Significance of Myocardial $\alpha$- and $\beta$-Adrenoceptors in Catecholamine-Induced Cardiac Hypertrophy

W. Zierhut and H.-G. Zimmer

The role of $\alpha$- and $\beta$-adrenoceptors in the development of catecholamine-induced cardiac hypertrophy in vivo was investigated. Rats received a constant intravenous infusion of norepinephrine or sodium chloride (control) for 3 days. The norepinephrine infusion was combined with the $\alpha$-blocker prazosin, the $\beta$-blocker metoprolol, or both blockers. For modulation of the work load of the heart, the calcium channel blocker verapamil was added to the norepinephrine infusion. A further group of animals was treated with the $\alpha$-adrenergic stimulator norfenephrine, which also was combined with prazosin or verapamil. Norepinephrine induced significant increases in mean aortic pressure, left ventricular $dP/dt$, heart rate, and total peripheral resistance. The left ventricular weight/body weight ratio was significantly elevated and was accompanied by an increase in the RNA concentration and the RNA/DNA ratio. Prazosin as well as metoprolol partially antagonized the increase in left ventricular weight and RNA concentration, whereas simultaneous prazosin and metoprolol treatment prevented the norepinephrine-induced alterations. Although combination of norepinephrine with verapamil resulted in considerable reduction of all functional parameters, the development of cardiac hypertrophy and the elevated RNA/DNA ratio were not significantly influenced. Stimulation of $\alpha$-receptors with norfenephrine elicited an increase in total peripheral resistance and in left ventricular weight, which was abolished by prazosin. Verapamil did not affect the norfenephrine-induced cardiac hypertrophy, although it normalized essentially all functional parameters. Thus, the rapid development of cardiac hypertrophy in the norepinephrine model seems to be directly mediated by stimulation of myocardial $\alpha$- and $\beta$-adrenoceptors rather than by hemodynamic changes. (Circulation Research 1989;65:1417-1425)

In spite of intensive investigations, the signal linking increased work load of the heart with the development of cardiac hypertrophy is still a subject of controversy. Many authors consider the catecholamine norepinephrine (NE) to play an important role in this context. In a number of pathophysiological conditions leading to cardiac hypertrophy, the activity of the sympathetic nervous system is enhanced, resulting in increased release of NE from the sympathetic nerve endings within the myocardium. Furthermore, administration of catecholamines can induce cardiac hypertrophy, and in addition, exercise-induced cardiac hypertrophy can be prevented by chemical sympathectomy. However, in other studies using different models, hypertrophy occurred independently of the adrenergic system. Thus, the significance of catecholamines seems to be dependent on the model used for initiation of cardiac hypertrophy.

The stimulus responsible for mediation of cardiac hypertrophy by NE may be via activation of $\alpha$- and $\beta$-adrenoceptors. $\alpha_1$, $\beta_1$, and $\beta_2$-adrenoceptors have been shown to exist on the mammalian myocyte. Furthermore, it is well established that activation of myocardial $\beta$-receptors induces cardiac hypertrophy in vivo. More recently, the hypertrophic response of cultured neonatal myocytes was related to an $\alpha_1$-adrenergic effect, while $\beta$-stimulation did not result in cellular hypertrophy. Whether $\alpha_1$-receptors are important mediators of cardiac hypertrophy in vivo is difficult to assess, since $\alpha$-stimulation in the intact animal leads to an increase in work load of the heart due to an increase in peripheral resistance, which per se could induce cardiac hypertrophy. The present study was undertaken to clarify the role of myocardial $\alpha$- and...
β-receptors in the catecholamine-induced hypertrophy in vivo as compared with the peripheral circulatory alterations evoked by NE. Therefore, we developed a model in which cardiac hypertrophy was induced after 3 days of intravenous infusion of NE. The short time period made possible administration of NE and specific antagonists as constant intravenous infusion, which provided an excellent control of the dose.

Our experimental model shows that direct stimulation of α1- and β-adrenoceptors leads to the development of cardiac hypertrophy and that the concomitant hemodynamic changes seem to play a minor role.

