Chamber-Specific Alterations of Norepinephrine Uptake Sites in Cardiac Hypertrophy

Michael Böhm, Maurizio Castellano, Markus Flesch, Christoph Maack, Marianne Moll, Martin Paul, Frank Schiffer, Oliver Zolk

Abstract—The present study investigated local differences of sympathetic activation and sympathetic neuroeffector defects in nonhypertrophied right and hypertrophied left ventricles in a rat model with renin-induced pressure overload [TG(mREN2)27]. As judged from the depletion of myocardial norepinephrine stores, sympathetic activation was more pronounced in the left than in the right ventricles. In addition, norepinephrine uptake carriers were reduced in left but unchanged in right ventricles. Gene expression of the carrier was unchanged in stellate ganglia. An increase of \( G_i \) expression and a heterologous adenylyl cyclase desensitization occurred only in the left but not in the right ventricles, whereas a reduction of \( \beta \)-adrenergic receptors was observed in both chambers. We concluded that general sympathetic activation can lead to \( \beta \)-adrenoceptor downregulation but that pressure overload further increases sympathetic activation involving norepinephrine uptake mechanisms in the left ventricles, resulting in heterologous \( \beta \)-adrenergic desensitization. (Hypertension. 1998;32:831-837.)

Key Words: hypertrophy, cardiac \( \bullet \) G proteins \( \bullet \) adenylyl cyclase \( \bullet \) catecholamines

In the failing myocardium, an activation of the tissue renin-angiotensin system (RAS) occurs. In addition, sympathetic stimulation\(^1\) produces a decrease of cardiac \( \beta \)-adrenoceptors\(^2,3\) and increased expression of inhibitory G-protein \( \alpha \)-subunits (\( G_{\alpha} \))\(^4,5\) resulting in a desensitization of the adenylyl cyclase and a blunted response to cAMP-increasing positive inotropic agents.\(^3\) In the failing human heart, reduced catecholamine stores\(^5\) and reduced norepinephrine uptake, carrier sites\(^6\) have been observed. It is not resolved whether the general sympathetic activation or local mechanisms of catecholamine release and a decreased uptake of norepinephrine within the myocardial chambers contribute to the extent of postsynaptic alteration of \( \beta \)-adrenergic signal transduction. Recently, it became evident that in left ventricular myocardium in hypertensive cardiac hypertrophy, adenylyl cyclase desensitization also occurs.\(^7,8\) Therefore, sympathetic activation with consecutive changes of \( \beta \)-adrenergic signal transduction could occur already in the stage of pressure overload–induced cardiac hypertrophy, thereby contributing to the progression of compensated cardiac hypertrophy to heart failure.\(^1\) However, it is not resolved whether a local activation of cardiac RAS or pressure overload could increase local sympathetic activation. Transgenic rats harboring the mouse renin gene \( Ren-2d \) [TG(mREN2)27] overexpress the transgene in several tissues and develop fulminant hypertens.\(^7\) A reduction of \( \beta \)-adrenergic receptors and an increase of \( G_{\alpha} \) proteins have been observed before in TG(m-REN2)27.\(^10\) The present study investigated whether pressure overload imposed on the left ventricles directly augments sympathetic neuroeffector defects by altering local concentrations and uptake mechanisms of norepinephrine. \( \beta \)-Adrenergic signal transduction alterations in the hypertrophied and overloaded left ventricles and in the nonhypertrophied right ventricles of TG(mREN2)27 were analyzed. Gene expression of uptake carrier molecules was studied in sympathetic cervical ganglia.

