β-Adrenergic signal transduction following carvedilol treatment in hypertensive cardiac hypertrophy

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1. Introduction

In chronic heart failure, neuroendocrine activation occurs [1], which is characterized by increased circulating norepinephrine concentrations [2] and an increased release of norepinephrine from the heart [3]. The circulating norepinephrine concentrations, as well as the release of norepinephrine, correlate with the poor prognosis of this syndrome [2]. Treatment of heart failure with β1-selective blockers, such as metoprolol [4] or bisoprolol [5], have been reported to improve symptoms and prognosis, in particular, in patients with dilated cardiomyopathy. Recently, carvedilol, a nonselective β-blocker with vasodilatory properties due to α-adrenoceptor antagonism, has been reported to improve prognosis in patients with ischemic
and dilated cardiomyopathy [6]. The mechanisms by which β-blockers exert these beneficial effects are not completely elucidated. One potential key mechanism could be the restoration of β-adrenergic effects on myocardial force of contraction. The blunted positive inotropic effects of catecholamines are due to a downregulation [7] and uncoupling of β-adrenoceptors [8,9] and an increase of inhibitory G-protein α-subunits [10,11], and are important mechanisms for contractile dysfunction in heart failure [12]. In endomyocardial biopsies from patients with heart failure due to dilated cardiomyopathy, treatment with metoprolol for six months increased the number of β-adrenoceptors and restored the positive inotropic effect of the β-adrenoceptor agonist, dobutamine [13]. Interestingly, treatment with carvedilol produced a similar increase in ejection fraction compared to metoprolol, but had no effect on the density of β-adrenergic receptors, as determined by right ventricular biopsies [14]. The use of myocardial biopsies for receptor ligand binding studies is hampered by a number of technical problems. Contamination with endocardial fibrous tissue can interfere with the exact determination of β-adrenoceptors in myocardial membranes. In addition, the amount of tissue is small. Thus, the alterations of the other signal transduction defects, like the increase in inhibitory G-protein α-subunits and consecutive alterations of adenylyl cyclase activity, cannot be detected.

In order to study the effects of carvedilol on the pathobiochemistry of β-adrenergic signalling, we investigated the effects of carvedilol on myocardial neuropeptide Y concentrations, β-adrenoceptors, Gia and adenylyl cyclase activity in a model of compensated cardiac hypertrophy with pathobiochemical alterations similar to those observed in the failing human heart, namely a downregulation of β1-adrenoceptors and an increase of Gia-proteins [15].

2. Methods

2.1. Transgenic animals

Transgenic animals [TG(mREN2)27] [15] were housed and treated in the animal laboratory of the Department of Clinical Pharmacology, University of Groningen, Netherlands. Sprague-Dawley (SD) control rats were the animals into which the transgene was originally introduced. Animals were obtained from Møllegard (Denmark). Animals were housed according to the guidelines of animal care of The Netherlands. Only male rats were used. The animals were held on a standard laboratory animal diet (Hope-Farms, Woerden, Netherlands) and tap water ad libitum. Treatment was started at the age of seven weeks for 11 weeks. Carvedilol was mixed to the chow at 1 g/kg. This is equivalent to an average dose of 30 mg/kg/day. The rats were exposed to alternating 12 h dark and light cycles at 20–22°C. Before sacrifice, blood pressures and heart rates were taken using the tailcuff method. Heart rate was also measured in vitro following sacrifice. Twelve rats were included in each group. Animals were killed by a blow on the head and were decapitated. Animals were bled from the carotid arteries. Serum was prepared from the sampled blood, frozen in liquid nitrogen and stored at −80°C until the humoral measurements were performed. Hearts were quickly removed. Hearts of TG(mREN2)27 exhibited concentric hypertrophy, but no dilatation. No signs of venous congestion were observed in any other organ. The hearts were retrogradely perfused according to the Langendorff technique and the in vitro heart rate was taken. After perfusion with Tyrode solution (in mmol/l: NaCl, 119.8; KCl, 5.4; CaCl2, 1.8; MgCl2, 1.05; NaH2PO4, 0.42; NaHCO3, 22.6; Na2EDTA, 0.05; ascorbic acid, 0.28 and glucose, 5.0) for 5 min, the spontaneous heart rate was taken for 5 min. The preparations achieved equilibrium heart rate within this time. Ten minutes after sacrifice, left ventricles were immediately prepared, split into three pieces from the basis to the apex and immediately frozen in liquid nitrogen. These techniques allowed us to study adenylyl cyclase activity, G-proteins and receptors in defined areas of the hearts.

