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Abstract

Objectives: In human end-stage heart failure as well as in experimental animal models of heart failure, G-protein-coupled receptor kinase activity (GRK) is increased while β-adrenoceptor responsiveness is diminished. In animal studies, β-adrenoceptor blockers reverse the GRK-mediated desensitization and down-regulation of myocardial β-adrenoceptors. The aim of this study was to investigate whether alterations in GRK activity are an early or late accompaniment of human heart failure and whether also in humans β-adrenoceptor blocker treatment is able to influence myocardial GRK activity.

Methods: We assessed in right atria, obtained from patients at different stages of heart failure, treated with or not treated with β-adrenoceptor blockers, and in the four chambers of explanted hearts, obtained from patients with end-stage heart failure, β-adrenoceptor density (by (-)-[125I]-iodocyanopindolol binding) and GRK activity (by an in vitro rhodopsin phosphorylation assay).

Results: With increasing severity of heart failure, plasma noradrenaline levels increased while myocardial β-adrenoceptor density decreased with a maximum in GRK activity in end-stage heart failure. However, in relation to the progression of heart failure, we found that GRK activity transiently increased at an early stage of heart failure (NYHA I and II) but decreased back to control values in patients at NYHA III and IV. β-Adrenoceptor blockers were able to reduce the early increase in GRK activity at NYHA I and II to control levels, whereas in those patients who did not have increased GRK activity (NYHA III and IV), they had only a marginal effect.

Conclusion: According to our results, an increase in GRK activity is an early and transient event in the course of heart failure that can be prevented by β-adrenoceptor blocker treatment.

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Keywords: β-Adrenoceptor; Heart failure; β-Arrestins

1. Introduction

Human heart failure is characterized by impaired myocardial β-adrenoceptor responsiveness mainly caused by two different alterations: a decrease in receptor density (down-regulation) and a functional uncoupling of the remaining receptors from the Gαs-protein-adenyl cyclase pathway (desensitization) [1,2]. The extent of this reduction in β-adrenoceptor density and function seems to be directly related to the severity of heart failure and an attribute to the compensatory and chronically elevated activity of the sympathetic nervous system, indirectly indicated by increased plasma noradrenaline levels [3].

However, several studies have provided evidence that these higher levels of catecholamines could be responsible for an increase in the expression and enzymatic activity of G-protein-coupled receptor kinases (GRKs), which phosphorylate only agonist-occupied receptors and by this facilitate their binding to β-arrestins. β-Arrestins, on the other hand, not only prevent further receptor-G-protein activation, but are also involved in the control of receptor

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endocytosis. Consequently, it was suggested that the up-regulation of GRKs may be somewhat connected to the desensitization and down-regulation of the β-adrenoceptors in the failing heart [4,5].

To date, β-adrenoceptor blocker treatment, in combination with diuretics and ACE-inhibitors, has become an established therapy for heart failure. Chronic β-adrenoceptor blockade improves left ventricular systolic dysfunction, heart failure symptoms and survival in patients with heart failure [6]. Clinical and experimental data suggest that β-adrenoceptor blockers directly interfere with components of the β-adrenoceptor-G-protein-adenylyl cyclase pathway. These changes involve up-regulation of the down-regulated β1-adrenoceptors, re-coupling of the uncoupled β2-adrenoceptors, prevention of further remodeling and reversal of existing remodeling and improvement in left ventricular systolic function by the restoration of maladaptive defects in myocardial Ca2+ signaling [2,7,8]. However, the mechanisms by which these components are altered are still poorly understood.

Recently, it was suggested that β-adrenoceptor blocker-induced alterations in GRK expression and activity could be one possible mechanism to explain the observed changes in the β-adrenoceptor-G-protein-adenylyl cyclase pathway. Whereas in mice sustained infusion of isoprenaline-induced myocardial hypertrophy associated with an impaired β-adrenoceptor signaling and increased GRK levels, the infusion of atenolol as well as carvedilol led to decreased GRK expression and enhanced β-adrenoceptor signaling [9]. Likewise in pigs, chronic treatment with bisoprolol resulted in increased left ventricular β-adrenoceptor density associated with decreased GRK activity [10]. Also in rats, with reflexory and chronically increased sympathetic nervous system activity due to salt-deprived diet, atenolol was able to reverse the GRK-mediated desensitization and down-regulation of myocardial β-adrenoceptors [11].