Materials and Methods

The experiments were done on female Sprague-Dawley rats (200–250 g body wt) fed a control rat chow diet (Altromin C 1000 Altromin GmbH, Lage, FRG) with free access to water. All substances were given as constant, intravenous infusions for 3 days via a catheter (Vygon, Aachen, FRG) positioned in the left jugular vein. The catheter was connected to a 20-ml syringe placed in an infusion pump (Infor AG, Basel, Switzerland). The infusion rate was 4 ml/kg/hr. The animals could move around freely in their cages during the infusions. NE was administered in a dose of 0.2 mg/kg/hr; sodium chloride–infused animals served as controls. NE was combined with the α1-blocker prazosin (0.1 mg/kg/hr), the β1-blocker metoprolol (1 mg/kg/hr), both prazosin and metoprolol, or the calcium-channel blocker verapamil (1 mg/kg/hr). In addition, a further group of animals received the α-adrenergic stimulator norfenephrine (2 mg/kg/hr), which was also combined with prazosin or verapamil. Thus, the animals were divided into nine groups: 1) control (sodium chloride), 2) NE, 3) NE+prazosin, 4) NE+metoprolol, 5) NE+prazosin+metoprolol, 6) NE+verapamil, 7) norfenephrine, 8) norfenephrine+prazosin, and 9) norfenephrine+verapamil. Although the affinity of metoprolol to the β1-receptor is considerably higher than to the β2-receptor, the dose of 1 mg/kg/hr should be sufficient to block both β1- and β2-adrenoceptors. To test this assumption, a further group of animals was treated with NE in combination with the nonselective blocker propranolol (1 mg/kg/hr). All substances were dissolved in 0.9% NaCl. For prevention of oxidation of the catecholamines, 100 mg/l ascorbic acid was added to the solutions and the syringes were protected against light.

(--)-Norepinephrine HCl was purchased from Sigma Chemical, München, FRG. L-(-)-Ascorbic acid was obtained from Merck, Darmstadt, FRG. Norfenephrine was kindly supplied by Godecke, Freiburg, FRG. Verapamil HCl was provided by Knoll, Ludwigshafen, FRG, and prazosin HCl was donated by Pfizer, Karlsruhe, FRG. Metoprolol tartrate was obtained from Ciba-Geigy, Wehr, FRG, and propranolol HCl was donated by Rhein-Pharma, Plankstadt, FRG.

Hemodynamic Measurements

After 3 days hemodynamic parameters were measured during drug infusion in the anesthetized closed-chest animal (Inactin® 80 mg/kg i.p., Byk Gulden, Konstanz, FRG) according to the method described previously. An ultraminiature catheter tipmanometer (model PR 249, Millar Instruments, Houston, Texas) was inserted into the right carotid artery and advanced into the left ventricle. During a period of 20 minutes left ventricular systolic pressure (LVSP), left ventricular rate of change of pressure (LVdP/dtmax), and heart rate (HR) were recorded simultaneously on a Brush 2600 recorder (Gould, Cleveland, Ohio). Thereafter, the catheter was placed in the aorta to obtain systolic, diastolic, and mean aortic pressure (MAP). Then the tipmanometer was replaced by a thermosensitive 1.5 F implantable microprobe (Columbus Instruments, Columbus, Ohio) for the determination of cardiac output (CO) with the thermodilution method. For this procedure, 0.1 ml of cold saline (18° C) was injected through a polyethylene tube (i.d. 0.58 mm) via the right jugular vein into the right atrium. The microprobe was connected to a Cardiomax II computer (Columbus Instruments). Each CO value was calculated as the mean of five consecutive measurements. Total peripheral resistance (TPR) was estimated by dividing MAP by CO. After the hemodynamic measurements, the hearts were rapidly excised and the right ventricular free wall was trimmed away. The ventricles were weighed and stored under liquid nitrogen until the determinations of RNA and DNA were made.

