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Effects of metoprolol on myocardial β-adrenoceptors and $G_{i\alpha}$-proteins in patients with congestive heart failure

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Abstract *Objective*: In human heart failure downregulation of β-adrenoceptors and upregulation of G_i-protein α-subunits ($G_{i\alpha}$) results desensitization of the myocardial β-adrenergic signal transduction pathway and reduced positive inotropic effects of catecholamines. Metoprolol treatment has been shown to restore the reduced β-adrenoceptor density in dilated cardiomyopathy. The main objective of the present study was to investigate whether metoprolol also decreases the elevated inhibitory $G_{i\alpha}$ levels in patients suffering from congestive heart failure.
Methods: Total $G_{i\alpha}$ was determined by pertussis toxin-catalysed ADP ribosylation and β_1- and β_2-adrenoceptor densities by radioligand binding in right ventricular myocardial biopsies of 18 patients with dilated or ischaemic cardiomyopathy (NYHA II–IV) before and after 3 months of therapy. Nine controls were treated with conventional therapy only [diuretics, digitalis, nitrates, angiotensin-converting enzyme (ACE) inhibitors], and nine received the β_1-selective blocker metoprolol in addition (mean 98 ± 12 mg daily).
Results: In biopsies from patients treated with metoprolol, $G_{i\alpha}$ significantly decreased to 74% of predrug value and total β- adrenoceptor increased by a selective increase in β_1- adrenoceptors (44.7 vs 34.0 fmol $\cdot$ mg^{-1} protein). These effects were accompanied by significantly increased oxygen uptake at the anaerobic threshold (8.65 vs 6.95 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}). In the control group no significant changes in biochemical and clinical parameters occurred.
Conclusion: Metoprolol partly reverses $G_{i\alpha}$-upregulation and β-adrenoceptor downregulation in heart failure, which might contribute to the clinical improvement of patients treated with β-blockers.

Key words Metoprolol, Cardiomyopathy, G-proteins; β-adrenoceptor density

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Introduction

In congestive heart failure due to idiopathic dilated (IDC) or ischaemic cardiomyopathy (ISDC), the compensatory increase in the sympathetic drive [1, 2] leads to a chronic overstimulation of the heart. This results in desensitization of the myocardial β-adrenergic signal transduction pathway [3–5] and reduced positive inotropic effects of β-adrenoceptor agonists [6, 7]. Furthermore, catecholamines are supposed to support the progression of heart failure [8]. In order to stop the vicious circle of adrenergic overstimulation, β-adrenoceptor blockers are under evaluation in the therapy of congestive heart failure [9]. Waagstein et al. [10] were the first to introduce this therapeutic approach in 1975. Their observation of a beneficial effect of long-term β-adrenoceptor blocker therapy was confirmed by two recent multicentre studies (MDC [11] and CIBIS [12] trials). Haemodynamics of patients with congestive heart failure especially due to dilated cardiomyopathy were improved and the rate of hospitalization was reduced [11, 12]. However, the overall mortality in these trials remained unchanged.

The molecular mechanisms by which β-adrenoceptor blockers act in congestive heart failure are not fully understood. In the myocardium of patients with ischaemic and dilated cardiomyopathy, β-adrenoceptor density is reduced and inhibitory G-proteins are increased [13–16]. Both molecular alterations are most likely to be secondary to the increased adrenergic drive

[17, 18]. Metoprolol treatment has been shown to restore the reduced β-adrenoceptor density in dilated cardiomyopathy [19]. The present study was performed to investigate whether metoprolol also decreases the elevated inhibitory G-protein levels in patients suffering from congestive heart failure.