These changes in β-adrenoceptor density and function underline the pivotal role of GRKs not only for the agonist-induced desensitization and down-regulation, but also for the antagonist-induced restoration or even up-regulation of the receptors in heart failure.

However, whether these alterations in myocardial GRK expression and activity due to β-adrenoceptor blocker treatment hold also true in patients with chronic heart failure (CHF) is still unclear. Furthermore, although it has conclusively been reported that GRK activity is increased in end-stage human heart failure, several aspects concerning function and regulation of GRKs during the development of myocardial dysfunction in human heart failure are still unclear [4,12,13]. Therefore, the aim of this study was to investigate whether alterations in GRK activity are an early or late accompaniment of human heart failure and whether also in humans β-adrenoceptor blocker treatment is able to influence myocardial GRK activity.

2. Methods

2.1. Patients

Right atrial appendages were obtained from total 117 patients (21 female/96 male) with coronary artery disease undergoing coronary artery bypass grafting with signs of apparent heart failure. Patients were classified according to the New York Heart Association (NYHA) into NYHA functional classes I to IV. Additionally, we investigated right atria from patients without any signs of apparent heart failure (controls, see Table 1). One hundred and one of those patients had received either angiotensin-converting-enzyme-(ACE)-inhibitors (n=62), angiotensin II AT-1 receptor antagonists (n=13), nitrates (n=11), calcium antagonists (n=8), diuretics (n=72), statins (n=84), acetyl salicylic acid (n=14), and occasionally digitalis glycosides (n=5), alone or in combination. Fifty-six of these patients were additionally treated with β1-adrenoceptor-selective blockers (metoprolol (n=26), bisoprolol (n=22), nebivolol (n=2)) or the β1- and β2-adrenoceptor-nonselective blocker carvedilol (n=6), whereas 16 patients at NYHA II had received neither anti-adrenergic nor anti-renin-angiotensin-system drugs. In addition, myocardial tissue was obtained from four male patients with end-stage heart failure (NYHA IV), due to ischemic (n=1) or dilated cardiomyopathy (n=3) undergoing heart transplantation (see Table 1). Those patients were on anti-failure medication including digitalis glycosides, diuretics, ACE-inhibitors and carvedilol and additionally received inotropic support by dobutamine/dopamine-infusion for at least 30±12 days (initial doses: 1.5±0.3 µg/kg body weight/min dobutamine/1.2±0.3 µg/kg body weight/min dopamine; doses on the day before

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<th>BMI</th>
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Controls—patients without any signs of apparent heart failure, NYHA—patients with signs of apparent heart failure classified according to the New York Heart Association (NYHA) into NYHA functional classes I-IV, n—number of patients, EF—ejection fraction (%), BMI—body mass index, C/B/M/N—patients treated with carvedilol or bisoprolol or metoprolol or nebivolol, HT—heart transplant recipients.
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transplantation: 5±2 μg/kg body weight/min dobutamine/3±2 μg/kg body weight/min dopamine).

Anesthesiological pre-medication and surgery was carried out as described elsewhere [14,15]. Right atrial appendages were removed after installation of the cardiopulmonary bypass. Immediately after excision, specimen were either quickly frozen in liquid nitrogen and stored at -80 °C (for determination of β-adrenoceptor density and GRK activity or noradrenaline transporter (uptake1) density) or were transferred into carbogenated (95% O₂/5% CO₂) Tyrode solution (mmol/l: NaCl 119.8; KCl 5.4; CaCl₂ 1.8; MgSO₄ 1.05; NaH₂PO₄ 0.42; NaHCO₃ 23.6; glucose 5.05; EDTA 0.05; ascorbic acid 0.28; at room temperature) and transported to the laboratories (for determination of uptake1 activity).

