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Differential effects of carvedilol and metoprolol on isoprenaline-induced changes in β-adrenoceptor density and systolic function in rat cardiac myocytes

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Abstract

Objective: β-Blockers improve cardiac function and survival in heart failure patients. The underlying mechanisms are not completely elucidated. Differences between agents might be important for the development of more specific therapeutical approaches. This study investigated whether metoprolol or carvedilol alter β-adrenergic signaling differently. **Methods:** β-Adrenoceptor density and systolic function were determined in rat adult ventricular cardiac myocytes. **Results:** 12 h isoprenaline-treatment (Iso, 1 μmol/l) reduced β-adrenoceptor density by 33% ($P<0.01$). The effect was abolished by incubation with isoprenaline plus metoprolol (3 μmol/l), but was more pronounced after coincubation with carvedilol (0.003 μmol/l, $P<0.05$ Carv vs. Iso). Metoprolol alone had no effect on β-adrenoceptor density, but carvedilol induced a decrease in receptor density even in absence of isoprenaline ($P<0.05$ Carv vs. ctr.). The isoprenaline (0.0003–10 μmol/l) induced concentration-dependent increase in myocyte shortening was blunted after 12 h preincubation with Iso (1 μmol/l, $P<0.001$). This reduction was abolished or partly prevented by coincubation with metoprolol or carvedilol, respectively. Carvedilol decreased the number of receptors which had to be occupied by isoprenaline in order to obtain 50% and 90% increase in myocyte cell shortening. Comparison of guanine nucleotide-dependent binding characteristics of isoprenaline, carvedilol and metoprolol revealed β-receptor agonist like binding characteristics for carvedilol, but antagonist like binding characteristics for metoprolol. **Conclusion:** Metoprolol but not carvedilol prevents isoprenaline-induced downregulation of myocyte β-adrenoceptors. The difference might be due to specific binding properties of the β-blockers. Restoration of isoprenaline responsiveness by carvedilol might be due to improved coupling of β-receptors to postreceptor effects.

Keywords: Adrenergic (ant)agonists; Heart failure; Myocytes; Receptors

1. Introduction

Neuroendocrine activation plays an important role for the pathophysiology of congestive heart failure. In patients with chronic heart failure, catecholamine release is enhanced. Plasma catecholamine levels are inversely correlated with prognosis [1,2]. Treatment of patients with β-blockers and thereby protection of the myocardium from excessive adrenergic stimulation [3,4] leads to an improvement of symptoms and prognosis [5–8]. Especially, improved ejection fraction and reduction in mortality have been demonstrated for the β_1-selective β-blockers metoprolol and bisoprolol as well as for the nonselective β-blocker carvedilol [6–8].

The mechanisms by which β-blockers exert their effects have remained unexplained. Effects of catecholamines on

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cardiac myocytes include induction of cardiac myocyte hypertrophy [9], enhanced necrosis [10] and apoptosis [11] and increased susceptibility of the heart to arrythmias [12]. Possible mechanisms contributing to the effects of β-blockers include cardiac protection from toxic catecholamine effects [3,4,13,14] including apoptosis [15], but also reduction in heart rate leading to lower myocardial energy expenditure [16], prolonged diastolic filling [17], increased myocardial blood flow [18] and protection from cardiac arrhythmias.

One consequence of excessive catecholamine stimulation is an increased β-adrenergic receptor kinase activity [19] leading to uncoupling of the decreased left ventricular myocardial β-adrenoceptor population in failing myocardium [20]. Together with an increase in inhibitory G-proteins [21,22], this results in a decreased inotropic responsiveness of the heart to catecholamines. It is unknown whether β-blockers improve β-adrenergic signal transduction. Treatment of heart failure patients with metoprolol led to increased inotropic responsiveness to dobutamine accompanied by an increased β-adrenoceptor density [23]. In contrast, improved left ventricular function in carvedilol treated patients was not accompanied by restoration of myocardial β-adrenoceptor density [24].

In this study, effects of carvedilol and metoprolol on β-adrenoceptor density and on contractility were examined in isoprenaline treated cardiac myocytes used as a model for the catecholamine desensitized myocardium. The principal question was how myocyte contractility could be restored after β-blocker treatment despite a missing restoration in β-adrenoceptor density.

2. Methods

2.1. Isolation and cultivation of rat adult ventricular cardiac myocytes

Ventricular myocytes were isolated from 12-week-old male Sprague–Dawley rats [25]**.** Animal handling conforms with the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23). Hearts were perfused retrogradely at 37°C with oxygenated Powell medium (in mmol/l: NaCl 110, KCl 2.5, KH_2PO_4 1.2, $MgSO_4$1.2, $NaHCO_3$ 25.0, glucose 11.1), followed by collagenase perfusion (0.07%, Type II, 243 U/mg, Worthington, NJ, USA, 5 ml/min) for 20 min and further incubation for 10 min. After nylon mesh (200 μm) filtration, cells were centrifuged at 25 *g* for 3 min, resuspended in Powell medium (200 μmol/l $CaCl_2$), centrifuged at 25 *g* for 2 min, resuspended in Powell medium (400 μmol/l $CaCl_2$). Albumin gradient centrifugation (15 *g*,1 min) led to an increase in the proportion of rod-shaped cells. Cells were suspended in medium M199 with Earle's salts (Gibco, Berlin, Germany) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), insulin (10 μg/ml), non essential amino acids (1:100, Gibco) and vitamins (1:50, Gibco), plated on laminin (1 $\mu g/cm^2$) coated dishes and maintained at 37°C in 95% O_2–5% CO_2. After 4 h, culture dishes were washed twice in order to remove non-adherent cells.

