Agonistic Anti-β₁-Adrenergic Receptor Autoantibodies From Cardiomyopathy Patients Reduce the β₁-Adrenergic Receptor Expression in Neonatal Rat Cardiomyocytes

Svenia Podlowski, PhD; Hans Peter Luther, MD; Rosemarie Morwinski, PhD; Johannes Müller, MD; Gerd Wallukat, PhD

**Background**—Autoantibodies directed against the β₁-adrenergic receptor have been described in patients with dilated cardiomyopathy. These autoantibodies exert an agonistic, chronotropic effect on spontaneously beating cultured neonatal rat cardiomyocytes. We studied the effect of such antibodies on β₁-adrenergic receptor expression.

**Methods and Results**—Cardiomyocytes were incubated with either the β₁-adrenergic agonist isoproterenol or autoantibodies for 72 hours. β₁-Adrenergic receptor expression was studied on the mRNA level with semiquantitative reverse transcription–polymerase chain reaction and on the protein level with immunoblotting. Isoproterenol downregulated both mRNA and β₁- and β₂-adrenergic receptor protein subtypes, whereas the anti–β₁-adrenergic receptor autoantibodies decreased only the β₁-adrenergic receptor mRNA and protein. Long-term incubation of cultured cardiomyocytes with isoproterenol or the anti–β₁-adrenergic receptor autoantibodies reduced the acute stimulatory effect of isoproterenol on the myocytes. These effects were prevented by incubating the cells with isoproterenol in the presence of propranolol or with anti–β₁-adrenergic receptor autoantibodies in the presence of bisoprolol. Bisoprolol also abolished the reduction of the β₁-adrenergic receptor expression caused by longer-term incubation with isoproterenol and the autoantibodies.

**Conclusions**—We conclude that after longer-term treatment with the anti–β₁-adrenergic receptor autoantibodies, the rat cardiomyocytes showed a β₁-adrenergic receptor expression similar to that observed in failing hearts from patients with dilated cardiomyopathy. (Circulation. 1998;98:2470-2476.)

**Key Words:** receptors, adrenergic, beta antibodies cardiomyopathy myocytes heart failure

The pathogenesis of dilated cardiomyopathy (DCM) is poorly understood; however, autoimmune mechanisms may be in part responsible. Autoantibodies against various cardiac structures have been detected in DCM patients, including the mitochondrial ADP/ATP carrier, laminin, myosin heavy chain, and the β₁-adrenergic receptor (β₁-AR). The failing heart is characterized by a reduced responsiveness to β₁-adrenergic agonists due to persistently elevated circulating norepinephrine levels, which desensitize the β₁-adrenergic receptor (β₁-AR) response and foster adrenergic receptor uncoupling to G proteins. Functional impairment to β₁-adrenergic stimulation could also be caused by an increase in Gₛ activity or expression. There is evidence that alterations of both β₁-AR and Gₛ processes contribute to the loss of response. Several investigators showed a downregulation of the β₁-AR. In contrast, the expression of the β₂-AR was unchanged or only slightly decreased.

Primary cultured rat cardiomyocytes are a convenient model to study adrenergic receptors and their signal transduction pathways. Short-term stimulation by the β₁-adrenergic agonist isoproterenol causes an increase in the beating frequency of the cardiomyocytes, accompanied by a desensitization and downregulation of the β₁-AR after long-term treatment. DCM patient sera containing anti–β₁-AR autoantibodies increase the beating frequency of the cells. The chronotropic activity differs from the effect of isoproterenol in that the β₁-AR is not desensitized in short-term experiments. We proposed earlier that chronic β₁-AR stimulation by anti–β₁-AR autoantibodies may trigger the development of DCM. Evidence for this hypothesis was supplied by Dörffel et al, who removed the anti–β₁-AR autoantibodies in their DCM patients. These results supported the notion that disappearance of anti–β₁-AR autoantibodies is associated with improved cardiac function. To further elucidate the relationship between anti–β₁-AR autoantibodies and β₁-AR and their regulation, we studied the long-term effect of these autoantibodies on both the β₁-AR and β₂-AR subtypes.
Methods