Full informed consent was obtained from all patients before participating in the study. The study was approved by the local ethic committee and the investigation conforms with the principles outlined in the Declaration of Helsinki.

2.2. Determination of myocardial GRK activity and β-adrenoceptor density

Cytosolic and membraneous GRK activity was determined in 66 right atria from patients without any signs of heart failure (controls) and from patients at NYHA I–IV without and with β-adrenoceptor blocker treatment by in vitro phosphorylation of rhodopsin exactly as recently described [13]. To date no GRK-subtype selective in vitro phosphorylation assay is available, however, rhodopsin is phosphorylated with the relative order of: GRK2 > GRK3= GRK5 [16]. Therefore, we will refer to our in vitro rhodopsin phosphorylation assay as GRK activity, although it mostly measures GRK2. In 54 of the 66 right atria, we additionally determined myocardial β-adrenoceptor density by (-)-[125I]-iodocyanopindolol-([125I]-CYP) binding, exactly as described elsewhere [14].

With the same methods, cytosolic and membraneous GRK activity as well as β-adrenoceptor density were also determined in all four chambers of four explanted hearts from patients at end-stage heart failure undergoing heart transplantation.

2.3. Determination of plasma noradrenaline levels

For determination of the plasma noradrenaline level from 52 of the 66 patients, venous blood was drawn through a catheter into ice-cold EDTA-tubes before surgery. Samples were centrifuged with 600 g for 10 min at 4 °C, plasma was removed, quickly frozen in liquid nitrogen and stored at -20 °C until further use. Plasma noradrenaline level was assessed by high-pressure-liquid-chromatography (HPLC) and electrochemical detection (ChromSystems, Instruments and Chemicals GmbH; Munich, Germany).

2.4. Determination of myocardial noradrenaline transporter (uptake1) activity and density

The activity of uptake1 was assessed in slices of right atria obtained from 5 patients not treated with anti-adrenergic and anti-renin–angiotensin-system drugs, 6 patients treated with ACE-inhibitors but not treated with β-adrenoceptor blockers and in 8 patients treated with ACE-inhibitors and β-adrenoceptor blockers by accumulation of [3H]-noradrenaline into tissue slices. Furthermore, uptake1 density was determined in right atria from 11 patients not treated with anti-adrenergic and anti-renin–angiotensin-system drugs, 9 patients treated with ACE-inhibitors but not treated with β-adrenoceptor blockers and in 12 patients treated with ACE-inhibitors and β-adrenoceptor antagonists by [3H]-nisoxetine binding, details have been recently described [15].

2.5. Statistics

Experimental data given in text, figures and table are expressed as means±S.E.M. of (n) experiments. Equilibrium dissociation constant (K_D) and maximal number of binding sites (B_max) for [125I]-CYP binding and [3H]-nisoxetine binding were calculated by nonlinear regression analysis (hyperbolic function y=B_max×x/(K_D+x)) using the iterative curve fitting Prism program (Graph-Pad Software, San Diego, CA, USA). Significance of differences was estimated by one-way ANOVA—with Dunnet’s post-test for comparison of all columns vs. controls or Bonferroni’s post-test for comparison of selected pairs of columns. All statistical calculations were performed with the Prism program. A p-value <0.05 was considered to be significant.

2.6. Drugs used

(-)-[125I]-iodocyanopindolol ([125I]-CYP, specific activity 2200 Ci/mmol) and γ-[32P]-ATP, D.L.-[7-3H(N)]-noradrenaline hydrochloride (specific activity: 13.5 Ci/mmol) and [N-methyl-3H]-nisoxetine (specific activity: 80 Ci/mmol) were purchased from NEN™ Life Science Products, Boston, MA, USA. Purified bovine rhodopsin and the βγ-subunits for the GRK-assay were kindly provided by Prof. Dr. M. J. Lohse (University of Würzburg, Germany). All other chemicals were of purest grade commercially available.