2.2. Contractility measurements

Cardiac myocytes were superfused at 0.6 ml/min in a 400-μl Perspex chamber with modified Tyrode's solution (in mmol/l: NaCl 119.8, KCl 5.4, $CaCl_2$ 1.0, $MgCl_2$ 1.05, $NaHCO_3$ 22.6, $NaHPO_4$ 0.42, glucose 5.0, ascorbic acid 0.28 and EDTA 0.05), oxygenated with 95% O_2–5% CO_2 at 32°C. Cells were electrically stimulated at 0.5 Hz with 5 ms pulses (Stim2, Scientific Instruments, Heidelberg, Germany). Contraction amplitudes were measured using a phase-contrast microscope (Diaphot 300, Nikon, Tokyo, Japan) connected to a one-dimensional camera (ZK4, Scientific Instruments). Signals were digitalized and analyzed by computer (Mucell, Scientific Instruments). Twitch amplitude was calculated as maximal shortening length subtracted from resting length and standardized by dividing by resting length [26].

2.3. Myocardial membrane preparation

Left ventricular myocardial tissue was chilled in ice-cold 20 mmol/l Tris–HCl, 1 mmol/l EDTA, pH 7.4 and mechanically disrupted (Ultraturrax, Jahnke and Kunkel, Staufen, Germany). The homogenate was spun at 480 *g* (Beckmann J 218) for 15 min. The supernatant was diluted with an equal volume of 1 mmol/l of KCl and centrifuged at 100 000 *g* for 30 min. The pellet was resuspended in 50 volumes of homogenization buffer and centrifuged at 100 000 *g* for 30 min. This pellet was suspended in 50 volumes of 50 mmol/l Tris–HCl, 10 mmol/l $MgCl_2$, pH 7.4.

2.4. β-Adrenoceptor binding studies

β-Adrenoceptor density was determined in left ventricular membranes and isolated cardiac myocytes using ^{125}I-iodocyanopindolol (^{125}ICYP, specific activity of 2000 Ci/mmol).

In membrane preparations, β-adrenoceptor density (B_{max}) and dissociation constant (K_d) was determined in ^{125}ICYP saturation curves with eight increasing concentrations of ^{125}ICYP between 3 and 200 pmol/l. Propranolol (3 μmol/l) was used for determination of nonspecific binding. Cold ligand binding affinity was measured by ligand-^{125}ICYP competition curves using 25 pmol/l of ^{125}ICYP to maintain radioligand concentrations at approximate K_d. The assay was performed in a volume of 250 μl.

The protein amount used was 20–30 μg. Incubation at 25°C for 120 min allowed complete equilibration of β-adrenoceptors with radioligand. Reaction was terminated by rapid vacuum filtration through Whatman GF/C filters (Whatman, Clifton, NJ, USA). Filters were washed three times with 6 ml of ice-cold incubation buffer.

Binding studies were performed in the absence and presence of guanidinoimidophosphate (Gpp(NH)p, 100 μmol/l), a non-hydrolyzable guanine nucleotide analog converting receptors in a high to those in a low affinity state.

In isolated adult ventricular cardiac myocytes, B_{max} was determined by cell incubation with 50 pmol/l ^{125}ICYP ($2\times K_D$) for 60 min at 37°C. For determination of non-specific binding 3 μmol/l propranolol were used. Incubation was stopped by vacuum evacuation of the incubation solution followed by two washes with incubation buffer. Cells were lysed with trichloroacetic acid (10%) and bound radioactivity was determined.

All experiments were performed in triplicate.

2.5. Statistical analysis

Regression analysis was performed by computer analysis (GraphPad Software, San Diego, CA, USA). For determination of B_{max} and K_d, linear regression was performed according to Scatchard [27]. Competition curve slope (pseudo-Hill factor, n_H), the concentration at which 50% of the effect were achieved (EC_{50}) 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 high affinity (K_H) or low affinity receptor state (K_L) were calculated according to Cheng and Prussow [28]. $K_{i'}$ values were calculated from EC_{50} values as 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.

Data shown are mean±S.E.M., EC_{50} values are given with their range. For statistical analysis, Student's *t*-test and one-way ANOVA were used. $P<0.05$ was considered significant.

2.6. Materials

Carvedilol (racemic mixture of *R*-(+)- and *S*-(−)-enantiomers) was from SmithKline Beecham (King of Prussia, PA, USA), metoprolol (+)-tartrate salt from Astra Zeneca (Wedel, Germany). Guanylimidodiphosphate (Gpp(NH)p) was from Boehringer Mannheim (Mannheim, Germany), ^{125}ICYP from Amersham-Buchler (Freiburg, Germany), cell culture media from Gibco (Karlsruhe, Germany), other chemicals from Sigma (Deisenhofen, Germany).

3. Results

3.1. β-Adrenoceptor binding characteristis of carvedilol and metoprolol

Binding characteristics of isoprenaline, carvedilol and metoprolol were investigated in left ventricular membrane preparations. Isoprenaline exerted biphasic binding in absence of Gpp(NH)p indicating binding to two distinct receptor states with high and low affinity (62.0±7.6 and 38.0±7.6% of all receptors, respectively) (Fig. 1). Addition of Gpp(NHp) led to a rightward shift of the now monophasic binding curve with a Hill coefficient of 1.0 (±0.2) and a dissociation constant (K_{Low}=376 (224–630) nmol/l) similar to the dissociation constant of the low affinity state of the receptor in absence of Gpp(NH)p (K_{Low}=1650 (418–6356) nmol/l). Thus, in presence of Gpp(NHp) isoprenaline binds to low affinity receptors only, whereas in its absence it binds to high and low affinity receptors.