Neonatal Rat Cardiomyocytes

Neonatal rat cardiomyocytes were prepared as described earlier.\textsuperscript{4,15,18} About half the isolated cells were myocytes, and the rest were fibroblasts and other cells. Briefly, single cells were dissociated from the minced ventricles of 1- to 2-day-old Wistar rats by use of a 0.25% crude trypsin solution and were cultured in Halle SM201 medium containing 2 \( \mu \)M/L fluoroedeoxyuridine,\textsuperscript{8,19} which prevents proliferation of any nonmyocyte cells. Longer-term treatment with drugs or with anti-\( \beta_1 \)-AR autoantibodies was performed for 72 hours. During this time, we observed no dedifferentiation of the cultured cells. The control cells were timed controls. Isoproterenol, sodium deoxycholate, and aprotinin were purchased from Sigma. Propranolol was acquired from Isis-Porem Pharma. Bisoprolol was a gift from E. Merck.

Immunoglobulin Fraction Preparation

Serum was obtained from patients with idiopathic DCM. The diagnosis was verified by cardiac catheterization of all patients. Biopsies confirmed the diagnosis and ruled out other heart diseases. The serum immunoglobulin fraction was isolated by ammonium sulfate precipitation at a saturation of 40% and washed twice. The samples were dialyzed against 1 L dialyzed buffer (10 mmol/L sodium phosphate, 154 mmol/L NaCl solution, pH 7.2) at 4°C for 30 hours. The antibodies were taken up in dialyzed buffer, stored at \(-20^\circ\)C, and used in a dilution of 1:40.

RNA Preparation

Cardiomyocytes (\( 6 \times 10^6 \) per flask) were transferred from flasks to reaction tubes and centrifuged for 3 minutes at 3000 \( \times \)g at 4°C. After the medium was removed, total RNA was isolated as described by Chomczynski and Sacchi.\textsuperscript{30}

DNAse Treatment

Isolated RNA (18-\( \mu \)L) samples were mixed with 2.2 \( \mu \)L 10 \times \text{DNAse buffer} (40 mmol/L Tris-HCl, 6 mmol/L MgcCl\textsubscript{2}, pH 7.5)/2 \( \mu \)L DNAse I (Pharmacia Biotech) and heated at 37°C for 10 minutes. The RNA purification was performed with the RNeasy Kit (Qiagen GmbH). The RNA concentration was determined by UV absorption. The ratio of optical densities at 260 and 280 nm was between 1.8 and 2 in all cases.

Reverse Transcription

For reverse transcription (RT) into cDNA, 1 \( \mu \)g of total RNA was used. First, RNA was denatured together with 25 pmol random hexamers for 5 minutes at 70°C and then reverse transcribed by incubation for 60 minutes at 42°C in the presence of 0.5 mmol/L dNTP, 0.01 mmol/L dithiothreitol, 1 U Superscript reverse transcriptase, 1 U RNase H (both Gibco-BRL Life Technologies GmbH) in 50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, and 3 mmol/L MgCl\textsubscript{2}. The final volume was 20 \( \mu \)L.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) was performed with 2 \( \mu \)L (3 \( \mu \)L for \( \beta_1 \)-AR) cDNA in a final reaction volume of 50 \( \mu \)L. The assay mix contained 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L MgCl\textsubscript{2}, 0.2 mmol/L dNTP, 1 mmol/L of the respective primers, and 1.5 U of Taq DNA polymerase (Gibco-BRL Life Technologies GmbH) (Table). After initial denaturation at 95°C for 3 minutes and further denaturation for 1 minute, primer annealing was carried out at 60°C for 1 minute and extension at 72°C for 1 minute. The number of cycles was 27 for GAPDH, 33 for \( \beta_1 \)-AR, and 31 for \( \beta_2 \)-AR. The PCR products were analyzed after 2% agarose gel electrophoresis. We could not coamplify GAPDH with either \( \beta_2 \)-AR or \( \beta_2 \)-AR because the amplification rates of the products were not the same. During coamplification of any 2 sequences, competition occurs for available Taq polymerase, nucleotides, and magnesium. Consequently, the reaction condition may differ from PCR to PCR. We therefore carried out the PCR with aliquots of the same cDNA in separate tubes with GAPDH as an external standard. Because the \( \beta_2 \)-mRNA was weaker in preliminary experiments, a greater quantity of \( \beta_2 \)-mRNA was used in the reaction than \( \beta_2 \)-mRNA or GAPDH. DNA sequencing of both strands from the \( \beta_1 \)-AR and \( \beta_2 \)-AR PCR products was done by InViTek. To quantify the PCR products, agarose gels were filmed with a video camera, and the optical density was measured by densitometry (Raytest). The amount of the PCR products was normalized to the signal of the external standard GAPDH.