Methods

Transgenic Animals

Transgenic animals [TG(mREN2)27] were housed and bred in the animal laboratory of the Max Delbrück Centrum in Berlin, Germany. At the age of 5 weeks, animals were transferred to the animal laboratory of the Klinikum Großhadern, University of Munich (Germany). Sprague-Dawley (SD) control rats were obtained from the Laboratorium für Versuchstierkunde in Hannover (Germany). These were the animals into which the transgene was originally introduced. Animals were housed according to the guidelines of animal care of the University of Munich. Only male rats were used. The animals were maintained on a standard laboratory animal diet (Altromin) and tap water ad libitum. They were exposed to alternating 12-hour dark and light cycles at 20°C to 22°C. The experiments were performed at age 12 to 14 weeks. At this age, hypertension is fully established [ie, TG(mREN2)27 230±8 mm Hg versus SD 115±12 mm Hg]. Animals were killed by a blow on the head, and the hearts were rapidly removed. In TG(mREN2)27 compared with SD, there was a significant increase of absolute and relative heart weights [TG(mREN2)27: 1.82 0.03 g, 4.92 0.11 mg/g; SD: 1.42 0.03 g, 3.52 0.11 mg/g].
0.03 g, 3.78 0.08 mg/g; P < 0.001]. Hearts of TG(mREN2)27 exhibited concentric hypertrophy but no dilatation or any signs of heart failure such as excessive scarring. No signs of venous congestion were observed in any other organ. Hydroxyproline content and histological determination of scarring were similar in both groups and have been published recently.11

Preparation of Sympathetic Ganglia
The clavicle, pectoralis major as well as pectoralis minor muscles, and the ribs were transected along the ventral axillary line. The ventral thoracic wall was removed, exposing the cervical sympathetic trunk and the stellate ganglia, which were located beneath the first and second ribs. Pre- and postganglionic nerve fibers were dissected, and the ganglia were removed and immediately frozen in liquid nitrogen.

Adenylyl Cyclase Determinations
Adenylyl cyclase activity was determined according to Salomon et al12 with modifications as described recently.

Membrane Preparation for Receptor and G-Protein Determinations
Myocardial tissue was chilled in 30 mL ice-cold homogenization buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, 1 mmol/L DTT, pH 7.4). Connective tissue was trimmed away, myocardial tissue was minced with scissors, and membranes were prepared with a motor-driven glass-Teflon homogenizer for 1 minute. Afterward, the membrane preparation was homogenized by hand for 1 minute with a glass-glass homogenizer. The homogenate was spun at 484 g (rotor, Beckman JA 20) for 10 minutes. The supernatant was filtered through 2 layers of cheese cloth, diluted with an equal volume of ice-cold 1 mol/L KCl, and stored on ice for 10 minutes. This suspension was recentrifuged at 100 000 g for 1 hour at 20°C before the labeling reaction, as described earlier. 3

Pertussis Toxin–Induced 32 P-ADP Ribosylation
32P-ADP ribosylation of Gαs by pertussis toxin was performed for 12 hours at 4°C in a volume of 50 μL containing 100 mmol/L Tris-HCl, pH 8.0, at 20°C. 25 mmol/L DTT, 2 mmol/L ATP, 1 mmol/L GTP, 50 mmol/L 32P-NAD (800 Ci/mmol), and 20 μg/mL pertussis toxin that had been activated by incubation with 50 mmol/L DTT for 1 hour at 20°C before the labeling reaction, as described earlier. 3,10

Immunoblotting Techniques
Immunoblotting techniques were performed as described before.10 The polyclonal antiserum (MB 1) was raised in rabbits against the C-terminal decapetide of retinal transducin (KENLKDCGLF) coupled to keyhole limpet hemocyanine. The antiserum recognized Gαs and Gαt, but not Gαi, or Gαo (not shown). Blots were stained with an alkaline phosphatase–labeled goat anti-IgG antiserum.

RNA Preparation
Total RNA from frozen left ventricular tissue samples was prepared according to the protocol of Chomczynski and Sacchi.13 Between 50 and 100 μg of total RNA were obtained from 150 mg tissue. The mean yield did not significantly differ between myocardium from TG(mREN2)27 and SD.

Northern Blotting Techniques
Total RNA (10 μg) was separated on a 6% formaldehyde/1.2% agarose gel, blotted on nylon membranes (Schleicher and Schuell) by overnight capillary blotting, and fixed by UV irradiation. The techniques have been recently described by Flesch et al.11 The membrane was hybridized with a 1.75-kb cDNA fragment encoding for Gαs. Quantification of the signals was performed by densitometric analysis using the Image Quant Densitometric System (Molecular Dynamics).