Metoprolol did not reveal guanine nucleotide modifiable binding characteristics. Already in absence of Gpp(NH)p, the binding curve for metoprolol was monophasic and was not shifted after addition of Gpp(NH)p, indicating that metoprolol identifies low affinity binding sites only (K_D= 474 (262–857) nmol/l without Gpp(NH)p and K_D=392 (219–699) nmol/l) with Gpp(NH)p) (Fig. 1).

In absence of Gpp(NH)p, the binding curve of carvedilol was biphasic indicating binding to high and low affinity binding sites. In presence of Gpp(NH)p, the binding curve was shifted to the right and became monophasic with a Hill coefficient of approximately 1 (0.9±0.19) indicating binding to low affinity binding sites only (in absence of Gpp(NH)p: 60.1±12.5% receptors in high affinity state, 39.9±12.5% receptors in low affinity state, K_{High}=0.15 (0.05–0.46) nmol/l, K_{Low}=5.6 (0.7–47.1) nmol/l; in presence of Gpp(NH)p: 100% receptors in low affinity state, K_{Low}=1.0 (0.7–1.3) nmol/l).

3.2. Effect of catecholamines on cell size and viability

Mean cell length of cardiac myocytes after isolation was 125±6 μm. After 12 h incubation in isoprenaline (1 μmol/l) or in control medium mean cell length was 121±7 and 119±8 μm, respectively (n=10 per condition). The number of rod shaped cells was 77±5% after isoprenaline incubation and 79±4% after incubation in control medium. Creatine kinase activity in the cell culture medium after 12 h incubation time was 3.4±0.2 U/l for myocytes treated in control medium, 3.4±0.7 U/l for myocytes treated with isoprenaline, 3.4±0.5 U/l for myocytes treated with isoprenaline and carvedilol and

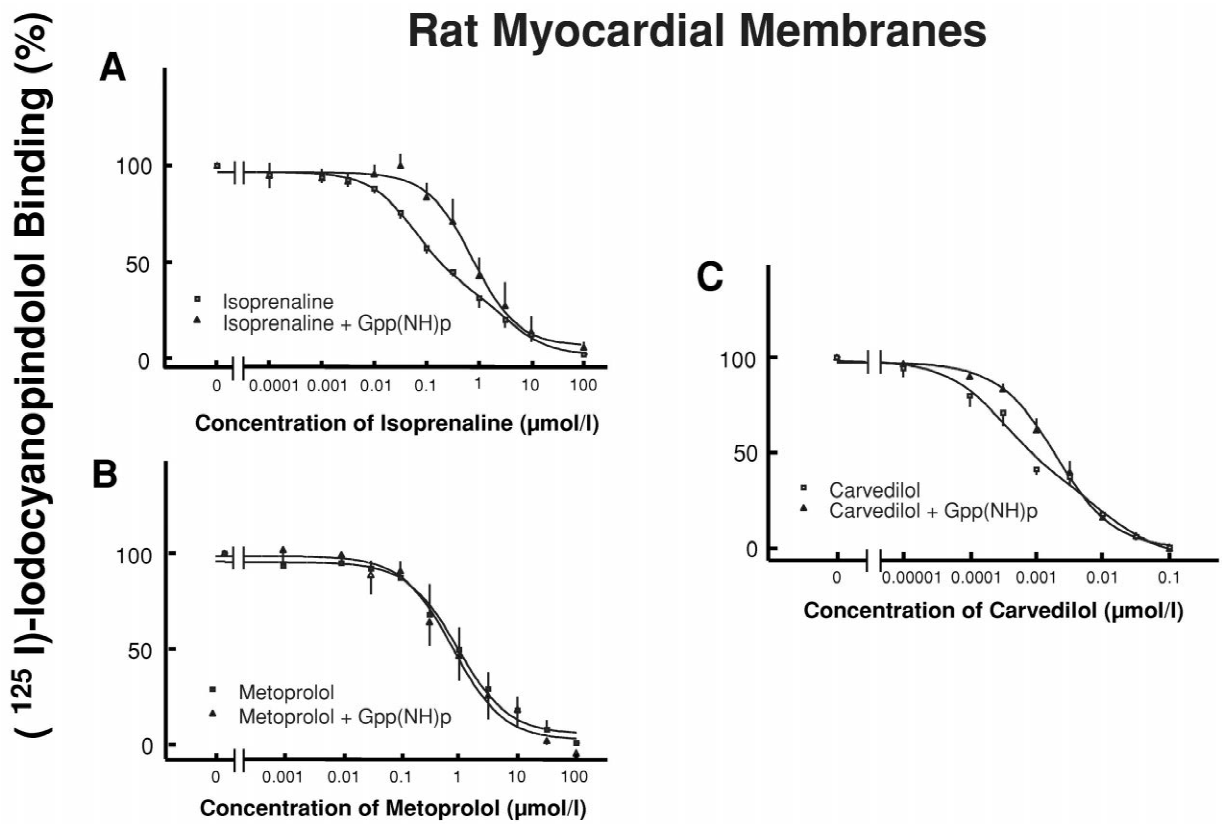

Fig. 1. Competition binding curves between ^{125}I-cyanopindolol (^{125}ICYP) and cold β-adrenoceptor ligands in isolated rat ventricular myocardial membrane preparations in absence or presence of Gpp(NH)p.

