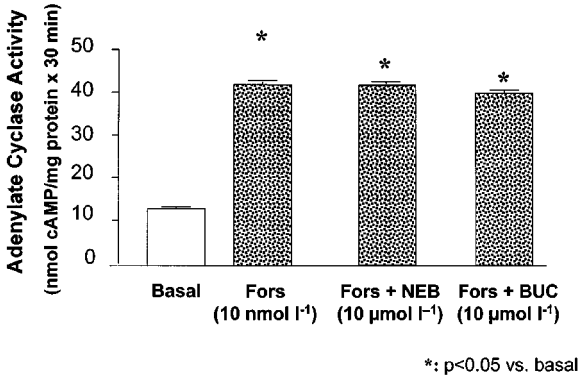

Figure 7 Influence of bucindolol and nebivolol on adenylate cyclase activity in crude membrane preparations of human left ventricular non-failing myocardium.

Table 2 Negative inotropic potency of metroprolol, carvedilol, and nebivolol in human left ventricular failing myocardium

	Basal FOC (mN mm^2)	+*Isoprenaline* (1 µmol l^{-1}) (mN mm^2)	(% basal FOC)	*Max. NIE* (30 µmol l^{-1}) (mN mm^2)	(% ISO FOC)	IC_{50} (µM)
Metoprolol ($n=5$)	9.0±2.1	18.1±4.7	+100±30	4.6±0.5	−70±5	0.60±0.18
Carvedilol ($n=4$)	9.3±0.7	17.3±2.1	+86±16	8.7±1.1	−48±7	4.14±1.90
Nebivolol ($n=6$)	11.6±3.2	23.1±5.2	+134±27	14.8±4.2	−34±10	7.02±3.33

FOC: force of contraction, Max. NIE: maximal negative inotropic effect, % basal FOC: percentage of basal force, IC_{50}: concentration necessary to achieve a 50% decrease in FOC after isoprenaline-prestimulation, % ISO FOC: percentage of isoprenaline-stimulated force.