Reverse Transcription–Polymerase Chain Reaction
Total RNA from stellate ganglia was extracted by a modification of the method of Chomczynski and Sacchi using the single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction using the RNA-Clean Kit (AGS) according to the manufacturer’s instructions. Tissues were homogenized in RNA-Clean with a glass-Teflon homogenizer, followed by phenol-chloroform extraction and 70% ethanol washing of precipitated RNA. cDNA was synthesized from total RNA (1 μg) by mouse lymphoma virus–derived reverse transcriptase (BRL) using a random hexamer primer (Boehringer-Mannheim). After incubations at 42°C for 5 minutes, reverse transcription was terminated by heating at 95°C for 5 minutes and, 2 μL from the resulting cDNA was removed for subsequent polymerase chain reaction (PCR). These reactions were performed in 10 mmol/L Tris-HCl, pH 8.3; 50 mmol/L KCl; 1.5 mmol/L MgCl2; 0.001% (wt/vol) gelatin; 0.2 mmol/L dATP, dCTP, dGTP and dTTP; Taq DNA polymerase (1.25 U, Boehringer-Mannheim); and 50 pmol of each primer. Synthetic oligonucleotide primers used were 5’-CGA TGT TTG GCG TTT CCC CTA TC-3’ and 5’-CGA CGA CCA TCA TCA GAC AGA GCA-3’, for the uptake, carrier according to Ungerer et al14 and 5’-ACC ACA GTC CAT GCC ATC AC-3’ and 5’-TCC ACC CTG TTG CTG TA-3’ for GAPDH as an external control; 34 cycles of 95°C for 45 seconds, 62°C for 45 seconds, and 72°C for 90 seconds followed by 7 minutes at 72°C were performed. This yielded fragments of 502 bp for the neuronal uptake carrier and 513 bp for GAPDH. Under these conditions, the amplification was linear. PCR products were separated on 1.5% (wt/vol) agarose gels and transferred by capillary blotting onto nylon membranes (Hybond N, Amersham Buchler). Southern blot hybridization was performed with the hybridization buffer containing 50% formamide, 100 mg/mL salmon sperm DNA, 6× SSC, and 0.5% SDS. 3P-labeled probes were prepared by the random primer method using the Prime-It II kit (Stratagene). The membranes were washed twice with 2× SSC at room temperature and once with 2× SSC, 0.1% SDS at 50°C for 60 minutes. Autoradiography was performed, and autoradiograms were quantified by laser densitometry (Image Quant). PCR amplification has been shown to be linear within a range of 28 to 40 cycles (not shown).

Reconstitution of Myocardial Gαs Into S49 Cyclophilin Membranes
Reconstitution assays were performed as described previously.10
Norepinephrine Determinations
For norepinephrine measurements, tissue samples were homogenized with a Polytron device in 0.1 mol/L Tris HCl at pH 7.4. After centrifugation (10 000g, 30 minutes), norepinephrine was extracted with alumina and determined by high-performance liquid chromatography with electrochemical detection as described by Beschi et al.15

Force of Contraction
Experiments were performed on electrically driven (1 Hz) rat papillary muscles. Papillary muscles of uniform size from the left ventricles of TG(mREN2)27 and control rats (diameter, <1.0 mm; length, 3 to 6 mm) were dissected in aerated bathing solution (for composition, see below) at room temperature. The preparations were attached to a bipolar platinum stimulating electrode and suspended individually in 75-mL glass tissue chambers for recording of isometric contractions. The bathing solution was a modified Tyrode’s solution containing (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. It was continuously gassed with 95% O2 and 5% CO2 to record concentration-response curves. Positive inotropic response to norepinephrine was determined with cumulative addition of the solution until complete mechanical stabilization. Positive inotropic responses were measured with an inductive force transducer (W. Fleck) attached to stainless-steel fibers and displayed on a Hellige Helco Scriptor or Gould recorder. Each muscle was stretched to the length at which contraction force was maximal. The resting force (approximately 5 mN) was kept constant throughout the experiment. Preparations were electrically paced at 1 Hz with rectangular pulses of 5 milliseconds in duration (Grass stimulator 78). The voltage was approximately 20% greater than threshold.