Materials and methods

Twenty-five patients with congestive heart failure (NYHA II–IV) were selected for the study. Included were patients with IDC or ISDC. The diagnosis was based on clinical as well as echocardiographic findings, left and right heart catheterization, coronary angiography and myocardial biopsy. The mean age was 55.6 (2) (35–68) years. Spiroergometry revealed a reduced anaerobic threshold [7.3 (0.46) ml · kg · min^{-1}], representing a moderate to severe limitation of exercise capacity (Weber classification D). Patients who were not able to perform an exercise test due to end stage heart failure or accompanying diseases such as gonarthrosis or claudicatio, those with contraindications for treatment with β-blockers and females of child-bearing age, were excluded. According to the study design, patients were randomized into a control group (n = 14) and a treatment group (n = 11). Patients in the control group received conventional medical treatment consisting of angiotensin-converting enzyme (ACE) inhibitors, digoxin, organic nitrates, diuretics, antiarrhythmic drugs, calcium channel blockers, antiplatelet agents and potassium supplements. In the treatment group metoprolol was additionally given 2 or 3 times a day with an average maintenance dose of 98 (12) mg (range 50–150 mg) after a titration period of 7 weeks. The initial dose was 10 mg, which was gradually increased unless a severe and/or symptomatic reduction in blood pressure and/or heart rate developed. Three months after the first investigation myocardial biopsy and the clinical and functional examinations except left and right heart catheterization were repeated. The study protocol was approved by the local ethics committee, and all patients gave their written informed consent. The physicians involved were blinded to the individual therapy.

Seven of the 25 patients selected for the study (four in the control group, three in the metoprolol group) had to be excluded for the following reasons: Four patients died before the second biopsy [two sudden deaths (control group), one progressive left heart failure (control group), one myocardial reinfarction (metoprolol group)]. One patient refused the second biospy (metoprolol group) and in two patients tissue samples were not sufficient (one in the control group, one in the metoprolol group). Baseline characteristics of the remaining 18 patients are shown in Table 1.

Spiroergometry

Standardized, symptom-limited spiroergometry started at a workload of 25 W, with stepwise increments of 10 W every minute. The effects of exercise on cardiopulmonary variables were quantified as described previously [20].

Radioligand binding experiments

Experiments were performed from endomyocardial biopsies as described previously [20] using (-)-[^{125}I]-iodocyanopindolol ([^{125}I]-ICYP) for total number of β-adrenoceptors, (±)-CGP 12177 (4-[3-tertiary butylamino-2-hydroxypropoxy]-benzimidazole-2-on) for non-specific binding, and the highly selective β_1- adrenoceptor antagonist (±)-CGP 20712A (1-[2-(3-carbamoyl-4-hydroxy)phenoxyethylamino]-3-[4(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxy1]-2-propanol methanesulphonate) for the relative amounts of β_1- and β_2- adrenoceptors.

Pertussis toxin catalysed ^{32}P-ADP ribosylation

Experiments were performed with crude ventricular homogenates prepared from 5–20 mg tissue wet weight as described previously [20] using 30 µg protein (crude homogenate) in triplicate, discontinuous sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for protein separation and densitometric analysis of autoradiographs.

Table 1 Baseline characteristics and drug therapy [mean values (SEM)] (*EF* ejection fraction, *PCWP* pulmonary capillary wedge pressure, PCWP-ISD in control and metoprolol group and CI-ISD in metoprolol group n = 1)

Characteristic	Control			Metoprolol		
		ISD	IDC		ISD	IDC
Number of patients	9	3	6	9	5	4
Male/female	9/0	3/0	6/0	8/1	5/0	3/1
Age (years)	59 (3)	62 (2)	57 (4)	56 (4)	59 (2)	51 (8)
Weight (kg)	81 (3)	86 (8)	79 (4)	75 (4)	71 (6)	80 (6)
Heart rate (beats · min^{-1})	95 (7)	90 (7)	102 (13)	97 (11)	71 (11)	110 (12)
NYHA functional class II	1	1	0	4	3	1
III	7	3	4	5	2	3
IV	1	1	0	0	0	0
EF angiographic (%)	32 (4)	42 (7)	29 (4)	32 (3)	33 (5)	31 (5)
Cardiac index [ml · (m^2)$^{-1}$]	2.7 (0.3)	2.0 (0.4)	2.8 (0.5)	3.0 (0.7)	3.0	2.4 (0.7)
PCWP (mmHg)	13 (2)	9	14 (2)	16 (2)	22	14 (2)
Drug therapy						
Digoxin	7	1	6	8	4	4
Diuretics	8	1	7	6	2	4
ACE inhibitors	7	1	6	8	4	4
Nitrates	5	3	2	3	3	0
Ca^{2+}-channel blockers	5	3	2	1	0	1
Antiplatelet agents	3	2	1	5	4	1
Potassium supplements	0	0	0	2	1	1
Dose of metoprolol (mg · day^{-1})	0	0	0	98(12)	65 (6)	131(19)