Protein Isolation

Cardiomyocytes were washed with PBS buffer (mmol/L: dibasic sodium phosphate 9.1, monobasic sodium phosphate 1.7, NaCl 150, pH 7.4). The cells were then scratched off with a rubber policeman, added to 0.1 mL RIPA buffer (PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS) with freshly added inhibitors (\( \mu \)g/mL: 10 mg/mL PMSF 10, aprotinin 30, 100 mmol/L sodium orthovanadate 10) and homogenized by passage through a 21-gauge needle. The lysate was incubated on ice for 1 hour and centrifuged at maximal speed for 30 minutes at 4°C. The protein concentration was measured according to the method of Lowry et al.\textsuperscript{21}

Electrophoresis and Western Blotting

Electrophoresis was performed with 7.5% SDS–polyacrylamide gels. Before they were loaded onto the gel, the samples were heated for 1 minute at 95°C. Thereafter, the separated proteins were blotted on nitrocellulose. The blots were then incubated with blocking buffer (5% skim-milk powder and TBST: 10 mmol/L Tris-HCl, 150 mmol/L NaCl, 0.05% Tween-20, pH 8.0) for 1 hour at room temperature, followed by incubation with 1 \( \mu \)g/mL anti–\( \beta_1 \)-AR or anti–\( \beta_2 \)-AR polyclonal rabbit antibodies (Santa Cruz Biotechnology Inc) in blocking buffer overnight at 4°C. The antibodies were directed against an 18-mer (\( \beta_1 \)-AR, 466 to 484) or 19-mer (\( \beta_2 \)-AR, 399 to 418) peptide of the carboxy terminus of the \( \beta_2 \)-AR. After 2 washes for 7 minutes with TBST incubation with horseradish peroxidase–conjugated anti-rabbit antibodies (Sigma) was carried out at a dilution of 1:12 000 for 1 hour at room temperature. The blots were then washed 3 times with TBST and once with TBS for 5 minutes. Detection of the proteins was performed with an ECL Kit (Amersham Buchler GmbH & Co KG). The immunostaining intensity was measured densitometrically. To check the signal specificity, \( \beta_1 \)-AR or \( \beta_2 \)-AR polyclonal antibodies were preincubated with the corresponding peptides (5 \( \mu \)g/mL for \( \beta_1 \)-AR; 20 \( \mu \)g/mL for \( \beta_2 \)-AR) for 5 hours at room temperature.

Immunoprecipitation

We used IgG fractions from 3 patients with DCM previously applied to the stimulatory test. These IgG fractions contained autoantibodies against the \( \beta_2 \)-AR. These autoantibody-containing IgG fractions had been collected by immunoadsorption, and their specificity had been tested in a functional test as described below. The autoantibodies were specifically directed against amino acids 197 to 222 of the second extracellular loop of the \( \beta_2 \)-AR. First, anti–\( \beta_1 \)-AR autoantibodies were linked to Fc-specific anti-human IgG and conjugated with magnetic beads by overnight incubation at 4°C on a rotator. After 3 washes with PBS, solubilized membrane proteins were incubated with the joined antibodies for 2 hours at 4°C and washed 3 times with RIPA buffer. The precipitated proteins were eluted with 70 \( \mu \)L SDS sample buffer by heating at 95°C for 3 minutes. Precipitated proteins were separated by 7.5% SDS-PAGE and blotted on nitrocellulose. We used a commercial peptide antibody for detection raised against the \( \beta_2 \)-AR C terminus (Santa Cruz).
**Functional Test**

Four-day-old neonatal cardiomyocyte cultures were used to determine the stimulatory effect of isoproterenol on the spontaneously beating rate as described elsewhere.\(^4,15,17,18\)

**Statistical Analysis**

Values are given as mean ± SEM. We used Student’s *t* test for paired and unpaired groups or 1-way ANOVA with the Bonferroni post hoc test for selected pairs.