3.6±0.1 U/l for myocytes treated with isoprenaline and metoprolol ($n=5$ per condition).

3.3. Effects of catecholamine and β-blocker treatment on β-adrenoceptor density in cardiac myocytes

Incubation of cardiac myocytes with isoprenaline (0.001–10 μmol/l) for 6–48 h led to a time- and dose dependent downregulation in β-adrenoceptor binding sites by maximally 33±3% ($n=6$, $P<0.05$ vs. ctr.) after 12 h compared to cells which were incubated in control medium without catecholamines (Fig. 2, upper panel). In order to address the question whether β-blockers prevent β-adrenoceptor downregulation, myocytes were coincubated with carvedilol (0.003 μmol/l) or metoprolol (3 μmol/l). At these concentrations β-adrenoceptor occupancy by carvedilol or metoprolol were within a similar range (90%) as demonstrated by binding experiments. Coincubation of cardiac myocytes with metoprolol abolished the effects of isoprenaline on β-adrenoceptor density (Fig. 2). In contrast, coincubation with carvedilol led to a further reduction in receptor density by 54±7% ($n=6$ per condition, $P<0.05$ vs. isoprenaline treated myocytes).

A similar result was obtained when myocytes were incubated with isoprenaline 6 h prior to addition of the β-blockers for another 6 h. This approach might mimic the situation in chronic heart failure in vivo more closely in which medical treatment is initiated when β-adrenoceptor downregulation is already established. In this set of experiments, β-adrenoceptor density in control mycoyte preparations 16 h after cell isolation was higher than in previous experiments. This might indicate possible differences between β-adrenoceptor densities in individual myocyte preparations or differences between individual cell isolations concerning myocyte attachment to the laminin matrix (Fig. 2, lower panel).

In order to examine whether β-adrenoceptor blockers themselves exert effects on β-adrenoceptor density, isolated cardiac myocytes were treated with metoprolol (3 μmol/l) or carvedilol (0.003 μmol/l) in absence of isoprenaline. Under these conditions, metoprolol had no effect on β-adrenoceptor density, whereas carvedilol reduced β-adrenoceptor binding by 80±2%. Coincubation of cardiac myocytes with both β-blockers led to downregulation similar to the effect of carvedilol alone (−74±5% vs. ctr., Fig. 3).

3.4. Effects of β-blocker treatment on β-adrenoceptor density in isolated myocardial membranes

In order to determine whether the reduction of β-adrenergic binding sites in carvedilol treated cardiac myocytes might be due to irreversible binding of the β-blocker, myocardial membrane preparations were incubated with

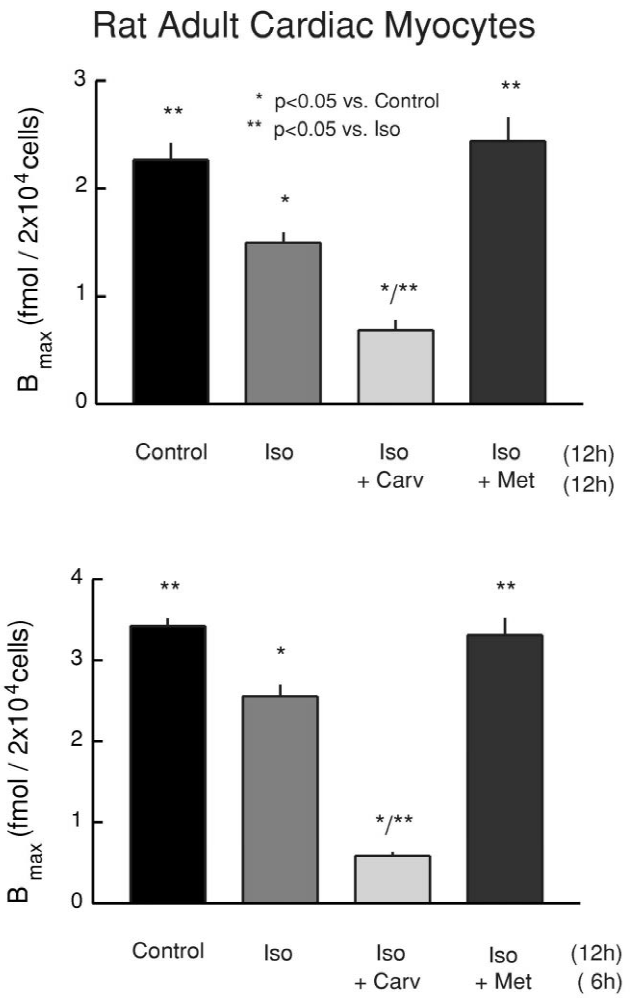

Fig. 2. β-Adrenoceptor density of isolated adult rat ventricular cardiac myocytes treated with isoprenaline (Iso), isoprenaline plus carvedilol (Carv) or isoprenaline plus metoprolol (Met) or incubated for the same time in medium 199 without catecholamine or β-blocker supplementation. Upper panel shows results for myocytes treated for 12 h with isoprenaline in presence of the respective β-blockers, lower panel demonstrates results for 12 h incubation with isoprenaline and addition of the respective β-blockers for the last 6 h of incubation only. Graph shows result of six independent experiments. Ordinate: B_{max} in fmol ^{125}I-cyanopindolol bound/2×10 [4] myocytes.

increasing concentrations of metoprolol or carvedilol for 1 h. Following incubation, membranes were vigorously washed before ^{125}ICYP binding was determined. Under these conditions, increasing concentrations of metoprolol (0.001–100 μmol/l) had no effect on the amount of ^{125}ICYP bound, whereas incubation with carvedilol (0.001–100 nmol/l) led to a concentration-dependent decrease in ^{125}ICYP binding by maximally 85±7% ($P<$ 0.001 for trend) (Fig. 4).