Table 2 Results of spiroergometries [Mean values (SEM)] (VO_{2AT} oxygen uptake at the anaerobic threshold, VO_{2max} maximum oxygen uptake, W_{AT} work load at the anaerobic threshold, W_{max} maximum work load)

Exercise testing	Control (n = a)		Metoprolol (n = a)	
	1st	2nd	1st	2nd
VO_{2AT} ($ml \cdot kg^{-1} \cdot min^{-1}$)	7.48 (0.26)	7.13 (0.56)	6.94 (0.91)	8.56 (1.07)*
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	11.87 (0.48)	11.43 (0.97)	13.07 (1.55)	15.60 (2.07)
W_{AT} (W)	49 (3)	51 (2)	47 (6)	58 (8)*
W_{max} (W)	85 (5)	81 (4)	80 (9)	90 (12)*

* Significant vs 1st ($P < 0.05$)

Statistical analysis

Values are presented as individual data or means with SEM. The equilibrium dissociation constant (K_D) and the maximal number of binding sites (B_{max}) were calculated from Scatchard plots [21]. Statistical significance was estimated using Student's *t*-test for paired and unpaired observations, or the Mann-Whitney (Wilcoxon) test. $P < 0.05$ was considered significant.

Results

Spiroergometry

The baseline spiroergometries (Table 2) revealed both in the β-adrenoceptor blocker-treated group and in the control group reduced oxygen uptake at the anaerobic threshold, indicating moderate to severe heart failure. Values between the groups were not significantly different. In the metoprolol group cardiopulmonary exercise capacity increased significantly by an average of 25% during 3 months of therapy (Fig. 1). Apart form the increased oxygen uptake at the anaerobic threshold, metoprolol also improved significantly the maximal workload and workload at the anaerobic threshold. After 3 months of β-adrenoceptor blocker therapy, heart rate at rest decreased significantly from 95 (7) to 76 (5) beats $\cdot$ min^{-1} and heart rate at maximum workload from 143 (1) to 127 (7) beats $\cdot$ min^{-1}.

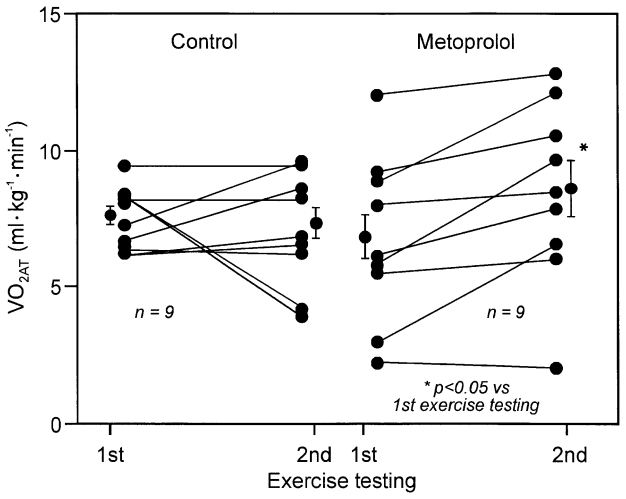


Fig. 1 Results of cardiopulmonary exercise testing. Individual data and means with SEM

There was no correlation between the increase in oxygen consumption at the anaerobic threshold and heart rate at rest.