2.2. Adenylyl cyclase determinations

Adenylyl cyclase activity was determined according to Salomon et al. [16], with modifications as described recently [17]. In brief, particulate washed membrane fractions (10 000 g sediment) were prepared from homogenates of rat hearts (at approx. 4°C). The activity of adenylyl cyclase was determined in a reaction mixture containing 50 μmol/l 32P-α-ATP (approximately 0.3 μCi per 100 μl), 50 mmol/l triethanolamine–HCl, 5 mmol/l MgCl2, 100 μmol/l EGTA, 1 mmol/l 3-isobutyl-1-methylxanthine, 5 mmol/l creatine phosphate, 0.4 mg/ml creatine kinase and 0.1 mmol/l cyclic adenosine monophosphate (cAMP) at pH 7.4 in a final volume of 100 μl. The reaction was started by the addition of the membrane suspension. The mixture was preincubated for 5 min at 37°C. The incubation time was 20 min at the same temperature. Reactions were stopped by the addition of 500 μl of 120 mmol/l zinc acetate. Next, the zinc acetate was neutralized by 600 μl Na2CO3 (144 mmol/l). After centrifugation for 5 min at 10 000 g, 0.8 ml of the supernatant was applied to a neutral alumina column that had been equilibrated with 0.1 mmol/l Tris–HCl, pH 7.5. The effluent was collected and the 32P-labeled cAMP was determined by measuring the radioactivity in a liquid scintillation spectrometer (LKB Wallac 1272 Clinigamma, Freiburg, Germany). In experiments with MnCl2, MgCl2 was substituted by MnCl2 (5 mmol/l) in the reaction mixture.
2.3. Membrane preparation for receptor and G-protein determinations

Myocardial tissue was chilled in 30 ml of ice-cold homogenization buffer (10 mmol/l Tris–HCl, 1 mmol/l EDTA, 1 mmol/l dithiothreitol, pH 7.4). Connective tissue was trimmed away and myocardial tissue was minced with scissors. Membranes were prepared with a motor-driven glass–teflon homogenizer for 1 min. Afterwards, the membrane preparation was homogenized by hand for 1 min using a glass–glass homogenizer. The homogenate was spun at 484 g (rotor, Beckman JA 20) for 10 min. The supernatant was filtered through two layers of cheese cloth, diluted with an equal volume of ice-cold 1 mol/l KCl and stored on ice for 10 min. This suspension was centrifuged at 100 000 g for 30 min. For radioligand binding experiments, the pellet was resuspended in 50 volumes of incubation buffer (50 mmol/l Tris–HCl, 10 mmol/l MgCl₂, pH 7.4) and homogenized for 1 min with a glass–glass homogenizer. This suspension was recentrifuged at 100 000 g for 30 min. The final pellet was resuspended in incubation buffer (50 volumes) and was stored at −80°C. All preparation steps were performed on ice in a cool room at 4°C. Centrifugation steps were also performed at 4°C. Previous experiments in our laboratory have shown that storage for up to three years at −70–80°C does not alter adenyl cyclase activity and G-protein or receptor content.

2.4. Radioligand binding studies

The assays were performed in a total volume of 250 μl incubation buffer (for composition, see above). The incubation was carried out at 25°C for 120 min. These conditions allowed complete equilibration of the receptors with the radioligand. The reaction was terminated by rapid vacuum filtration through Whatman GF/C filters, and filters were immediately washed three times with 6 ml each of ice-cold incubation buffer. All experiments were performed in triplicate. Myocardial β-adrenoceptors were studied using 125I-labeled cyanopindolol, as previously described [11,15] using a single concentration for B_max determination (50 mol/l).