**Results**

**RT-PCR**

RT-PCR resulted in single, specific products of the expected size (Figure 1, top). Sequence analysis was carried out with the corresponding known sequences of both receptor subtypes.\(^22,23\) For quantification, we optimized the reactions. The cycle number was titrated for sufficient but exponential amplification (Figure 1, bottom). Furthermore, RT was done with different amounts of RNA. The amplification of the \(\beta_1\)-AR and the \(\beta_2\)-AR was proportional to the amount of RNA (0.5 to 1.25 \(\mu\)g). For all further experiments, 1 \(\mu\)g of total RNA was used for the RT. GAPDH expression was unchanged in differently treated cells. We were therefore able to use GAPDH as an external standard.

Four-day-old neonatal rat cardiomyocytes were treated for 72 hours with isoproterenol. Both the \(\beta_1\)-AR and \(\beta_2\)-AR mRNAs were significantly reduced after treatment with isoproterenol (Figure 2), to the same level, compared with untreated cells (\(\beta_1\)-AR, 71.6%; \(\beta_2\)-AR, 73±7%). Incubation with anti–\(\beta_1\)-AR autoantibodies had different effects on the mRNA expression of the receptor subtypes (Figure 3). After 72 hours of treatment, the expression of \(\beta_1\)-AR mRNA was significantly decreased to 66±5% of control and corresponded to the isoproterenol-treated cells. In contrast, the \(\beta_2\)-AR mRNA was unchanged by the anti–\(\beta_1\)-AR autoantibody incubation (94±9%). To check whether or not the resulting downregulation of the \(\beta_1\)-AR mRNA was a specific anti–\(\beta_1\)-AR autoantibody–mediated effect, cells were treated with IgG fractions from healthy controls (negative IgG fraction). Figure 3 shows that the \(\beta_1\)-AR was expressed equally in untreated cells and in cells incubated with negative IgG fractions (110%). In comparison, the expression was markedly reduced in anti–\(\beta_1\)-AR autoantibody–treated cells.

**Western Blotting**

\(\beta_1\)-AR and \(\beta_2\)-AR immunodetection was carried out with polyclonal antibodies raised against a peptide of the carboxy terminus. For the \(\beta_1\)-AR, a 42-kDa protein was detected, and for the \(\beta_2\)-AR, a 65-kDa protein was detected. To check the specificity of the signals, antibodies were neutralized with the respective peptides before immunodetection (Figure 4). Furthermore, immunoprecipitation was performed for the \(\beta_1\)-AR using anti–\(\beta_2\)-AR autoantibodies of DCM patients. The precipitated proteins were electrophoretically separated, transferred to nitrocellulose, and detected by the Santa Cruz \(\beta_1\)-AR antibodies. Figure 4b shows that the anti–\(\beta_1\)-AR antibodies directed against the second extracellular loop precipitated a 42-kDa protein that was detected by the second \(\beta_1\)-AR antibodies directed against the carboxy terminus. The left lane

---

### Sequences of Forward and Reverse Oligonucleotide Primers Specific for Rat Sequences of \(\beta_1\)-Adrenergic Receptor, \(\beta_2\)-Adrenergic Receptor, and GAPDH

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Primer Orientation</th>
<th>Position in Coding Sequence</th>
<th>Distance, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta_1)-Adrenergic receptor</td>
<td>Forward</td>
<td>273</td>
<td>522</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>795</td>
<td></td>
</tr>
<tr>
<td>(\beta_2)-Adrenergic receptor</td>
<td>Forward</td>
<td>363</td>
<td>387</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>750</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>Forward</td>
<td>595</td>
<td>605</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>1201</td>
<td></td>
</tr>
</tbody>
</table>

The position of the primers in the coding sequence of the respective mRNAs, the primer orientation, and the length of the generated PCR products are indicated.