Total β-adrenoceptor density and subtype distribution

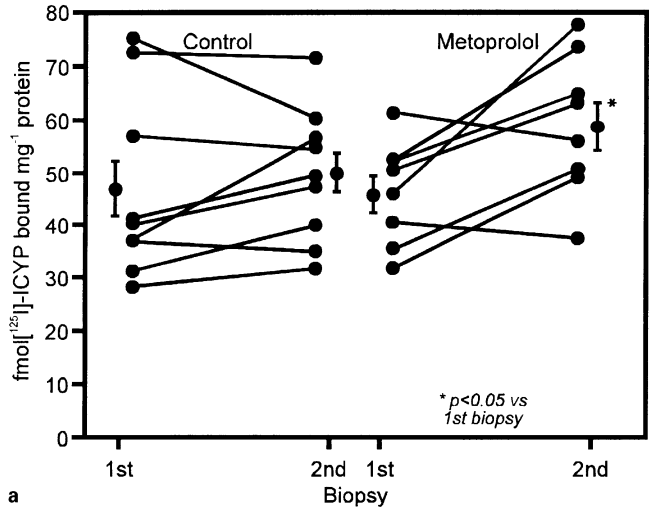
The β-adrenoceptor densities of all patients and their change under therapy are shown in Fig. 2a. In the first biopsy the total number of β-adrenoceptors was similar in the control and metoprolol group, with values of 46.4 (5.7) and 45.9 (3.2) fmol $\cdot$ mg^{-1}, respectively. The second biopsy revealed in the control group unchanged numbers of β-adrenoceptors [49.1(4.1) fmol $\cdot$ mg^{-1}]. In contrast, after 3 months treatment with metoprolol, total density of β-adrenoceptors increased significantly from 45.9 (3.2) to 58.0 (4.6) fmol $\cdot$ mg^{-1}. This represents an increase of 26% compared to the first biopsy. The increase was almost exclusively due to an increase in the β_1-adrenoceptor subtype (Fig. 2b). β_2-Adrenoceptor density remained unchanged (Fig. 2c). The equilibrium dissociation constant (Kd) values did not differ significantly between groups, ranging from 16.1 (1.5) to 19.9 (1.4) pmol $\cdot$ 1^{-1}.

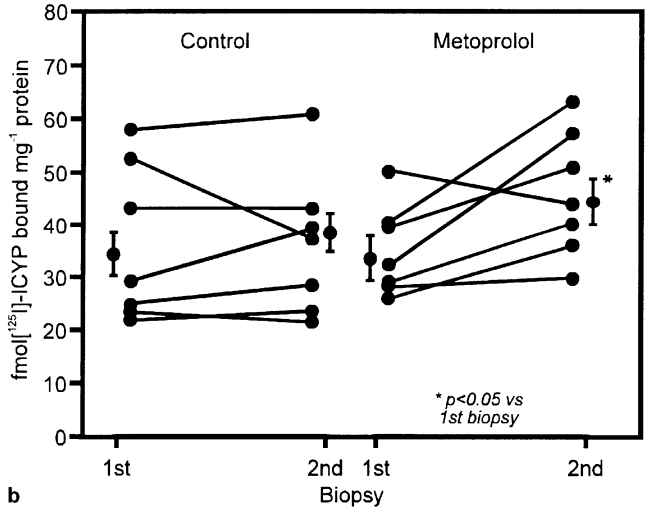
Pertussis toxin-catalysed ^{32}P-ADP ribosylation of $G_{i\alpha}$

Figure 3 shows the densitometric evaluation of autoradiographic signals of pertussis toxin-catalysed ^{32}P-ADP ribosylation of $G_{i\alpha}$-proteins. Baseline values in the metoprolol group were slightly but not statistically significantly higher than those in the control group [8570 (781) vs 6470 (800) density units]. Under metoprolol treatment, $G_{i\alpha}$ decreased significantly from 8570 (781) to 6510 (553) density units, whereas in the control group $G_{i\alpha}$ remained unchanged [6470 (800) to 6770 (554) density units] in the second biopsy.