Metabolic Measurements

RNA and DNA tissue concentrations of the left ventricle including the septum were determined photometrically according to a modified Schmidt-Thannhauser procedure. Briefly, the tissue samples were ground to a fine powder under liquid nitrogen and extracted with 0.3N perchloric acid (PCA). After centrifugation, the sediments were washed three times with 0.3N PCA. The lipids were extracted by use of 10% potassium acetate in 50% ethanol, followed by methanol, chloroform and methanol (2:1), benzene, and diethyl ether. For hydrolysis of RNA, the samples were incubated in 0.5N KOH for 14 hours at room temperature. After addition of 0.5 ml of 6N PCA, the samples were kept in an ice bath for 1 hour. The precipitate (containing DNA) was separated by centrifugation, and the supernatant (containing RNA) was decanted. For estimation of DNA, the sediments were suspended in 2 ml of 1N PCA and incubated in a water bath at 70° C for 1 hour. Then the supernatant was separated by centrifugation. For elimination of errors arising from the release of proteins, mainly in the RNA fraction, during the alkaline digestion in 0.5N...
TABLE 1. Changes in Functional and Metabolic Parameters After 3 Days of Continuous Intravenous Infusion of Prazosin (0.1 mg/kg/hr), Metoprolol, or Verapamil (each 1 mg/kg/hr)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Prazosin</th>
<th>Metoprolol</th>
<th>Verapamil</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>328±6</td>
<td>373±12*</td>
<td>243±21*</td>
<td>373±17</td>
</tr>
<tr>
<td>LVdP/dt\text{\text{max}} (mm Hg/sec)</td>
<td>9,740±309</td>
<td>14,257±981*</td>
<td>6,233±681*</td>
<td>7,500±901</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>123±2</td>
<td>113±3</td>
<td>108±8</td>
<td>105±6*</td>
</tr>
<tr>
<td>CO (ml/kg/min)</td>
<td>353±7</td>
<td>443±24*</td>
<td>303±21</td>
<td>391±20</td>
</tr>
<tr>
<td>TPR (Hg/kg/min/ml)</td>
<td>0.35±0.01</td>
<td>0.26±0.02*</td>
<td>0.37±0.04</td>
<td>0.27±0.01*</td>
</tr>
<tr>
<td>RNA (mg/g/DNA)</td>
<td>1.83±0.03</td>
<td>1.95±0.07</td>
<td>1.79±0.04</td>
<td>1.93±0.03</td>
</tr>
<tr>
<td>LVW/BW (mg/g)</td>
<td>2.35±0.02</td>
<td>2.35±0.06</td>
<td>2.43±0.05</td>
<td>2.44±0.05</td>
</tr>
</tbody>
</table>

Values are mean±SEM; number of experiments in parentheses. HR, heart rate; LVdP/dt\text{\text{max}}, left ventricular rate of change of pressure; MAP, mean aortic pressure; CO, cardiac output; TPR, total peripheral resistance; LVW/BW, left ventricular weight/body weight.

*p<0.05 vs. control.

KOH, the supernatants were measured at two wavelengths (RNA, 260 and 286 nm; DNA, 268 and 284 nm). The reliability of this method was tested by determination of the RNA and DNA concentrations of myocardial tissue enriched with calf thymus DNA (Sigma Chemical) and calf liver RNA (Sigma Chemical). The result was that the RNA concentration was slightly overestimated while the DNA concentration was measured correctly. The overestimation was slightly overestimated while the DNA concentration was measured correctly. The overestimation was less than 10% and of a comparable extent in all determinations so that comparison of the different experimental groups was possible.

Statistical Analysis

Each value is expressed as a mean±SEM. The data were first compared by analysis of variance (ANOVA). In case of significant differences, multiple r testing was done by use of the Bonferroni modification. A value of p<0.05 was considered significant. The significance of correlations was evaluated by calculation of the correlation coefficient rS according to Spearman.

Results

All substances were well tolerated by the animals. None showed signs of cardiac decompensation such as pleural or abdominal effusion. The left ventricular end-diastolic pressure did not differ significantly among the groups; it did not exceed 7 mm Hg. The end-diastolic pressure did not differ significantly as pleural or abdominal effusion. The left ventricular weight ratio was partially antagonized by either a- or B-blockade (Table 3). These changes in RNA metabolism were associated with an elevation in the LV weight/body weight ratio (Table 3, Figure 1A).

Effect of Concomitant a- and B-Adrenergic Blockade on Norepinephrine-Induced Alterations

Simultaneous administration of prazosin antagonized the NE-induced elevation in MAP, but hardly influenced the increase in HR and LVdP/dt\text{\text{max}} (Table 2, Figure 1B). CO was elevated, and TPR fell below the control level. Metoprolol attenuated the increase in HR and LVdP/dt\text{\text{max}}. In contrast with a-blockade, B-blockade reduced the CO and elevated TPR (Table 2, Figure 1C). Prazosin as well as metoprolol significantly attenuated the left ventricular RNA concentration and the RNA/DNA ratio to the same extent (Table 3). Likewise, the LV weight/body weight ratio was partially antagonized by either a- or B-blockade (Table 3). Simultaneous administration of both prazosin and metoprolol attenuated the NE-induced increase in the RNA/DNA ratio and abolished the elevation of the LV weight/body weight ratio (Table 3, Figures 1A and 1D). The functional parameters were different from the control values but in the opposite direction compared with the influence of NE alone (Table 2, Figures 1A and 1D). Combination of NE with propranolol instead of metoprolol revealed no differences in the functional and metabolic parameters between these two groups (data not shown).