2.5. Pertussis toxin-induced 32P-ADP-ribosylation

32P-ADP-ribosylation of Giα by pertussis toxin was performed for 12 h at 4°C in a volume of 50 μl containing 100 mmol/l Tris–HCl, pH 8.0 at 20°C, 25 mmol/l dithiothreitol, 2 mmol/l ATP, 1 mmol/l GTP, 50 mmol/l 32P-labeled NAD (800 Ci/mmole) and 20 μg/ml pertussis toxin that had been activated by incubation with 50 mmol/l dithiothreitol for 1 h at 20°C prior to the labeling reaction, as described earlier [17]. Samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) [10% (w/v) acrylamide, 16 cm total gel length]. Gels were stained with Coomassie blue and dried before autoradiography was performed.

2.6. Neuropeptide Y determinations

For neuropeptide measurements, tissue samples were homogenized with a Polytron device in 0.1 mol/l Tris–HCl, pH 7.4. Neuropeptide Y was determined using a commercially available radioimmunoassay (Amersham Buchler, Braunschweig, Germany).

2.7. Miscellaneous

Protein concentrations were determined according to Lowry et al. [18] using bovine serum albumin as the standard. SDS–PAGE was performed as described by Laemmli [19].

2.8. Materials

Forskolin was donated by Hoechst, Frankfurt, Germany. Guanosine-5’-triphosphate (GTP), guanylylimidodiphosphate [Gpp(NH)p], adenosine-5’-triphosphate (ATP), creatine phosphate and creatine kinase were purchased from Boehringer-Mannheim (Mannheim, Germany) and isobutylmethylxanthine (IBMX) was from EGA-Chemie (Steinheim, Germany). The ligand 125I-labeled cyanopindolol (Cyp) was from Amersham-Buchler (Braunschweig, Germany). Dithiothreitol was from Serva (Heidelberg, Germany). Pertussis toxin was from List Biological Laboratories (Campbell, USA). All other compounds used were of analytical grade or the best grade commercially available. Only deionized and double distilled water was used throughout. Carvedilol was provided by Boehringer-Mannheim (Mannheim, Germany).

2.9. Statistics

The data shown are means±SEM. Statistical significance was estimated with analysis of variance according to Wallenstein et al. [20]. A Bonferroni correction was used to correct for multiple comparisons. A p-value of less than 0.05 was considered to be significant. K_L values were determined graphically in each individual experiment.

3. Results

3.1. Blood pressure, heart rate and heart weight

As summarized in Table 1, systolic blood pressure and heart rate were significantly higher in TG(mREN2)27 compared to SD. Treatment with carvedilol significantly reduced the heart rate and blood pressure. Consistently, rate pressure product was increased in TG(mREN2)27 compared to SD but was significantly reduced following
treatment with carvedilol (Table 1). Fig. 1A shows the difference in heart rate in vivo and in vitro. The difference in heart rate was more pronounced in TG(mREN2)27 than in SD, as an indicator of increased catecholamine drive in vivo in transgenic rats. In carvedilol-treated rats, this difference was reduced to control values. Thus, excess stimulation was completely blocked by carvedilol. The heart rate in vitro, as determined in Langendorff isolated heart preparations, did not differ (Table 1). In contrast, the increase in relative left ventricular weights in hypertensive TG(mREN2)27 compared to SD was not significantly reduced by carvedilol (Table 1). The relative right ventricular weights did not differ (Table 1). Taken together, carvedilol treatment reduced the elevated heart rate in vivo and reduced blood pressure, but had no significant effect on left ventricular hypertrophy in TG(mREN2)27. Lung water was not significantly changed in TG(mREN2)27 (3.0±0.13 mg/g, n=11) compared to control rats (2.62±0.07 mg/g, n=14, n.s.). However, +dp/dt was similar in TG(mREN2)27 compared to SD (6685±379 mmHg/s, n=8 vs. 6000±212 mmHg/s, n=9, n.s.). In contrast, the rate of relaxation (−dp/dt) was significantly reduced in TG(mREN2)27 compared to SD (5771±371 vs. 7159±454 mmHg/s, n=9, p<0.05).