Miscellaneous
Protein was determined according to Lowry et al16 using bovine serum albumin as the standard. SDS-polyacrylamide gel electrophoresis was performed as described by Lämmli.17

Materials
Forskolin was donated by Hoechst AG (Frankfurt, Germany). GTP, guanylylimidodiphosphate (Gpp(NH)p), ATP, creatine phosphate, and creatine kinase were purchased from Boehringer-Mannheim; isobutylmethylxanthine (IBMX) was from EGA-Chemie. The ligands 125I-Cyp and 3H-nisoxetine were from Amersham Buchler. DTT was from Serva. Pertussis toxin was from List Biological Laboratories. All other compounds used were of analytical or best grade commercially available. Only deionized and double distilled water was used throughout.

Statistics
The data shown are mean±SEM. Statistical significance was estimated with ANOVA according to Wallenstein et al.18 A value of P<0.05 was considered significant. Ki values were determined graphically in each individual experiment.

Results
Heart Weights and Hemodynamic Changes
In TG(mREN2)27 rats, absolute and relative heart weights were significantly increased. The increase in total heart weight was due to an increase in relative and absolute weights of the left ventricles, whereas no significant differences were observed between the right ventricular weights of the 2 groups.

Adenyllyl Cyclase Activity
Basal (~64%–), Gpp(NH)p (~35%–), and isoprenaline (~55%–)–stimulated adenylyl cyclase activities were depressed in left ventricles but were unchanged in right ventricular membranes from TG(mREN2)27 compared with SD (Figure 1). The effect of forskolin was slightly depressed in left ventricular membranes but unchanged in the right ventricle of TG(mREN2)27.

β-Adrenergic Receptors
β-Adrenoceptors were determined by radioligand binding experiments using 125I-Cyp. β-Adrenergic receptors were reduced in right and left ventricles of TG(mREN2)27 compared with controls (not shown). The reduction was similar in both chambers (38±6 versus 19±4 fmol 125I-Cyp/mg protein, right ventricle, P<0.01; 39±6 versus 24±6 fmol 125I-Cyp/mg protein, n=8 to 10, left ventricles, P<0.01, not shown). The Ki values did not differ (not shown).

Stimulatory G-Protein α-Subunits
To study whether the function of stimulatory G-protein α-subunits (Gαs) is impaired in TG(mREN2)27, Gαs proteins solubilized from membranes were reconstituted into membranes from S49 cyn– mouse lymphoma cells, which genetically lack endogenous Gαs. After reconstitution, isoprenaline- and Gpp(NH)p-stimulated adenylyl cyclase activities were not different whether S49 cyn– membranes were supplemented with Gαs from TG(mREN2)27 or SD (not shown).

Figure 1. Basal, Gpp(NH)p (30 μmol/L), isoprenaline (100 μmol/L), and forskolin (30 μmol/L)–stimulated adenylyl cyclase activities in right (RV) and left (LV) ventricular membranes from transgenic rats [TG(mREN2)27] and age-matched Sprague-Dawley rats (SP) as controls.

Figure 2. Incorporation of radioactivity by pertussis toxin–catalyzed 32P-ADP ribosylation into approximately 40 kDa of right and left ventricular membranes from transgenic rats [TG(mREN2)27] and age-matched Sprague-Dawley rats (SP) as controls.
Inhibitory G-Protein α-Subunits

To study whether differential alterations of inhibitory G-protein α-subunits (G\textsubscript{i}α) in the right and in the left ventricles of TG(m\textsubscript{REN}2\textsuperscript{27}) could account for the differences in adenylyl cyclase activity, the levels of G\textsubscript{i}α were determined with pertussis toxin–catalyzed 32\textsuperscript{P}-ADP ribosylation. An increase by 45% of pertussis toxin substrates was observed in the left ventricles of TG(m\textsubscript{REN}2\textsuperscript{27}), whereas no change was observed in right ventricular preparations (Figure 2). We then asked whether the upregulation of G\textsubscript{i}α occurs at the pretranslational level. Steady-state mRNA levels were measured by Northern blot hybridization experiments. Northern blots of total mRNA with the cDNA fragment encoding for G\textsubscript{i}α hybridized to a single band at 2.4 kb. In left ventricles, the signal intensity was increased by 40% compared with that of controls (Figure 3). In the right ventricles, no differences were detected between TG(m\textsubscript{REN}2\textsuperscript{27}) and controls.