3.5. Effects of catecholamine or β-blocker treatment on cardiac myocyte contractility

In control myocytes which were incubated for 12 h in

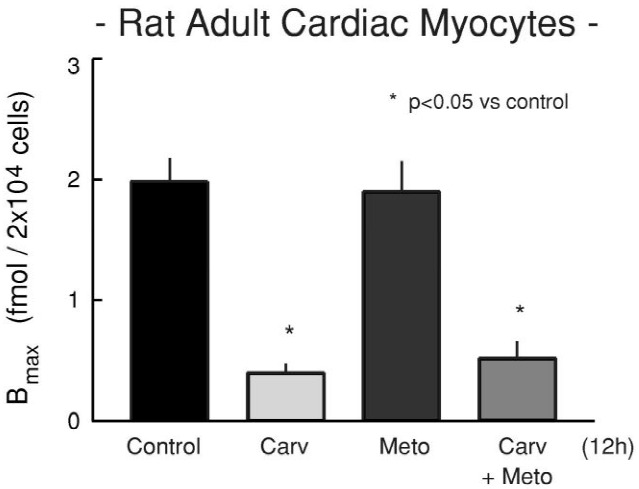

Fig. 3. β-Adrenoceptor density of isolated adult rat ventricular cardiac myocytes treated with carvedilol (Carv), metoprolol (Met) or carvedilol plus metoprolol for 12 h or of control incubated cardiac myocytes. Graph shows result of six independent experiments. Ordinate: B_{max} in fmol ^{125}I-cyanopindolol bound/2×10 [4] myocytes.

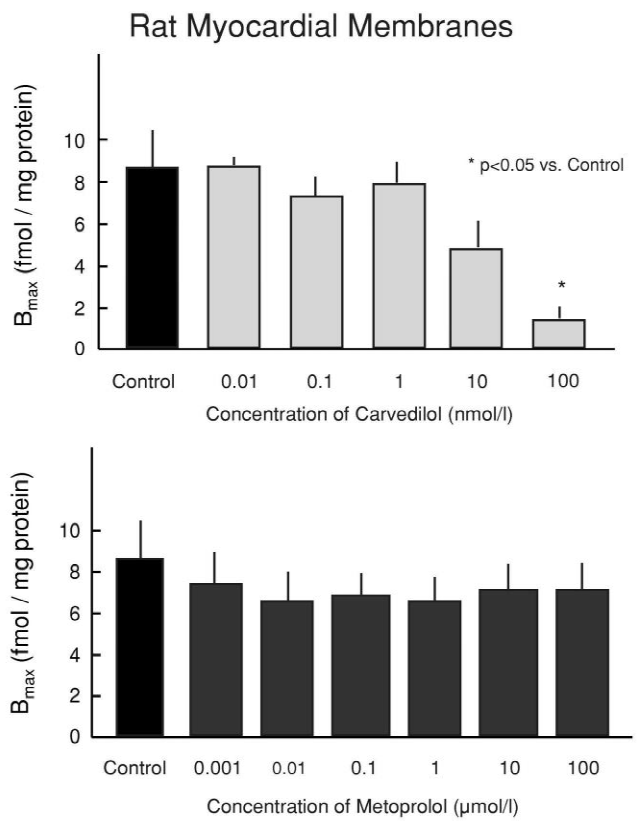

Fig. 4. Concentration-dependent effect of 1 h treatment of isolated left ventricular myocardial membrane preparations with carvedilol (upper panel) or metoprolol (lower panel) on β-adrenoceptor density. Graph shows result of three independent experiments. Ordinate: B_{max} in fmol ^{125}I-cyanopindolol bound/2×10 [4] myocytes.

medium 199 without catecholamine supplementation, acute addition of isoprenaline (0.0003–10 μmol/l) led to a concentration-dependent increase in systolic cell shortening by maximally 236±42% (Fig. 5, upper panel and Table 1). Treatment of cardiac myocytes for 12 h with isoprenaline (1 μmol/l) reduced the contractile response to isoprenaline to 53±18% of the maximum effect ($P<0.001$ isoprenaline treated vs. control cells). Catecholamine desensitization was completely prevented by coincubation of myocytes with metoprolol (3 μmol/l). Coincubation of cardiac myocytes with carvedilol (0.003 μmol/l) prevented the decrease in the maximum cell shortening response to isoprenaline and diminished the rightward shift of the isoprenaline concentration response curve, which occurred as a result of isoprenaline treatment (Fig. 5 and Table 1). Results were similar when myocytes were pretreated with isoprenaline for 6 h prior to the addition of β-adrenoceptor blockers for another 6 h (Fig. 6 and Table 1).