3. Results

3.1. Plasma noradrenaline levels and right atrial β-adrenoceptor density in patients at different stages of heart failure with and without β-adrenoceptor blocker treatment

In CHF-patients not treated with β-adrenoceptor blockers plasma noradrenaline levels gradually increased (max. threefold) with increasing severity of heart failure (as judged
by NYHA classification) (Fig. 1); concomitantly right atrial β-adrenoceptor density gradually decreased (Fig. 2). In CHF-patients treated with β-adrenoceptor blockers, however, plasma noradrenaline levels were at each NYHA class lower (Fig. 1) and right atrial β-adrenoceptor density higher (Fig. 2) than in not treated CHF-patients.

### 3.2. Right atrial GRK activity in patients at different stages of heart failure with and without β-adrenoceptor blocker treatment

In CHF-patients not treated with β-adrenoceptor blockers activity of cytosolic and membraneous GRK increased gradually in early stages of heart failure from controls to NYHA II, but in more severe heart failure (NYHA III and IV) GRK activity declined to or below values obtained in controls (Fig. 3). In CHF-patients treated with β-adrenoceptor blockers cytosolic and membraneous GRK activity was at each NYHA class lower than in not treated CHF-patients (Fig. 3). This difference reached statistical significance in patients at NYHA II.

### 3.3. β-Adrenoceptor density and GRK activity in all four chambers of explanted hearts obtained from patients undergoing heart transplantation

In explanted hearts β-adrenoceptor density in right and left atria and ventricles was rather low (ranging from 12 to 25 fmol/mg protein, Fig. 4A); right atrial β-adrenoceptor density was $23.7±4.1$ fmol/mg ($n=4$) which was significantly lower than in right atria from controls independent whether they were treated ($63.5±6.2$ fmol/mg, $n=3$, Fig. 2) or not treated with β-adrenoceptor blockers ($44.5±8.1$ fmol/mg, $n=3$, Fig. 2). In contrast, cytosolic and membraneous GRK activities were quite high (ranging from 38,500 to 45,400 and 85,600 to 103,700 cpm/mg protein, respectively); right atrial GRK activities were $44,672±3,329$ and $103,727±2,456$ cpm/mg protein, respectively (Fig. 4B), and that was significantly higher than values for right atrial GRK activities in controls not treated with β-adrenoceptor blockers ($28,917±1202$ and $72,715±6464$ cpm/mg protein, respectively, $n=4$; Fig. 3), but nearly identical to right atrial GRK activities of CHF-patients at NYHA II not treated with β-adrenoceptor blockers.

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** Plasma noradrenaline levels in relation to severity of heart failure. Controls—patients without any signs of apparent heart failure, NYHA—patients with signs of apparent heart failure classified according to the New York Heart Association (NYHA) into NYHA functional classes I–IV, open columns—patients not treated with β-adrenoceptor blockers, filled columns—patients treated with β-adrenoceptor blockers, pNA plasma noradrenaline levels in pg/ml, (n) number of patients; (*) $p<0.05$ vs. controls not treated with β-adrenoceptor blockers, (#) $p<0.05$ vs. controls treated with β-adrenoceptor blockers.

![Fig. 2](https://example.com/fig2.png)

**Fig. 2.** Right atrial β-adrenoceptor density in relation to severity of heart failure. Controls—patients without any signs of apparent heart failure, NYHA—patients with signs of apparent heart failure classified according to the New York Heart Association (NYHA) into NYHA functional classes I–IV, open columns—patients not treated with β-adrenoceptor blockers, filled columns—patients treated with β-adrenoceptor blockers, [125I]-CYP (-)[125I]-iodocyanopindolol, (n) number of patients; (*) $p<0.05$ vs. controls not treated with β-adrenoceptor blockers, (#) $p<0.05$ vs. controls treated with β-adrenoceptor blockers.
3.4. Right atrial noradrenaline transporter (uptake1) density and activity in patients at NYHA II