Myocardial Norepinephrine Concentrations

To study whether sympathetic activation is different in right and left ventricles, myocardial norepinephrine concentrations were studied in both chambers. Myocardial norepinephrine levels were reduced by 73% in the left ventricles but by only 28% in the right ventricles (Figure 4, top). Taken together, as judged from the myocardial norepinephrine concentrations, sympathetic activation appears to be stronger in the pressure-overloaded left ventricles than in the nonhypertrophied right ventricles. Corresponding to this, circulating norepinephrine (110.6±24.1 {[n=8]} versus 71.2±11.4 {[n=10]} pmol/mL) serum concentrations were not significantly changed in TG(m\textsubscript{REN}2\textsuperscript{27}) compared with controls.

Norepinephrine Uptake\textsubscript{1} Carrier Sites

To investigate whether an alteration of myocardial norepinephrine uptake\textsubscript{1} carrier sites could play a role in the differential alterations of norepinephrine concentrations in the ventricular chambers, densities of these carrier proteins were determined by radioligand binding experiments using the carrier-specific ligand \textsuperscript{3}H-nisoxetine. Binding of \textsuperscript{3}H-nisoxetine was monophasic and saturable (not shown). Figure 4, bottom, summarizes the densities in the right and left ventricles. The decline in uptake\textsubscript{1} carrier sites was 55% in left ventricles, whereas the density was similar in right ventricles. To study the mechanisms of the decline in norepinephrine uptake\textsubscript{1} sites, we investigated uptake\textsubscript{1} carrier mRNA by semiquantitative PCR in the stellate ganglia. Figure 5 shows the PCR product, which was expected given the primers used. Sequence analyses revealed a 100% identity of the sequence of the PCR product with the predicted sequence. No PCR product was detected in the heart. Analyses showed that there was no difference between TG(m\textsubscript{REN}2\textsuperscript{27}) and control rats (Figure 5).

At this stage, the question remained whether a 55% decline of norepinephrine uptake sites in left ventricles is sufficient to have functional consequences. To address this issue, left ventricular papillary muscle experiments were performed, and the sensitivity for the positive inotropic effect of norepinephrine (110.6±24.1 {[n=8]} versus 71.2±11.4 {[n=10]} pmol/mL) serum concentrations were not significantly changed in TG(m\textsubscript{REN}2\textsuperscript{27}) compared with controls.
nephrine was determined. The maximal positive inotropic effect of norepinephrine was reduced, possibly due to the $\beta$-adrenergic desensitization in TG(mREN2)27 (Figure 6A). However, the effect of norepinephrine was more potent in TG(mREN2)27, indicating that the reuptake of norepinephrine from the synaptic cleft was impaired (Figure 6B).

**Figure 5.** RT-PCR for uptake, carrier mRNA and for the housekeeping gene GAPDH from sympathetic cervical ganglia of transgenic rats [TG(mREN2)27] and Sprague-Dawley rats (SP) as controls. cDNA template from RT reaction was amplified for 32 cycles. Amplification of uptake, and GAPDH cDNA yielded fragments of 513 bp and 612 bp, respectively. Bar graphs show uptake/GAPDH ratio in ganglia from TG(mREN2)27 and SP as controls.

**Figure 6.** Concentration-response curves for the effect of norepinephrine on force of contraction of isolated electrically driven papillary muscle strips from transgenic rats [TG(mREN2)27] and Sprague-Dawley rats (SP) as controls. Graphs show the increase of force of contraction in millinewtons (A) and the positive inotropic effect of norepinephrine in percentage of the maximal increase (B).

**Discussion**

Force of contraction is regulated by norepinephrine that is released from sympathetic nerves on stimulation. The inactivation mechanism is mainly a reuptake of norepinephrine into presynaptic stores by uptake1, carrier proteins.$^{19}$ In situations of neuroendocrine activation, such as in heart failure$^1$ or hypertensive cardiac hypertrophy, the sympathetic drive is increased, producing a desensitization of adenylyl cyclase$^{7,8,20}$ by a decline of $\beta_1$-adrenergic receptors$^{2,8}$ and an increase of $G_i$ proteins.$^{3,5}$ These alterations have also been demonstrated in several models of hypertensive heart disease.$^{7,8}$ It is not completely clear whether circulating catecholamine concentrations or the increased release of norepinephrine from the failing heart is responsible for the desensitization. A decline in uptake, carrier proteins has been suggested to be of relevance$^6$ because the reduction of norepinephrine uptake sites has been reported to increase the interstitial norepinephrine concentrations.$^{21,22}$ Indeed, tracer techniques have shown that increases in interstitial norepinephrine concentrations are negatively correlated with the density of $\beta$-adrenergic receptors.$^{23}$