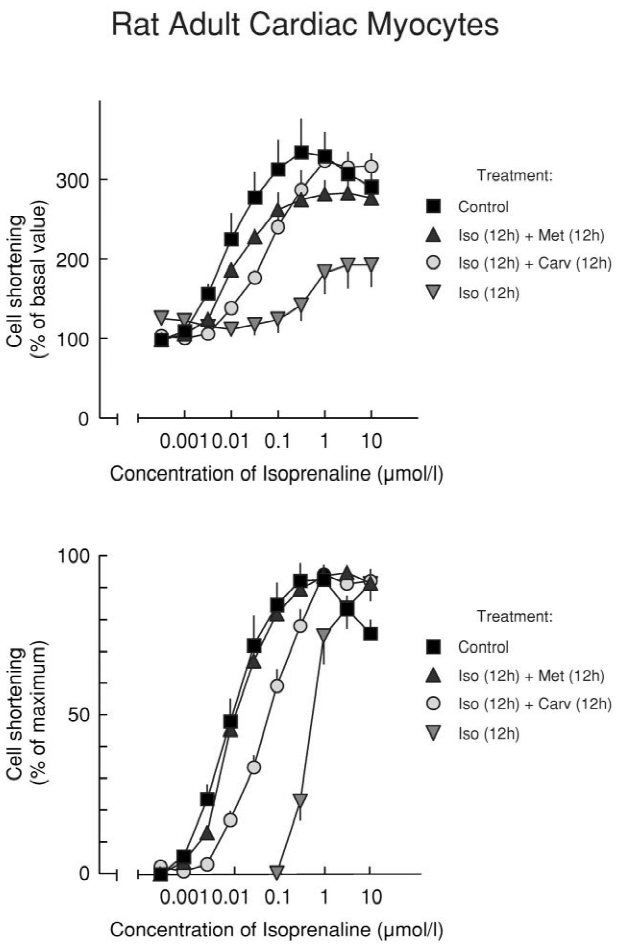

Fig. 5. Effect of isoprenaline on cell shortening of isolated cardiac myocytes incubated for 12 h with isoprenaline (Iso), isoprenaline plus carvedilol (Carv) or isoprenaline plus metoprolol (Met) or in medium 199 without catecholamine or β-blocker supplementation ($n=12$ per group). Cell shortening in percentage of basal value (upper panel) or in percentage of maximum effect (lower panel).

3.6. Modulation of β-receptor effector pathway coupling by metoprolol and carvedilol

The efficacy of the interaction between the single agonist-occupied β-adrenoceptor and the resulting contractile response was calculated by plotting the concentration-dependent effect of isoprenaline on isolated myocyte cell shortening against the relative β-adrenoceptor occupation as calculated from radioligand binding studies (Fig. 7). Whereas in isoprenaline treated cells, 43% receptor occupation was necessary to obtain 50% of the maximum effect and 62% occupation was necessary to obtain 90% of the maximum effect of isoprenaline on cell shortening, only 21 and 41% receptor occupation were required to obtain these effects after treatment with carvedilol. In control as well as in metoprolol treated myocytes, 4 and 16% receptor occupation were required to obtain 50 or 90% of the maximum effect.

4. Discussion

The present study addressed the question whether restoration of myocardial β-adrenoceptor density might explain the improvement in left ventricular function in response to β-blocker treatment.

In isoprenaline treated cardiac myocytes, used as a model for the catecholamine desensitized myocardium, β-blocker treatment prevented a decrease in the contractile responsiveness to isoprenaline. However, only treatment with metoprolol prevented β-adrenoceptor downregulation, whereas receptor downregulation in response to isoprenaline was even more pronounced after coincubation with carvedilol.

The present results confirm findings in right ventricular biopsies of heart failure patients treated with either metoprolol or carvedilol in which only metoprolol, but not carvedilol increased β-adrenoceptor density [24]. Similarly, no increase in β-adrenoceptor density in response to carvedilol treatment was observed in an in vivo model for renin-induced hypertensive cardiomyopathy [30]. Also, carvedilol as well as bucindolol decreased β-adrenoceptor density in chicken heart cells [31,32].

One explanation for divergent effects of carvedilol and metoprolol on myocyte β-adrenoceptor density might be differences concerning their interaction with β-adrenoceptors. Although carvedilol lacks agonist activity [32], its binding properties resemble a β-receptor agonist more than an antagonist. Similar to the agonist isoprenaline, carvedilol binds to high and low affinity binding sites in absence of guanine nucleotides, but binds to low affinity sites only when guanine nucleotides are present. In contrast, metoprolol binding is not influenced by Gpp(NH)p. These binding characteristics might be associated with different effects on the intrinsinc activity of β-adrenoceptors. Even in absence of an agonist, β-adrenoceptors

Table 1
Cardiac myocyte cell shortening after treatment with isoprenaline, isoprenaline and carvedilol or metoprolol and under control conditions[a]

	Basal cell shortening (% of diastolic cell length)	Maximum response to isoprenaline (% of diastolic length)	EC_{50} for inotropic response to isoprenaline (μmol/l)
12 h treatment with isoprenaline + β-blocker			
Control ($n=12$)	3.5±0.4	11.2±0.9[a]	0.0069 (0.0036–0.0129)
Isoprenaline ($n=12$)	6.6±0.8*	8.8±0.6*	0.3330 (0.0255–4.343)*
Isoprenaline + carvedilol ($n=12$)	3.4±0.1[+]	10.5±0.6[a]	0.0578 (0.0449–0.0744)*[+]
Ispoprenaline + metoprolol ($n=12$)	3.4±0.2[+]	9.8±0.8[a]	0.0112 (0.0089–0.0144)[+]
6 h treatment with isoprenaline followed by 6 h treatment with isoprenaline + β-blocker			
Control ($n=12$)	3.7±0.4	11.4±0.8[a]	0.0072 (0.0040–0.0129)
Isoprenaline ($n=12$)	6.5±0.8*	8.8±0.6*[a]	0.3418 (0.03906–2.992)*
Isoprenaline + carvedilol ($n=12$)	3.5±0.6[+]	11.1±0.6[+a]	0.1648 (0.1100–0.2470)*
Ispoprenaline + metropolol ($n=12$)	3.7±0.4[+]	10.9±0.4[+ a]	0.0066 (0.0030–0.0144)[+]