In a final set of experiments, we assessed right atrial uptake1 because this is reduced in CHF and may, by this, contribute to β-adrenoceptor desensitization and increases in GRK activity [17]. Uptake1 activity was assessed only in right atria from patients at NYHA II because these patients showed the largest changes in GRK activity (Fig. 3). The patients were divided into three groups: (A) not treated with anti-adrenergic or anti-renin–angiotensin-system drugs (n=16); (B) treated with ACE-inhibitors but not with β-adrenoceptor blockers (n=15); and (C) treated with ACE-inhibitors and β-adrenoceptor blockers (n=20). Uptake1 density was in group B significantly higher than in group A and increased further in group C (Fig. 5A). On the other hand, uptake1 activity was not different between groups A and B, but was significantly increased in group C (Fig. 5B).

4. Discussion

In the present study, in CHF-patients plasma noradrenaline levels increased and right atrial β-adrenoceptor density decreased in relation to the severity of the disease (judged by NYHA functional classification). These results are in agreement with numerous studies that showed in CHF-patients a decrease in β-adrenoceptors (mainly β1-adrenoceptors) and an increase in sympathetic activity (as...
indirectly indicated by increased plasma noradrenaline levels) [1,2,17]. In addition, cardiac GRK – the enzyme that phosphorylates agonist-occupied receptors – is increased in CHF; this has been shown in various animal models of CHF and also in end-stage human heart failure (in explanted hearts) [4,5,12,13,18]. The present data confirm these findings: in all four chambers of the explanted hearts of patients undergoing heart transplantation because of end-stage heart failure GRK activity was markedly enhanced apparently without regional differences.

In addition, however, we assessed GRK activity in right atria from CHF-patients in relation to the course of the disease. In these patients, right atrial GRK activity was increased only in early (i.e., moderate, NYHA I-II) heart failure while in more severe heart failure (NYHA III–IV), it declined back to nearly control levels.

It is generally believed that one of the strongest stimuli to activate GRK is stimulation of β-adrenoceptors (for example by endogenous catecholamines) [5]. In the present study, however, plasma noradrenaline levels had only marginally increased in patients with NYHA I and II, and it is rather unlikely that this marginal increase might be sufficient to strongly activate GRK. On the other hand, the present data clearly show that in patients with NYHA II (those patients having the largest increase in right atrial GRK activity), right atrial uptake1 is significantly decreased. This should result in increased synaptic cleft noradrenaline concentrations that could well be high enough to strongly activate right atrial β-adrenoceptors, and by this GRK. Taken together, the data of this study indicate that the increase in right atrial GRK activity in early heart failure is rather due to local than to systemic effects of increased catecholamines.

In the present study, in CHF-patients, chronic treatment with β-adrenoceptor blockers resulted in all NYHA classes in higher β-adrenoceptor densities compared to patients of the same NYHA class not treated with β-adrenoceptor blockers. Since our patients were predominantly treated with the β₁-adrenoceptor-selective blockers metoprolol or bisoprolol, it is likely that this increase in β-adrenoceptors is mainly an increase in β₁-adrenoceptors [19,20] although we could not measure that directly. On the other hand, in CHF-patients treated with β-adrenoceptor blockers cytosolic and membraneous GRK activity was at each NYHA class lower than in not-treated CHF-patients (cf. Fig. 3). This difference reached statistical significance in patients at NYHA II presumably because in these patients, the largest increase in GRK activity was observed. From these results, we can conclude that (1) the increase in GRK is an early event in the development of CHF, and (2) chronic β-adrenoceptor blockade can prevent such increases in GRK in early heart failure.