3.2. Myocardial neuropeptide Y concentrations

Neuropeptide Y is costored and coreleased with norepinephrine from sympathetic nerve terminals [21]. During chronic stimulation, myocardial sympathetic nerve terminals become depleted of neuropeptide Y. Fig. 1B shows that myocardial neuropeptide Y concentrations are reduced by about 47% in TG(mREN2)27 compared to control rats. The reduction of blood pressure by carvedilol did not alter myocardial concentrations of neuropeptide Y.

3.3. β-Adrenergic receptors

The density of myocardial β-adrenergic receptors was determined by radioligand experiments in myocardial membranes. As shown in Fig. 1C, the number of β-adrenergic receptors was significantly reduced in hearts from TG(mREN2)27 compared to untreated controls. In carvedilol-treated TG(mREN2)27, the receptor density was even lower than in untreated TG(mREN2)27.

3.4. Inhibitory G-protein α-subunits

Besides the downregulation of β-adrenergic receptors, the increase of inhibitory G-protein α-subunits has been identified as one cause of adrenergic subsensitivity in cardiac failure and hypertrophy. As shown in original autoradiographs (Fig. 2, upper panel), hearts of TG(mREN2)27 exhibited an increase in pertussis toxin-catalyzed incorporation of 32P-ADP-ribose into a 40-kDa substrate, presumably Giα. Treatment with carvedilol did not reduce pertussis toxin substrates in TG(mREN2)27 compared to SD (Fig. 2).

3.5. Adenylyl cyclase activity

Fig. 3 summarizes the effect of carvedilol treatment on adenylyl cyclase activity. Basal adenylyl cyclase was reduced in TG(mREN2)27 compared to SD (Fig. 3A). Also, the effects of isoprenaline, Gpp(NH)p or forskolin on adenylyl cyclase activity were depressed (Fig. 3B–D). Carvedilol treatment did not significantly increase adenylyl cyclase activity compared to values in TG(mREN2)27 controls. In order to assess whether or not depressed forskolin-stimulated enzyme activity is due to an alteration of the catalyst activity of adenylyl cyclase, experiments were performed in the presence of 5 mmol/1 MnCl2 and in the absence of MgCl2. Under this condition, the adenylyl cyclase is uncoupled from the influence of G-proteins [22]. In the presence of manganate ions, there was no difference between SD and TG(mREN2)27, independent of whether rats were treated with carvedilol or not (not shown). Thus, the catalyst activity is not altered in TG(mREN2)27 and is not affected by carvedilol treatment.

Table 1
Blood pressure, heart rate in vivo or ex vivo and rate pressure product of transgenic rats TG(mREN2)27 (n=12–14) and control Sprague-Dawley rats (SD) (n=10–14)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SD</th>
<th>TG(mREN2)27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure systolic (mmHg)</td>
<td>139±4</td>
<td>192±14*</td>
</tr>
<tr>
<td>Heart rate in vivo (beats/min)</td>
<td>361±10</td>
<td>405±6*</td>
</tr>
<tr>
<td>in vitro (beats/min)</td>
<td>293±12</td>
<td>270±9</td>
</tr>
<tr>
<td>Rate pressure product (mmHg/min)</td>
<td>43.6±1929</td>
<td>71.212±4600*</td>
</tr>
<tr>
<td>Relative heart weight (mg/g)</td>
<td>3.7±0.1</td>
<td>5.1±0.3*</td>
</tr>
<tr>
<td>Relative LV weight (mg/g)</td>
<td>3.0±0.07</td>
<td>4.3±0.26*</td>
</tr>
<tr>
<td>Relative RV weight (mg/g)</td>
<td>0.8±0.03</td>
<td>0.8±0.05</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>482±8.7</td>
<td>492±32.8</td>
</tr>
</tbody>
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LV, left ventricle; RV, right ventricle.
*p<0.01 vs. SD; †p<0.05 vs. control TG(mREN2)27.
3.6. Interaction of carvedilol with β-adrenergic receptors