Discussion

An important feature of progressive heart failure is the neurohumoral activation including catecholaminergic overstimulation of the heart. The resulting down-regulation of the β-adrenoceptors [4, 13, 14] and the increase in the inhibitory G-proteins [13–16] lead to a





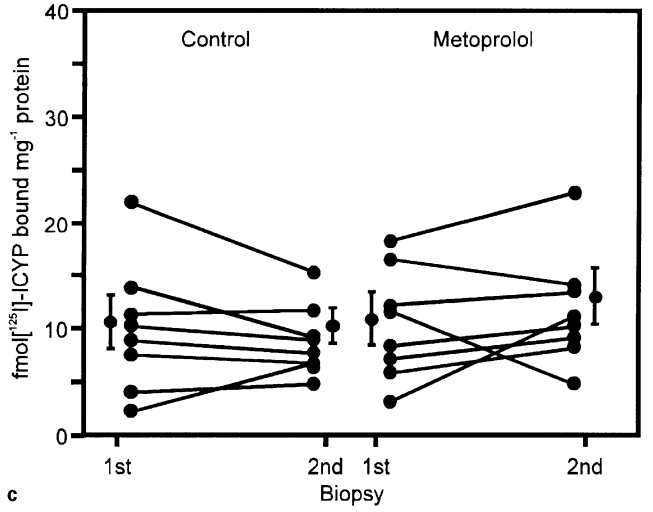


Fig. 2a–c β-Adrenoceptor densities, **b** β_1-adrenoceptor subtype densities and **c** β_2-adrenoceptor subtype densities. Individual data and means with SEM. In one patient in the metoprolol group insufficient cardiac tissue was obtained for measurement of β-adrenoceptors

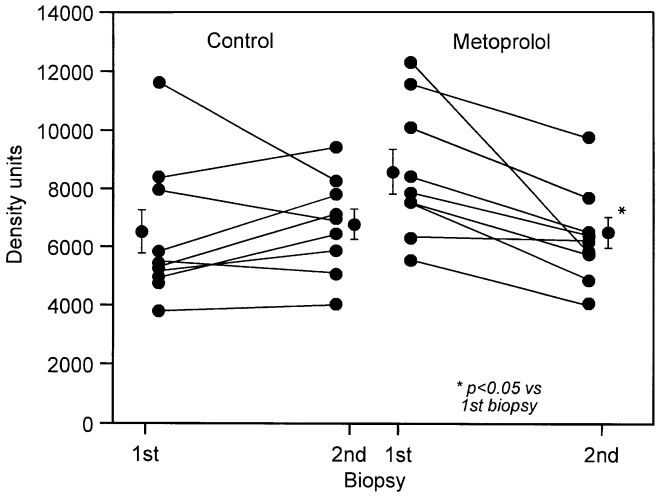


Fig. 3 Pertussis toxin-catalysed ^{32}P-ADP ribosylation, $G_{i\alpha}$-proteins. Individual data and means with SEM

desensitization of the β-adrenergic signal transduction pathway [3–5] and a reduced positive inotropic effect of β-adrenoceptor agonists [6, 7]. β-Adrenoceptor blocker therapy in congestive heart failure reduces plasma noradrenaline levels [22–24] and thus protects, in addition to the direct blockade of the receptors, the myocardium against the toxic effect of catecholaminergic overstimulation. It seems reasonable that by this mechanism β-blocker therapy leads to a resensitization of the β-adrenergic system in failing myocardium [19].

In accordance with previous studies by Heilbrunn et. al. [19], we found a significant increase in the myocardial β-adrenoceptor density after 3 months treatment with metoprolol in patients with congestive heart failure due to ischaemic or dilated cardiomyopathy. The present study extends these findings by showing that the increase in β-adrenoceptor density is almost exclusively due to an increase in the β_1-adrenoceptor subtype. The β_1-selectivity of the increase is in agreement with previous studies in patients with coronary heart disease in which treatment with β_1-selective blockers was shown to lead to a selective increase in (atrial) β_1-adrenoceptors. Unexpectedly, this went along with a potentiation of the adenylyl cyclase-stimulating effects of β_2-adrenergic agonists [25]. Whether a similar potentiation also exists in the β-adrenergic system in ventricular myocardium in heart failure (this study) remains to be studied.