Several studies in animal models of heart failure have shown that an increase in GRK expression and activity is deleterious for the heart, whereas blockade of GRK activity by βARKct (a peptide derived from the Gβγ-protein binding site in the C-terminus of bovine GRK2) or by β-adrenoceptor blockers is beneficial for animals with heart failure [9–11,21,22]. β-Adrenoceptor blockers are now standard therapy in CHF-patients [23]; thus, according to the present findings, one mechanism of their beneficial effects might be that they can prevent increases
in GRK and, by this, slow down development of chronic heart failure.

Moreover, chronic β-adrenoceptor blocker treatment appears to restore uptake activity (cf. Fig. 5); this should result in a decrease of the pathophysiologically enhanced noradrenaline content in the synaptic cleft and by this at the receptor site. Accordingly, desensitization process is reduced and should lead to a restoration of β-adrenoceptor density—and this is, as shown in Fig. 2, indeed the case.

Interestingly, we found that GRK activity in all four chambers of the explanted hearts from patients undergoing heart transplantation was markedly increased (to a similar extent as in NYHA II class CHF-patients) although these heart transplant recipients were treated with the nonselective β-adrenoceptor blocker carvedilol. The reason for this divergent behavior of GRK (increases despite β-adrenoceptor blocker treatment in explanted hearts vs. reduction towards control levels in CHF-patients treated with β-adrenoceptor blockers) is not known. However, as discussed above, the strongest stimulus to activate GRK is β-adrenoceptor stimulation; in CHF-patients, this is caused by the (local) increase in noradrenaline in (patho)physiologic concentrations, whereas patients undergoing heart transplantation are treated for “bridging to transplant” with high (pharmacological) doses of dobutamine and dopamine. Thus, it might be possible that β-adrenoceptor blockers can prevent β-adrenoceptor stimulation, and by this GRK-activity, by (patho)physiologic concentrations of β-adrenoceptor agonist (endogenous catecholamines) while pharmacological doses of dobutamine may overcome β-adrenoceptor blockade in the explanted human heart, and by this lead to activation of GRK.

In the present study, however, right atrial GRK activity increased only transiently during the course of heart failure being elevated in early (NYHA I and II), but reduced to nearly control values in late (severe) heart failure (NYHA III and IV). This is in contrast to data from several animal models [9,11,24–26] but in agreement with the findings of Ping et al. [10,27] in a porcine model of pacing-induced heart failure and by Vinge et al. [28] in rats subjected to ligation of coronary artery, who also found an early increase in GRK that later in the course of the disease declined back to basal levels. We do not know the reason for the transient increase of GRK activity in the human failing heart and can only speculate: it might be that in early heart failure (locally) increased catecholamines activate GRK via β-adrenoceptor stimulation and simultaneously initiate the β-adrenoceptor desensitization process. In late heart failure β-adrenoceptors are markedly desensitized and now the (local) increase in catecholamines is not sufficient to stimulate the (desensitized) β-adrenoceptors.

4.1. In conclusion

The data of the present study show that in the course of human heart failure the increase in GRK activity is an early and transient event—most likely due to local rather than systemic alterations in sympathetic nervous system—that can be prevented by β-adrenoceptor blocker treatment.

4.2. Limitation of the study

We have measured the β-adrenoceptor signal transduction pathway in right atria, and extrapolate these data to the left ventricle because left ventricular tissue was not available. For the following reasons, we believe that this extrapolation is valid: (a) a decrease in β-(mainly β1) adrenoceptors has been shown in numerous studies not only in ventricles, but also in atria of CHF-patients (for review, see [2]); (b) a decrease in uptake1 as we found in atria, has been also demonstrated in left ventricles of CHF-patients [17], and (c) the fact that in explanted hearts GRK activity was in atria as well as in ventricles increased to a very similar extent favors the idea that changes of right atrial GRK activity mirror in fact changes of GRK in ventricles.

In addition, we have measured activity of right atrial GRK but did not measure—because of limited access to tissue—expression of GRK-subtypes by immunoblotting. However, it is very likely that we have measured mainly GRK2, since rhodopsin is phosphorylated with the relative order of GRK2 ≫ GRK3 ≈ GRK5 [16].

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References