One would expect that β-blocker treatment restores desensitized adenylyl cyclase activity. Since this is not the case, this finding points towards an atypical interaction of carvedilol with β-adrenergic receptors. In order to investigate the interaction of carvedilol-occupied β-adrenergic receptors with G-proteins, competition experiments with carvedilol for 125I-Cyp-binding to myocardial membranes in the absence and presence of Gpp(NH)p were performed. Identical experiments were performed with the agonist isoprenaline and the β1-selective antagonist metoprolol (Fig. 4). Fig. 4A shows that binding of isoprenaline was biphasic, with a high and a low affinity component of
binding in the absence of Gpp(NH)p. In the presence of the guanine nucleotide, the curve shifts to the right and becomes monophasic, with a Hill coefficient close to one (Table 2). In contrast, binding of the \(\beta\)-adrenoceptor antagonist metoprolol was not affected by Gpp(NH)p (Fig. 4B). Binding characteristics of carvedilol are shown in Fig. 4C. Competition by carvedilol for \(^{125}\)I-Cyp-binding was also compatible with binding to two sites with high and low affinity. As with the agonist isoprenaline, carvedilol competition curves were shifted to the right by Gpp(NH)p and became monophasic. Differences in the distribution of high and low affinity states between the receptor subtypes were not determined, due to the scatter of the binding curves, which allows only a qualitative comparison. Taken together, binding characteristics of the \(\beta\)-blocker antagonist carvedilol are atypical and resemble in some aspects those of \(\beta\)-adrenoceptor agonists.

4. Discussion

\(\beta\)-Adrenergic neuroeffector transmission was characterized in transgenic rats with compensated cardiac hypertrophy following treatment with the \(\beta\)-adrenergic receptor antagonist carvedilol. Carvedilol reduced rate-pressure-product and significantly decreased, but did not normalize, blood pressure. Left ventricular weights were not significantly reduced. In hypertensive rats, there was a significant depletion of neuropeptide Y stores, evidence for a local activation of cardiac sympathetic nerves, which was insensitive to carvedilol. As reported before, adenylyl cyclase activity was desensitized by an increase in inhibitory G-protein \(\alpha\)-subunits and a downregulation of \(\beta\)-adrenoceptors in TG(mREN2)27 [15]. Carvedilol treatment did not resensitize adenylyl cyclase, but even decreased myocardial \(\beta\)-adrenergic receptor numbers. Treatment had no effect on the amount of myocardial \(G_{\text{i}}\). In radioligand binding experiments, carvedilol behaved in a manner similar to that of the agonist isoprenaline, but different from that of metoprolol, because it exhibited guanine-nucleotide-modifiable binding.

4.1. \(\beta\)-Adrenergic signal transduction in cardiac failure and hypertrophy

The failing human heart exhibits a desensitization of adenylyl cyclase with a consecutive blunted response to cAMP-elevating positive inotropic agents [7–9,23]. The
syndrome [26]. According to the Framingham study, arterial hypertension producing chronic pressure overload on the heart is one prominent cause of heart failure [27]. In several models of pressure overload, including the hearts of patients with hypertensive heart disease, a desensitization of β-adrenergic effects by variable alterations of β-adrenergic receptors and G-proteins has been identified [28]. These findings have attracted the idea that β-adrenergic desensitization could be a mechanism that occurs before the manifestation of heart failure in compensated hypertrophy due to pressure overload and could contribute to the progression from compensated hypertrophy to overt heart failure.

It is important to note that all animal models have limitations for extrapolation of the data generated to the situation in chronic heart failure in humans. In particular, strong differences occur in β-adrenergic signal transduction. In models with renal failure, corticosteroid hypertension and Goldblatt-hypertension, adenylyl cyclase is reduced, due to postreceptor events rather than to a down-regulation of β-adrenergic receptors [28]. Among the various models, transgenic rats harbouring the mouse renin gene exhibit alterations of β-adrenergic signal transduction in the heart, which are similar to those observed in human cardiomyopathic tissue [28]. These rats develop fulminant hypertension with myocardial hypertrophy [29]. Myocardial norepinephrine and neuropeptide Y concentrations are depleted, whereas plasma concentrations of neuropeptide Y and norepinephrine are increased [15]. Adenylyl cyclase is desensitized, due to a reduction in β-receptors by 50% and an increase of inhibitory G-proteins by 40%. In addition, the velocities of contraction and relaxation are reduced in isolated cardiac preparations. This is due to a depressed gene and protein expression of the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase, and of phospholamban [30]. The biochemical alterations contributing to contractile alterations clearly correlate to those observed in human heart failure [31]. Nevertheless, since, in contrast to the situation in human heart, failure of systolic function is not affected to these extents, the models of compensated cardiac hypertrophy are mostly, if not exclusively, suitable for biochemical characterization rather than for determining the effects on contractile performance. This transgenic model has been chosen to study the effects of carvedilol on β-adrenergic signal transduction.