**Chamber-Specific Alterations in Myocardial Disease**

In patients with dilated cardiomyopathy and predominant left ventricular failure, $\beta$-adrenergic receptors are similarly downregulated in both chambers, but an increase of immunodetectable $G_i$ and a decrease in guanine nucleotide–stimulated adenylyl cyclase activity has been observed only in the left but not in the right ventricles.$^5$ Consistently, myocardial norepinephrine and neuropeptide Y were more depleted in the left than in the right ventricles.$^5$ In patients with primary pulmonary hypertension and isolated right ventricular failure, $\beta$-adrenoceptor downregulation, neurotransmitter depletion, and adenylyl cyclase desensitization occurred only in the right ventricles.$^5$ Therefore, there might be local differences in the regulation of sympathetic neuroeffector mechanisms. When left ventricular heart failure is experimentally induced by destruction of the aortic valve, the reduction of norepinephrine concentrations and of $\beta$-adrenoceptor downregulation is observed in the left ventricle only.$^{24}$ However, when norepinephrine uptake is impaired by chemical denervation of the heart by treatment of animals with 6-hydroxydopamine, $\beta$-adrenergic desensitization is enhanced and the chamber-specific alterations are abolished.$^{24}$ These observations emphasize that norepinephrine-induced uptake mechanisms are critical for $\beta$-adrenergic desensitization in the postsynaptic membranes. Furthermore, when heart failure is induced by systemic application of adriamycin, the reduction of norepinephrine stores and $\beta$-adrenergic receptors occurs in both ventricles to a similar extent.$^{25}$ Thus, mechanical performance of either ventricle can induce alterations of $\beta$-adrenergic signal transduction.

The present findings add one pathological condition to those in which defective norepinephrine uptake plays a role, ie, in the pressure-loaded ventricle in hypertensive cardiac hypertrophy. Candidates that produce differential alterations of presynaptic or postsynaptic sympathetic neuroeffector mechanisms could be a locally different sympathetic activa-
tion or direct stretch and hemodynamic load–induced alterations of the respective ventricle. In the present study, we have taken advantage of the TG(mREN2)27 model, which at the studied age is characterized by compensated cardiac hypertrophy. TG(mREN2)27 develop adenylyl cyclase desensitization due to a downregulation of β-adrenergic receptors and an increase of $G_m$ already in the stage of compensated cardiac hypertrophy. Right ventricular weights were unchanged, whereas there is a marked increase in the left ventricular weights. This model allows the study of the effect of pressure overload on norepinephrine uptake carriers and adenylyl cyclase desensitization in comparison to the non-loaded right ventricle.

**Norepinephrine Concentrations in TG(mREN2)27**

In left ventricles from TG(mREN2)27, there was a stronger decline of myocardial norepinephrine stores than in the right ventricles. Although these measurements cannot discriminate between the norepinephrine concentrations in the presynaptic stores and the synaptic cleft, this observation points toward an impairment of norepinephrine uptake or stronger sympathetic activation in the pressure-overloaded left compared with right ventricles. These findings are similar to the data in the right ventricles with predominant left ventricular failure. In TG(mREN2)27, the transgene is expressed in various tissues in the presence of a suppressed plasma renin activity. Angiotensin II facilitates the release of norepinephrine from sympathetic nerve terminals. Therefore, local formation of angiotensin II by overexpression of the transgene could be involved in sympathetic activation in the heart. The stronger activation in the left ventricle can be explained by an additional sympathetic stimulation due to mechanical overload. Interestingly, norepinephrine plasma concentrations were not significantly changed in TG(mREN2)27 compared with controls. This indicates that the locally released norepinephrine is a stronger determinant of postsynaptic signal transduction defects than circulating plasma catechols. In support of this notion, a report indicated a significant correlation of norepinephrine spillover from the heart but not of plasma concentrations of norepinephrine to the prognosis of patients with heart failure.