[a] Cell shortening given in percentage of diastolic length. *, $P<0.05$ vs. control cells, [+], $P<0.05$ vs. isoprenaline incubated cells, [a], $P<0.05$ vs. basal cell shortening. Data were obtained during six independent experiments per group. EC_{50} values are given with range.

can be in an active state enabling GDP–GTP exchange by $G\alpha_s$ and adenylyl cyclase stimulation [33,34]. β-Blocking agents typically transfer active receptors into an inactive state. Whereas metoprolol exhibits a high degree of inverse agonism, labetolol or bucindolol exhibit only very small or no inverse agonism [35,36]. Referral of a β-adrenoceptor into an active or inactive state by different β-blockers might be of importance for receptor regulation since the activated receptor is substrate for phosphorylation by β-adrenergic kinase [37].

On the other hand, determination of ^{125}I-cyanopindolol binding sites in isolated myocardial membranes treated with either metoprolol or carvedilol indicates that β-adrenoceptor densities might be underestimated. Since there is no degradation of β-adrenoceptors in isolated membranes, the observed concentration-dependent decrease in β-adrenergic binding sites in membrane preparations in response to carvedilol treatment despite extensive washout of the antagonist can only be explained by nearly irreversible occupation of β-adrenoceptor binding sites by carvedilol. In contrast, metoprolol binding to the β-receptor was completely reversible. However, downregulation and irreversible blockade of receptors should affect the inotropic responsiveness to catecholamines similarly. Indeed, one may hypothesize that alterations in β-adrenoceptor density in response to β-blocker treatment are of no relevance at all for the contractile performance of the failing heart. Independent from divergent effects on receptor density [24], almost all β-blockers used in heart failure trials led to an improvement of left ventricular function [29].

However, exercise capacity is not improved by carvedilol [29,38] or bucindolol [39], but some findings indicate an improvement with metoprolol [5,29,40]. These differences might reflect differences in β-adrenergic responsiveness in patients treated with particular β-blockers. Experimental results in this study are in accordance with this hypothesis because metoprolol prevented both the decrease in the contractile efficacy and in the potency of isoprenaline in catecholamine-treated isolated cardiac myocytes. In this context, efficacy is defined as an unchanged maximum cell shortening response of the myocytes to isoprenaline. Potency changes are indicated by alterations in the EC_{50} value and a right- or leftward shift of the isoprenaline concentration response curve. In contrast to metoprolol, carvedilol completely restored the efficacy, but only partly restored the potency of isoprenaline in catecholamine treated cardiac myocytes.

On the other hand, there is evidence that carvedilol increases the sensitivity of the single β-adrenoceptor to agonist binding. This is indicated by a decrease in the relative amount of receptors, which have to be occupied by isoprenaline after treatment with carvedilol in order to obtain 50 or 90% of the maximum contractile response. This effect of carvedilol is even more impressive if one keeps in mind that the absolute number of accessible receptors in carvedilol treated cells is further decreased compared to myocytes treated with isoprenaline only. Thus, carvedilol improves the coupling of the single β-adrenoceptor to its effector pathway. This might be one mechanism by which the decreased receptor number is compensated.

One explanation for this phenomenon might be a decreased β-adrenergic receptor kinase activity in carvedilol treated cardiac myocytes leading to increased cAMP formation despite β-adrenoceptor downregulation [41]. An alternative explanation might be that blockade of α-adrenergic receptors by carvedilol increases myocyte

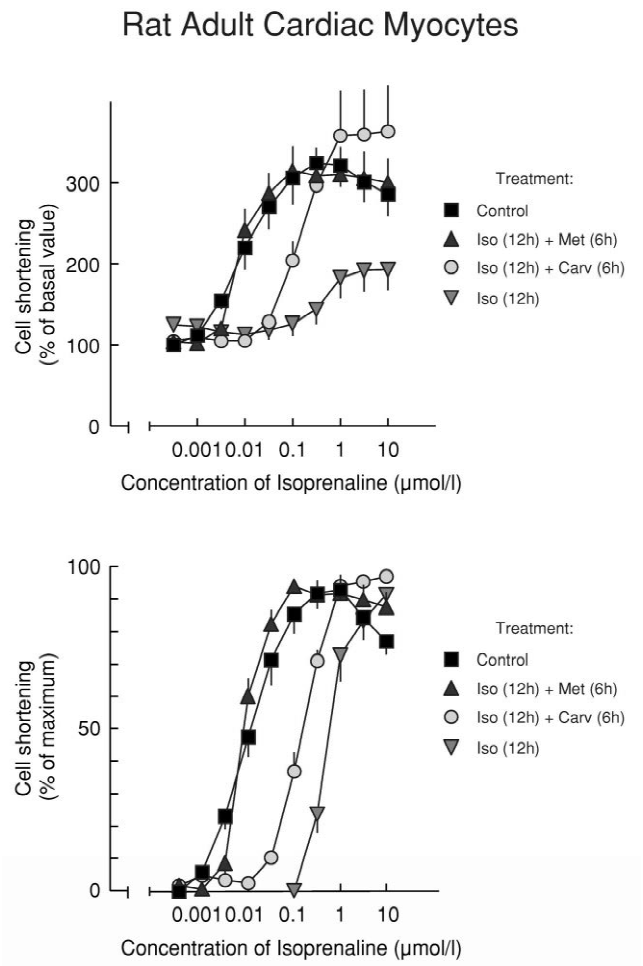

Fig. 6. Effect of isoprenaline on cell shortening of isolated cardiac myocyte treated for 12 h with isoprenaline (Iso), or treated with isoprenaline for 12 h with addition of carvedilol (Carv) or metoprolol (Met) for the last 6 h or treated under control conditions ($n=12$ per group). Ordinate: cell shortening in % of basal value (upper panel) or in percentage of maximum effect (lower panel).