4.2. Effects of β-blockers

A variety of studies have shown that β-blocker treatment improved ejection fraction, submaximal exercise tolerance and left ventricular filling pressures [12–14]. Metoprolol treatment has been reported to reduce the need for cardiac transplantation in dilated cardiomyopathy [4]. Bisoprolol improved the prognosis in patients with dilated cardiomyopathy [5]. Recently, a reduction in mortality by
about 65% has been reported for both ischemic and dilated cardiomyopathy in the US Carvedilol Heart Failure Trials [6]. The mechanisms by which β-blocker treatment favourably act are poorly understood. One candidate mechanism is the restoration of β-adrenergic receptor–effector coupling, with improvement of cardiac performance. Indeed, metoprolol treatment for six months increased ejection fraction and increased the number of β-adrenergic receptors in right ventricular biopsies [13]. Recently, Gilbert et al. [14] reported similar increases of ejection fraction by treatment with metoprolol or carvedilol. Interestingly, the number of β-adrenergic receptors increased after metoprolol, but not after carvedilol, treatment. This finding pointed towards an atypical effect of carvedilol on myocardial β-adrenergic receptors.

The measurement of β-adrenergic receptors in endomyocardial biopsies is hampered by several biological and technical difficulties, due to the small amount of sample and due to contamination by large amounts of fibrous tissue. Therefore, we set out to determine the effects of carvedilol in a model with similar pathobiochemical alterations as in the failing human heart.

4.3. Effects of carvedilol on β-adrenergic signal transduction

Carvedilol is a β-adrenergic receptor antagonist with a low selectivity to β₁-adrenoceptors, which, in vivo, is of no, or only minor, importance [32]. In addition, it produces vasodilatation by α₁-adrenoceptor antagonism and exhibits antioxidative [33] and antiproliferative effects in smooth muscle cells [34]. In TG(mREN2)27, carvedilol reduced the rate pressure product to values similar to those in control rats. Thus, β-blockade by carvedilol was sufficient. In TG(mREN2)27, the number of β-adrenergic receptors was significantly reduced. Carvedilol did not restore, but even reduced, β-adrenergic receptor numbers. In the same model, antihypertensive treatment with captopril and the AT₁-receptor antagonist, Bay 10-6734, restored adenylyl cyclase [35]. This effect has been attributed to an increase in the number of β-adrenergic receptors, normalization of G-proteins and was accompanied by a partial restitution of neuropeptide Y concentrations in the heart. In the present study, carvedilol had neither an effect on myocardial neuropeptide Y-concentrations nor did it increase the density of β-adrenergic receptors. The reduction of β-adrenoceptor density is even different from previous observations in humans. Carvedilol did not increase the number of β-adrenergic receptors in patients with heart failure [14], whereas the β₁-adrenoceptor antagonist metoprolol significantly upregulated β-adrenoceptor numbers in myocardial biopsies [13,14]. Both studies, at least, did not show an increase of β-adrenoceptors as one would expect. The reduction in rats but not in humans could be explained by the relatively higher dose producing a larger receptor occupation, which cannot be obtained in humans. Interestingly, carvedilol reduced the transcardiac gradient of released norepinephrine, whereas metoprolol had no significant effect on norepinephrine release in the failing human hearts [14]. Therefore, the lack of effects of carvedilol on β-adrenergic receptor densities cannot be explained by a failure of the compound to reduce myocardial norepinephrine release. Altogether, the data point towards an atypical effect of carvedilol on β-adrenergic receptors, which, unlike with other β-blockers, prevents upregulation of β-adrenergic receptors [14] or can even downregulate the β-adrenoceptors (these experiments). As shown in experiments on myocardial membranes, carvedilol binds to β-adrenergic receptors with two affinity
states. The high affinity state of binding sites can be transformed to a low affinity status by the application of the nonhydrolysable guanine nucleotide, Gpp(NH)p. This indicates that the binding properties of carvedilol are similar to those of the agonist isoprenaline [36], although carvedilol has no intrinsic sympathomimetic activity. Similar effects have been reported in human myocardial membranes [35]. These peculiarities of the compound could explain why β-adrenoceptor numbers do not recover, but are even more downregulated, in TG(mREN2)27 following carvedilol treatment. In support of this notion are data on chicken cardiomyocytes, in which carvedilol also downregulated β-adrenergic receptors [32].