**β-Adrenergic Desensitization**

If sympathetic activation is different in right and left ventricles from TG(mREN2)27, one would suggest that postsynaptic alterations of the β-adrenergic receptor/adenylyl cyclase system would also be different. The reduction of β-adrenergic receptors was similar in both right and left ventricular tissue, even though the norepinephrine concentrations were more strongly reduced in the left ventricles. The stimulation of adenylyl cyclase by saturating concentrations of isoprenaline was unchanged in the right but reduced in the left ventricles. The preserved maximal effect in the right ventricle can be explained by the well-documented presence of a receptor reserve in the rat myocardium, which maintains the maximal effect despite a certain degree of receptor downregulation. The depression of the isoprenaline effect in left ventricular membranes is due to the slightly more pronounced relation of β-adrenergic receptors and the identified postreceptor event, i.e., an increase of $G_m$ in the left ventricle. However, $G_m$ was only increased on mRNA and protein level in the left but not the right ventricles. After treatment of rats with isoprenaline, the amount of $G_m$ protein is increased. In neonatal rat cardiomyocytes, it has been shown that the increase of $G_m$ only occurs at high norepinephrine concentrations and after prolonged exposure to norepinephrine, whereas the decline of β-adrenoceptors is more sensitive to norepinephrine. Therefore, the increase of $G_m$ mRNA, protein levels, and $G_m$–related pertussis toxin substrates is most likely mediated by the stronger increase of β-adrenergic drive in the left ventricles than in the right ventricles of TG(mREN2)27.

**Norepinephrine Uptake, Carrier Sites**

Norepinephrine uptake is inversely related to the number of β-adrenergic receptors in a model of experimental heart failure. Thus, alterations of uptake carrier sites could contribute to the different degree of norepinephrine depletion and β-adrenergic desensitization in right and left ventricles. There was a 55% decline of norepinephrine uptake sites. The decline in uptake carriers apparently is due to an increase in protein degradation, reduced translation, or altered posttranslational processing rather than to a reduced gene expression of the protein because gene expression was not altered in stellate ganglia. The observed reduction of uptake, carrier sites is functionally relevant, as demonstrated by the increased sensitivity of the hypertrophied ventricles to the positive inotropic effect of norepinephrine. The decline in uptake sites and the left shift of the concentration–response curve of norepinephrine corresponds to the observations made previously in failing human myocardium. Infusion of norepinephrine into nondiseased dogs with a presumably intact norepinephrine uptake did not reduce β-adrenergic receptor number even though norepinephrine plasma concentrations were increased by a factor of 15. This findings emphasize a role for an intact norepinephrine uptake mechanism to protect the heart from β-adrenergic desensitization. Evidence for a chamber-specific regulation has been provided by data in rats on right heart failure due to monocrotaline treatment. In these rats, uptake carrier sites were reduced in the right but not left ventricles. In support of these data are recent observations in rats with left ventricular myocardial infarction. In the noninfarcted regions of the left ventricles, but not in the right ventricles, isoprenaline- and Gpp(NH)p-stimulated adenylyl cyclase activity was reduced. This was associated with an increased turnover of norepinephrine in left but not right ventricles.

**Conclusions**

Degree and mechanisms of β-adrenergic desensitization in cardiac hypertrophy due to pressure overload and in heart failure can be different in both chambers, indicating that not only systemic but also local control of cardiac contractility is altered in these pathological conditions. The reduction of uptake carrier sites appears to be pathophysiologically relevant in altering recapture of norepinephrine at sympathetic nerve endings, thus potentiating or prolonging receptor activation and promoting β-adrenergic desensitization. It most likely is induced by pressure overload of the left ventricles.
Because the presynaptic alterations are chamber-specific and occur already before the occurrence of overt heart failure, they could contribute to the progression of left or right ventricular dysfunction in various pathological conditions such as heart failure, cor pulmonale, or ventricular dysfunction in various valve diseases. Presynaptic alterations of the sympathetic nerve terminals could provide additional targets for the pharmacological treatment of these disabling conditions.

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References

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