---

**Figure 1.** Top, 2% agarose gel electrophoresis of GAPDH, \(\beta_1\)-AR, and \(\beta_2\)-AR RT-PCR products. After RT of 1 \(\mu\)g total RNA from neonatal rat cardiomyocytes, PCRs were performed for 24 (GAPDH), 33 (\(\beta_1\)-AR), and 31 (\(\beta_2\)-AR) cycles. Bottom, Semiquantitative display of GAPDH, \(\beta_1\)-AR, and \(\beta_2\)-AR RT-PCR vs number of PCR cycles. OD indicates optical density.
revealed that additional signals at 55 kDa were due to cross-reactions with the second polyclonal anti-rabbit antibodies. The quantification ranges for both receptor subtypes were determined by protein dependency experiments (Figure 4). The signals increased proportionally with the protein concentrations. For semiquantitative analysis of the β₁-AR, 20 μg of protein was sufficient, whereas 40 μg of protein was loaded for the β₂-AR experiments.

Isoproterenol treatment (Figure 5) led to a significant reduction of both β-AR protein levels (β₁-AR, 50±12%; β₂-AR, 79±5%). Incubation with anti–β₁-AR autoantibodies (Figure 6) decreased only the β₁-AR protein level (65±13%), but not the β₂-AR (117±5%). This subtype-specific effect of the anti–β₁-AR autoantibodies confirmed the mRNA expression data (Figure 3) shown earlier. In contrast, non–anti–β₁-AR autoantibody–containing IgG fractions did not influence the β₁-AR

![Figure 2](image-url)

**Figure 2.** Expression of the β₁-AR and β₂-AR mRNA in 4-day cultures of neonatal cardiomyocytes after incubation with isoproterenol 10⁻⁵ mol/L for 72 hours. Total RNA was prepared from cardiomyocytes, and semiquantitative RT-PCR was carried out. Isoproterenol decreased both β-adrenergic receptor subtypes significantly. Values were obtained by densitometric analysis of PCR products, normalized to GAPDH signal. **P<0.01, ***P<0.001 (Student’s t test).

![Figure 3](image-url)

**Figure 3.** Effect of isoproterenol, anti–β₁-AR autoantibodies, and IgG fraction from normal subjects on mRNA expression of β₁-AR and β₂-AR. After 72 hours of treatment, both isoproterenol 10⁻⁵ mol/L and anti–β₁-AR autoantibodies decreased β₁-AR mRNA level significantly (a). Anti–β₁-AR autoantibodies had no effect on β₂-AR mRNA expression (b). Nonspecific antibodies did not change β₁-AR mRNA expression (c). AAB indicates autoantibody. **P<0.01, ***P<0.001 (1-way ANOVA with Bonferroni post hoc test). OD indicates optical density.

![Figure 4](image-url)

**Figure 4.** Determination of β₁-AR and β₂-AR on protein level in neonatal rat cardiomyocytes. Solubilized proteins were separated by 7.5% SDS-PAGE, blotted on nitrocellulose, and incubated with polyclonal peptide antibodies. a, For β₁-AR, immunoreaction revealed a single band at molecular weight of ~42 kDa. Preincubation of antibodies with respective oligopeptide led to disappearance of signal. Protein amount was plotted against mean optical density (OD) (n=3). b, Immunoprecipitation of β₁-AR protein. Anti–β₁-AR autoantibodies directed against second extracellular loop of β₁-AR were linked to anti-human IgG, conjugated with magnetic beads, and incubated with solubilized cells. Precipitated proteins were detected by polyclonal peptide antibodies against carboxy terminus of β₁-AR (lane 1). Lane 2 revealed nonspecific signals due to polyclonal anti-rabbit antibodies. c, Detection and protein dependence of β₂-AR protein.
protein expression. Because isoproterenol and the anti-β₁-AR autoantibodies reduced the β₁-AR, we tested whether or not a specific β₁-adrenergic blocker would abolish the β₁-AR down-regulation. Cardiomyocytes were treated with isoproterenol and bisoprolol simultaneously for 72 hours. We applied anti-β₁-AR autoantibodies and bisoprolol to the cells in the same manner and found that bisoprolol partly prevented the isoproterenol-induced β₁-AR reduction (Figure 7). The effect of the anti-β₁-AR autoantibodies was completely blocked by bisoprolol.