Besides a downregulation of β-adrenergic receptors, an increase of Giα-proteins has been described in the failing heart [10,11] and in left ventricular myocardium of TG(mREN2)27 [15]. This pathobiochemical change is most likely induced by chronic β-adrenoceptor stimulation of the heart [37]. The increase in Giα-related pertussis toxin substrates induced by norepinephrine was due to an increase in the transcription rate of the Gio2-gene, as determined by nuclear run-on assays [38] and was sensitive to the β-adrenoceptor antagonists [37,38]. One would expect that β-adrenoceptor blockade or treatment with agents that reduce sympathetic activation would normalize the elevated levels of β-proteins. In myocardial biopsies from patients with heart failure and treated with metoprolol for six months, a reduction of pertussis toxin substrates has been observed [39]. In spontaneously hypertensive rats, treatment with fosinopril reduced sympathetic activation and reduced the elevated levels of Giα-proteins [40]. However, carvedilol had no effect on G-protein levels and, consistently, did not restore guanine nucleotide-dependent stimulation of adenylyl cyclase activity. Consistent with the lack of upregulation of β-adrenergic receptors and Giα-proteins, neuropeptide Y concentrations were not affected. Taken together, carvedilol does not influence β-adrenergic signal transduction, although it does produce antiadrenergic effects in vivo.

From this and previous studies, one has to assume that carvedilol does not influence β-adrenergic signal transduction, although it has marked effects on outcome in patients with moderate and also severe [6,41] heart failure. It has been shown that norepinephrine has strong toxic effects on cardiomyocytes [42]. Therefore, a blockade of β-adrenoceptors and antiadrenergic effects in the failing heart is much more complete when β-adrenergic receptors are downregulated. In addition, carvedilol possesses additional effects, such as antioxidant [33] and antiproliferative [35] properties. Whether or not these effects are relevant for the in vivo situation is an open question at present.

4.4. Potential limitations

These experiments have been performed in an animal model, which exhibits a transgenic overexpression of the mouse renin, ren24, gene. The consequence is a fulminant hypertension and a strong sympathetic activation. The pathobiochemical alterations are a reduction of β1-adrenergic receptors and an increase of Giα-proteins. Compared with other models of experimental or hereditary left ventricular pressure overload, TG(mREN2)27 have the greatest similarities concerning their rigorously studied β-adrenergic signal transduction defects to those observed in the failing human heart [15,28,29]. Nevertheless, we would like to point out that this is not a model for heart failure. It just has close pathobiochemical similarities to this condition. Thus, it seems worthwhile to study pharmacological interventions that have the potential to delay or prevent progression in such a model. The investigation can only provide insights into the pathobiochemistry and the effects of carvedilol thereon. Since TG(mREN2)27 exhibit a more or less preserved systolic function, functional determination following pharmacological treatment seems to be of no value.

5. Conclusions

In the used model of pressure overload and sympathetic activation, adenylyl cyclase is desensitized, due to a decrease in β-adrenoceptors and an increase in Giα-proteins, and in the presence of unchanged Gsα or catalyst activities. Carvedilol treatment did not reverse the biochemical changes despite its antiadrenergic effects in vivo. An atypical guanine nucleotide-sensitive interaction of carvedilol with β-adrenergic receptors could be responsible for these atypical effects. These findings support evidence for an effectiveness of carvedilol in the treatment of heart failure that is independent of β-adrenergic signal transduction. Identification of these mechanisms would provide insights into the mechanisms of heart failure and the development of new therapeutic strategies for this malignant condition.

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References