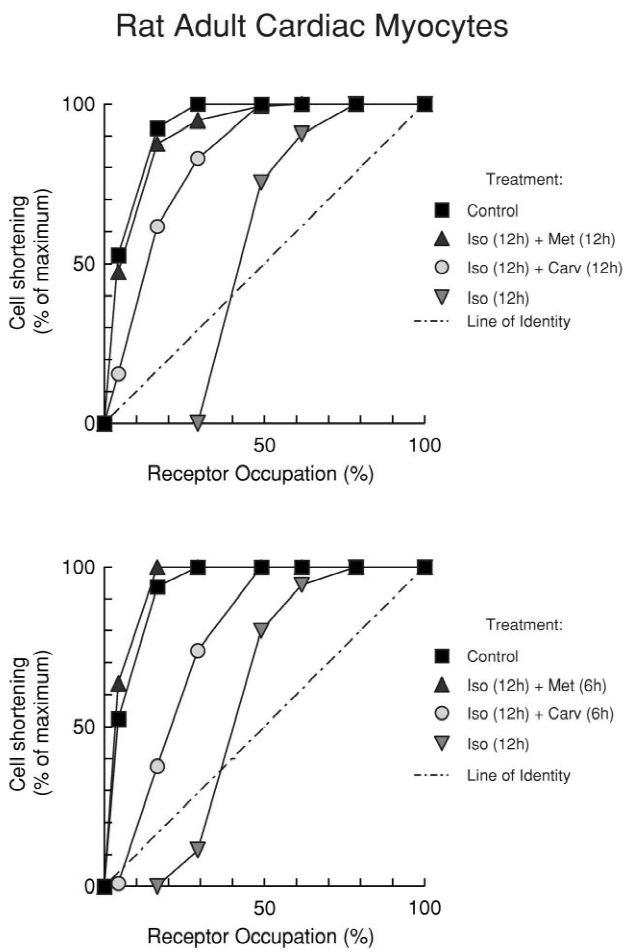

Fig. 7. Plots of percentage of β-adrenoceptor occupation by isoprenaline versus concentration-dependent effect of isoprenaline on systolic shortening of electrically stimulated cardiac myocytes treated with isoprenaline (Iso), isoprenaline plus metoprolol (Met) or isoprenaline plus carvedilol (Carv) for 12 h or incubated under control conditions (upper panel). Lower panel shows results for cells treated with isoprenaline for 12 h, but treated with the respective β-blockers only for the last 6 h. Measurements were performed on 12 isolated cardiac myocytes per group. Ordinate: Mean cell shortening in percentage of maximum response, 100% being determined for each group separately. Abscissa: Receptor occupancy in percentage as determined from ^{125}I-cyanopindolol-isoprenaline competition binding experiments in left ventricular myocardial membrane preparations.

contractility, e.g. due to blockade of α-receptor mediated activation of inhibitory G-proteins [42]. However, there is also contradictory evidence that α-adrenergic stimulation increases cardiac myocyte contractility, and this positive inotropic effect would be blocked by carvedilol [43].

Obviously, in vitro models have to be interpreted with some caution when referred to the situation in vivo. There were no indications for a significant toxic effect of isoprenaline, but we did not check for other cellular alterations which are characteristic for the failing myocyte. In addition, effects of specific and unspecific β-blockers on heart rate and peripheral vessels cannot be addressed in this model. Also, whether an improved coupling of the single β-receptor to its effector plays the same important role in heart failure patients has to be discussed with caution. In this context, it has to be emphasized that the human heart contains only few or no spare receptors, and a reduction in the number of available receptors is immediately translated into reduced β-adrenoceptor-mediated effects [44]. Another limitation of this study is that β-adrenoceptor subtypes were not studied separately. Most likely, the downregulation of total β-adrenoceptor density reflects decreased β_1-adrenoceptors as holds true for most models of cardiac hypertrophy and for human failing myocardium [45]. Whether remaining β_2-adrenoceptors are important for the regulation of myocyte contractility is open [46,47]. However, a β_2-adrenoceptor mediated increase in myocyte contractiliy is unlikely to occur after carvedilol treatment due to the unselective blockade of all β-adrenoceptor subtypes by this antagonist.

In summary, metoprolol but not carvedilol restores β-adrenoceptor density in catecholamine treated cardiac

myocytes. Increased rat cardiac myocyte contractility in response to metoprolol treatment might be due to an increase in β-adrenoceptor density. Increased myocyte contractility in response to carvedilol treatment despite a further β-receptor downregulation might be explained by an improved coupling of the single receptor to its effector pathway. Whether these different effects of β-blockers on β-receptor density and catecholamine responsiveness lead to different effects in heart failure patients, will have to be answered by ongoing comparative clinical trials, but might prepare the stage for the development of new and more specific pharmacological substances used in the therapy of chronic heart failure.

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