**Functional Tests**

Isoproterenol stimulation was measured in untreated cells and in cells preincubated with 10 μmol/L isoproterenol or anti-β₁-AR autoantibodies for 72 hours. A significant reduction in the stimulatory effect (Figure 8) was observed with both isoproterenol and anti-β₁-AR autoantibodies ($P<0.001$). When isoproterenol was applied together with the nonselective β-adrenergic antagonist propranolol for 72 hours, the decrease in number of beats was completely blocked. Similar results were found after long-term treatment of anti-β₁-AR autoantibodies together with the β₁-specific antagonist bisoprolol.

**Discussion**

We showed that nonselective isoproterenol downregulated both β₁- and β₂-AR mRNA and protein in neonatal rat cardiomyocytes, whereas the selective anti-β₁-AR autoantibodies downregulated only β₁-AR mRNA and β₁-AR protein. These findings underscore the specific action of the anti-β₁-AR autoantibodies. Our observations were confirmed by experiments in which we incubated the cells with isoproterenol or anti-β₁-AR autoantibodies in the presence of bisoprolol. Bisoprolol, which occupied the β₁-AR, inhibited the receptor downregulation from both isoproterenol and anti-β₁-AR autoantibodies. Finally, our functional studies support
our conclusions in that propranolol blocked the desensitization afforded by preincubation with isoproterenol and bisoprolol blocked the desensitization afforded by preincubation with β1-agonistic anti–β1-AR autoantibodies.

Immunoblotting revealed bands at ≈42 kDa and 65 kDa for the β1-AR and β2-AR, respectively. Our molecular weight estimates for the β1-AR differ from those of other investigations. 21-23 Hebert et al 24 performed immunoblotting experiments and showed that the human β1-AR expressed in SF9 cells had a monomeric (43 to 50 kDa) and a dimeric (85 to 95 kDa) form. We demonstrated that our immunoblotting signal represented the β1-AR by showing that the 42-kDa protein was recognized by 2 different antibodies. One of these antibodies (from the patients) precipitates the 42-kDa protein, and the other (Santa Cruz) was used for the detection.

Figure 8. Stimulatory effect of isoproterenol on beating frequency of neonatal cardiomyocytes treated with isoproterenol 10−6 mol/L, anti–β1-AR autoantibodies, isoproterenol/propranolol 10−5 mol/L, and anti–β1-AR autoantibodies/bisoprolol 10−6 mol/L for 72 hours. Figure shows increase in beats per minute. *P<0.05, ***P<0.001 (1-way ANOVA with Bonferroni post hoc test). iso indicates isoproterenol; biso, bisoprolol; and prop, propranolol.

The effect of anti–β1-AR autoantibodies on β2-AR expression. Thus, the downregulation was indeed a specific effect of the anti–β1-AR autoantibodies.

The effect of anti–β1-AR autoantibodies on β2-ARs in neonatal rat cardiomyocytes displays similarities to effects observed in failing human hearts. Several studies show that only the β2-AR mRNA levels are reduced in failing hearts. 11,12,14 The expression of the β2-AR mRNA is unchanged or only slightly decreased. 13 The downregulation has been attributed to the effects of norepinephrine, which differs from isoproterenol in terms of β2-AR affinity. Norepinephrine is a potent β1-AR and α1-AR agonist and has little action on β2-AR, whereas isoproterenol has a powerful action on all β-AR. 31

Similarly, radioligand binding studies reveal that only the β1-AR but not the β2-AR density is lower in hearts from DCM patients. 12,13 A condition in which circulating β1-AR antibodies have been described. 15-17 Our functional data corresponded with the results on mRNA and protein levels. Longer-term treatment with isoproterenol and anti–β1-AR autoantibodies led to a loss of cardiomyocyte isoproterenol responsiveness. In failing hearts, the reduction of the β1-AR number is in accord with the decreased positive inotropic effect of isoproterenol. 7 Our findings elucidate the role of circulating β1-AR autoantibodies in DCM patients. We suggest that, like norepinephrine in any form of heart failure, the autoantibodies stimulate, downregulate, and reduce the responsiveness of contractile mechanisms.