Our data are the first to show that β-blocker therapy decreases the level of inhibitory G-proteins in failing human myocardium. One important aspect of this result is that metoprolol treatment revealed its positive effect on β-adrenoceptor density and $G_{i\alpha}$-proteins in patients pretreated with ACE inhibitors. In a previous study we showed that therapy with ACE inhibitors alone in patients with chronic heart failure also resulted in a selective upregulation of β_1-adrenoceptors, but failed to decrease elevated inhibitory G-proteins [20].

Data of this study confirm results of an animal model of heart failure produced by catecholaminergic overstimulation, where we found an increase in the inhibitory G-protein level in isoprenaline-treated rats [26]. Both in cultured cells and in the whole animal, noradrenaline- or isoprenaline-induced alterations in β-adrenoceptors and inhibitory G-proteins were fully prevented by the concomitant application of β-blockers [26–28]. Under these conditions, the β-blockers also restored a normal adenylyl cyclase-stimulating and positive inotropic effect of isoprenaline. The functional significance of the relatively small changes in $G_{i\alpha}$-proteins and β-adrenoceptors is in dispute. However, many experimental data clearly indicate that even small changes in β-adrenoceptors and inhibitory G-proteins have profound effects on the sensitivity of the cardiac adenylyl cyclase signalling pathway, which is the most important regulator of contractile performance (review in [28]). For example, the experimental inactivation of inhibitory G-proteins by pertussis toxin (PTX) in isolated ventricular cardiomyocytes from failing human hearts completely restored the blunted inotropic response to isoprenaline [29]. The upregulation of the β-adrenoceptors and downregulation of the inhibitory G-proteins under metoprolol treatment may therefore improve the regulation of myocardial inotropy in the failing human heart.

These molecular changes have to be viewed as occurring in the presence of the β-blocker. Hence, the increase in β-adrenoceptors may be functionally counteracted by the rightward shift of the dose-response curve of noradrenaline. However, this assumption is only valid (1) given that the β-blocker is permanently present at a sufficient concentration and (2) does not take into account the significant increase in noradrenaline levels with exercise. Therefore, it may be that the increase in myocardial β_1-adrenoceptors found in the present study does not play a major role under resting conditions, but may well contribute to the increase in exercise capacity also seen in our β-blocker-treated patients. In addition, the decrease in G_i-proteins now found for the first time to occur after chronic β-blockade will not be counteracted by the presence of the β-blocker and may, therefore, be even more important than the change in receptors for the beneficial effects of β-blockade in heart failure.

Nevertheless the question remains and cannot be answered in the present study whether the clinical improvement is the consequence, the cause of or just accompanies the trend towards normalization of β-adrenoceptors and inhibitory G-proteins. In addition, given the complexity of heart failure, the observed effects of β-blockade on receptor density and amount of inhibitory G-proteins cannot, of course, fully explain the beneficial clinical effects. Various other factors may contribute, e.g. slowing of heart rate, reduction of energy consumption and protection against toxic and arrhythmogenic effects of catecholamines. However, our results provide evidence that the partial restoration of β-adrenoceptors and inhibitory G-proteins is a contributory factor.

In summary, the present study shows for the first time that the clinical improvement of patients with congestive heart failure during metoprolol treatment in addition to standard medication is accompanied by a selective upregulation of β_1-adrenoceptors and a downregulation of inhibitory G-proteins. This finding indicates that common mechanisms are involved in both the downregulation of β- adrenoceptors and increase in G_i in human heart failure. The reversal of changes in β-adrenergic signal transduction proteins may well contribute to the resensitization of failing myocardium to catecholaminergic-positive inotropic stimulation. This could be one of the mechanisms of the clinical improvement of patients with chronic heart failure under β-adrenoceptor blocker therapy.

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