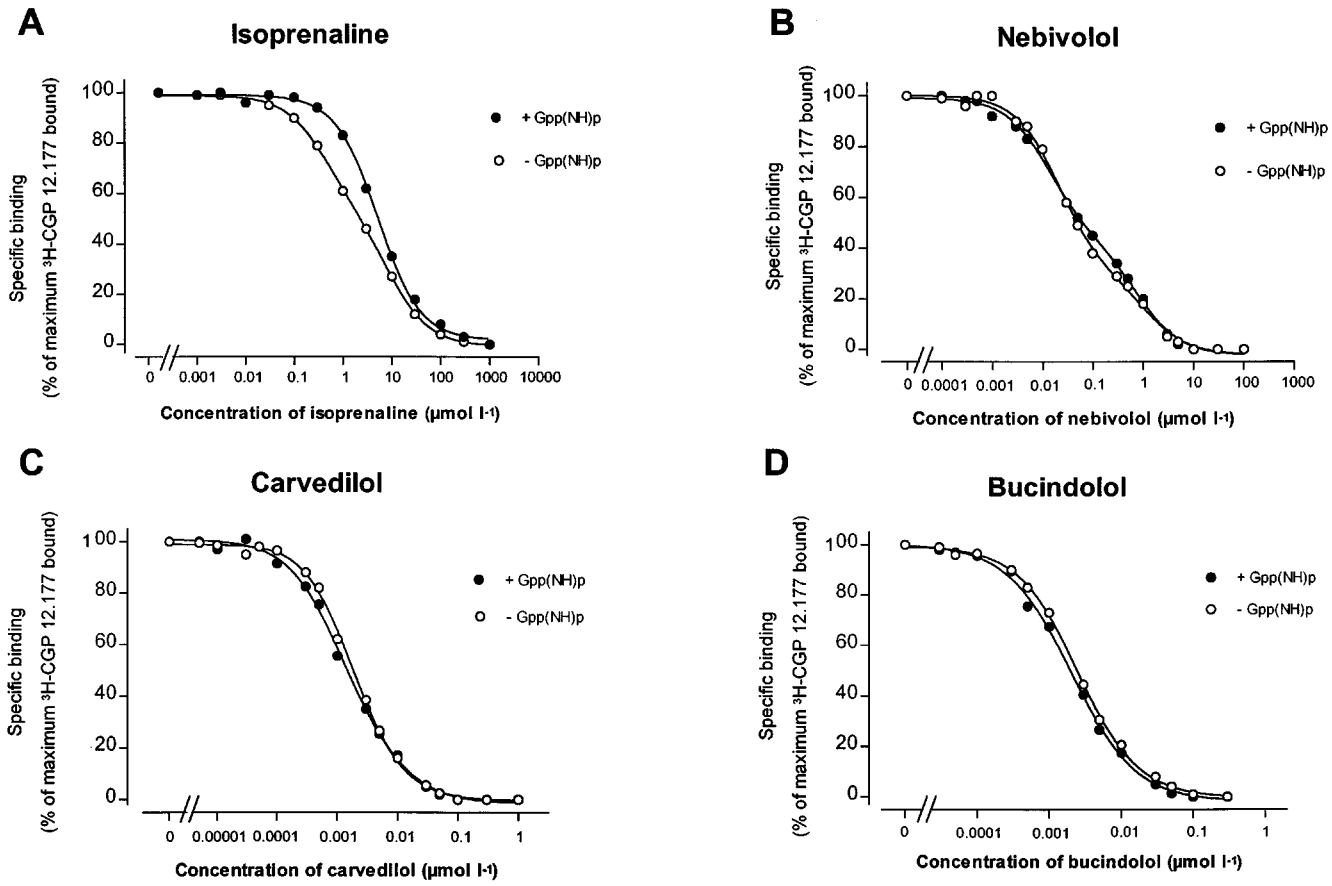

Figure 8 Effect of Gpp(NH)p on ^{3}H-CGP 12.177 binding experiments in the presence of isoprenaline, nebivolol, carvedilol and bucindolol in human non-failing left ventricular myocardium.

The question whether non-selectivity of carvedilol is critical to its benefit in chronic heart failure patients is currently being addressed in the Carvedilol and Metoprolol European Trial (COMET), comparing metoprolol and carvedilol. The influence of nebivolol-treatment on heart failure is currently being investigated in the SENIOR trial.

β_1-selectivity did not correlate with the negative inotropic potency or efficacy of the studied βAAs. The negative inotropic efficacy and potency of metoprolol, for example, is much more pronounced than that of carvedilol, although metoprolol is a β_1-selective, and carvedilol a non-selective βAA. Therefore, especially for the βAAs of the second and third generation, the direct cardiac action of these drugs may also be critically dependent on the additional mode of action provoked by these βAAs. Application of metoprolol, for example, has been shown to block the calcium current in ventricular myocytes of guinea-pigs (Sanchez-Chapula, 1992). Accordingly, initial application of metoprolol given in patients receiving background triple therapy for mild to severe heart failure produced significant decreases in cardiac output, cardiac index and stroke volume (Michel *et al.*, 1988). The minor cardio-depressant effects of nebivolol are in agreement with the favourable effects of nebivolol on left ventricular function seen in patients with dilated heart disease (Wisenbaugh *et al.*, 1993) and may be of importance for the therapeutical benefit in heart failure patients.

It has been shown recently that βAAs with ISA, like xamoterol, are contraindicated in human heart failure, because of the detrimental increase in heart rate (The Xamoterol in Severe Heart Failure Study Group, 1990). Previous studies showed that patients treated with β_1-selective antagonists without ISA had more adrenoceptors than control subjects (Motomura *et al.*, 1990) and that the adenylate cyclase activation by the β-adrenoceptor agonist isoprenaline is also enhanced in this situation (Trochu *et al.*, 1999). The ISA is therefore an important criterion for the therapeutical usefulness of βAAs in heart failure patients. In the present study no ISA was found for bucindolol and carvedilol in crude membrane preparations of human ventricular myocardium, which is in agreement with previous studies in rat as well as in human myocardium (Hershberger *et al.*, 1990; Bristow *et al.*, 1992). In human atrial myocardium, however, small amounts of partial agonism of bucindolol could be detected after the muscle had been depleted of catecholamines (Trochu *et al.*, 1999). These variable results may be due to the use of different experimental setups, with differences in the basal activation states of the β-adrenoceptors in the respective tissues (Schwinger *et al.*, 1990b). Nebivolol also did not reveal ISA

in the present study, which is in line with experiments in reserpinized dogs and spontaneously hypertensive rats. Thus, besides its high β_1-adrenoceptor selectivity and its small negative inotropy, the lack of ISA may be of special benefit for the therapeutical use of nebivolol in human heart failure.

Limitation of the study

The present study was performed under *in vitro* conditions, experiments were either performed on isolated trabeculae or on crude membrane preparations of human heart. It cannot be excluded that in the *in vivo* effects of the studied βAAs may differ from those observed *in vitro*. Due to the altered β-adrenoceptor-adenylate cyclase coupling, this study of the cardiac effects of βAAs may be of advantage compared to those performed in various animal models.

Conclusions

The cardiac effects of βAAs are varying in human cardiac tissue. The cardiodepressant effects of a βAA may not correlate with its β_1-selectivity but result from the combined effects of β_1-selectivity, intrinsic sympathomimetic properties and inverse agonism. The pharmacodynamical profile of nebivolol in human myocardium (high β_1-selectivity, lack of ISA and inverse agonistic activity) may be favourable for the treatment of heart failure patients. In consequence, whether or not nebivolol improves symptoms and outcome in patients with heart failure will be studied in a placebo controlled trial (SENIORS Study).

We are indebted to all colleagues of the Departments of Cardiothoracic Surgery of the Universities of Cologne and Munich for providing us with human myocardial samples. This work contains data from the doctoral thesis of A. Bundkirchen. Experimental work was supported by the Deutsche Forschungsgemeinschaft (DFG, Dr Schwinger) and the Leslee Fortune Project (Drs Schwinger and Brixius).

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