β-Blockers offer a further medical approach to heart failure therapy in DCM patients. 32 We showed that β1-AR downregulation with either isoproterenol or anti–β1-AR autoantibodies was prevented by β1-adrenergic blockers. In Western blotting experiments, bisoprolol partly abolished the isoproterenol-induced β1-AR downregulation and blocked the anti–β1-AR autoantibody–induced downregulation completely. Similarly, in the functional test, the chronotropic response was restored by propranolol and bisoprolol. Not only norepinephrine but also anti–β1-AR autoantibodies could be involved in the decrease of β1-AR number and β-adrenergic responses in DCM patients. The possibility that anti–β1-AR autoantibodies could play a role in the pathogenesis of DCM is underscored by the association between the autoantibodies and cardiac function. 15-17 In these patients, the removal of anti–β1-AR autoantibodies with immunoadsorption led to improved cardiac function. Randomized trials will be necessary to confirm these results; however, our present data may provide a mechanism by which these autoantibodies lead to and maintain DCM.

β1-AR downregulation probably depends on microtubules, because colchicine pretreatment inhibited β1-AR but not β2-AR downregulation in a glioma cell line. 33 The microtubule stabilizer taxol inhibited the effects of colchicine on isoproterenol-induced β1-AR downregulation. The effect appears to occur distal to the receptor at the level of Gs protein and/or via cAMP generation. 34 Hori et al 35 demonstrated that β-adrenergic stimulation with norepinephrine induced microtubular disassembly via the β1-AR. The process was accompanied by increased calcium influx, which may have inhibited tubulin polymerization, thereby disrupting cellular integrity. The β1-AR may be abolished by the β1-adrenergic blocker bisoprolol. The IgG fractions from healthy controls did not influence the β2-AR expression. Thus, the downregulation was indeed a specific effect of the anti–β1-AR autoantibodies.

Our data showed that prolonged isoproterenol treatment reduced both the β1-AR and β2-AR mRNA and protein levels, which is in accord with the observation that isoproterenol causes a downregulation of β2-AR and β2-AR mRNA levels in DDT-MF2 hamster cells, C6 glioma cells, and H9c2 cells. 28-30 Anti-β1-AR autoantibodies had an exclusive effect on the β1-AR gene and protein expression. In contrast, the β2-AR expression was nearly unchanged. The modulation of the β1-AR expression was
regulated by alternative mechanisms. For instance, in rat pulmonary cells, isoproterenol leads to downregulation of both β1- and β2-AR; however, dexamethasone selectively inhibits the downregulation of the β1- but not the β2-AR. This process involves the transcription factor, cAMP response element binding protein (CREB). We do not know to what extent altered regulation via microtubular function or differential regulation of the cAMP response element by CREB may have contributed to the phenomena we observed. Further investigations of β1- and β2-adrenergic signaling pathways and their regulation in cardiomyocytes will be necessary to elucidate these issues.

Acknowledgments

This work was financially supported by a Biomed-2 European Project (BMH4-CT95–1008) and by the Sparkassen Stiftung Medizin. We are grateful to Prof. A. Wollenberger for his scientific suggestions. We thank Prof. F.C. Luft for helping us with the manuscript. We thank Holle Schmidt, Monika Wegener, and Karin Karczewski for excellent technical assistance.

References


Agonistic Anti-β₁-Adrenergic Receptor Autoantibodies From Cardiomyopathy Patients Reduce the β₁-Adrenergic Receptor Expression in Neonatal Rat Cardiomyocytes
Svenia Podlowski, Hans Peter Luther, Rosemarie Morwinski, Johannes Müller and Gerd Wallukat

Circulation. 1998;98:2470-2476
doi: 10.1161/01.CIR.98.22.2470
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/98/22/2470

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/