Effect of Concomitant Verapamil Treatment on Norepinephrine-Induced Alterations

Verapamil normalized the NE-induced increase in MAP and TPR (Table 2, Figures 1A and 1E). HR was attenuated but did not reach the control level.
TABLE 2. Changes in Functional Parameters After 3 Days of Continuous Intravenous Infusion of Norepinephrine (200 μg/kg/hr) Norfenephrine (2 mg/kg/hr) Alone or in Combination With Prazosin (0.1 mg/kg/hr), Metoprolol, or Verapamil (each 1 mg/kg/hr)

<table>
<thead>
<tr>
<th>HR (beats/min)</th>
<th>LVSP (mm Hg)</th>
<th>LVPd/dt(_{max}) (mm Hg/sec)</th>
<th>MAP (mm Hg)</th>
<th>CO (ml/kg/min)</th>
<th>TPR (mm Hg-kg-min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=23)</td>
<td>328±6</td>
<td>142±2</td>
<td>9740±309</td>
<td>123±2</td>
<td>353±7</td>
</tr>
<tr>
<td>NE (n=13)</td>
<td>448±8*</td>
<td>152±4</td>
<td>21,338±882*</td>
<td>137±4*</td>
<td>330±15</td>
</tr>
<tr>
<td>NE+P (n=8)</td>
<td>411±5*</td>
<td>130±6†</td>
<td>19,900±845*</td>
<td>116±7</td>
<td>466±24*†</td>
</tr>
<tr>
<td>NE+M (n=8)</td>
<td>316±12†</td>
<td>136±7</td>
<td>12,038±756*</td>
<td>120±7</td>
<td>261±11*†</td>
</tr>
<tr>
<td>NE+P+M (n=10)</td>
<td>290±8*†</td>
<td>134±3†</td>
<td>11,290±304*</td>
<td>113±2†</td>
<td>407±12*†</td>
</tr>
<tr>
<td>NE+V (n=8)</td>
<td>382±18*†</td>
<td>136±4</td>
<td>17,638±1324*</td>
<td>116±4†</td>
<td>345±20</td>
</tr>
<tr>
<td>NORF (n=8)</td>
<td>435±10*</td>
<td>144±3</td>
<td>17,687±430*</td>
<td>127±2</td>
<td>285±9*</td>
</tr>
<tr>
<td>NORF+P (n=8)</td>
<td>386±14*</td>
<td>121±2*</td>
<td>12,713±923*</td>
<td>104±3</td>
<td>459±26*</td>
</tr>
<tr>
<td>NORF+V (n=8)</td>
<td>337±7</td>
<td>136±3</td>
<td>12,275±801*</td>
<td>118±3</td>
<td>337±8</td>
</tr>
</tbody>
</table>

Values are mean±SEM; number of experiments in parentheses. HR, heart rate; LVSP, left ventricular systolic pressure; LVPd/dt\(_{max}\), left ventricular rate of change of pressure; MAP, mean aortic pressure; CO, cardiac output; TPR, total peripheral resistance; NE, norepinephrine; P, prazosin; M, metoprolol; V, verapamil; NORF, norfenephrine.

*p<0.05 vs. control.
†p<0.05 vs. NE.

(Table 2, Figure 1E). The myocardial RNA/DNA and LV weight/body weight ratios were still elevated and were not significantly different from those in the NE group (Table 3, Figures 1A and 1E).

Effects of Norfenephrine Without and With Concomitant Infusion of Prazosin or Verapamil

Norfenephrine did not evoke significant changes in LVSP and MAP (Table 2). However, CO was depressed and TPR was higher than the control values (Table 2, Figure 2A). These norfenephrine effects were more pronounced than in the NE experiments. HR and LVPd/dt\(_{max}\) were increased (Table 2, Figure 2A) and the RNA/DNA and LV weight/body weight ratios were enhanced (Table 3, Figure 2A).

Concomitant administration of prazosin lowered MAP and TPR below the control level (Table 2, Figure 2B). There was still a moderate elevation of HR and LVPd/dt\(_{max}\), and CO was increased. The increases in RNA concentration and in LV weight/body weight ratio were abolished; however, there remained a slight elevation of the RNA/DNA ratio (Table 3, Figure 2A).

The results of the norfenephrine+verapamil group correspond well with those of the NE+verapamil group. Although functional parameters except for LVPd/dt\(_{max}\) were essentially normal (Table 2, Figure 2C), the hypertrophy elicited by norfenephrine was not influenced. The RNA concentration was diminished when compared with norfenephrine alone; however, the RNA/DNA ratio was elevated by about the same extent (Table 3, Figures 2A and 2C).

Relation Between Total Peripheral Resistance and Myocardial DNA Concentration

A significant correlation was found between increases in TPR and DNA concentration (Figure DNA concentration tended to be higher in group with elevated TPR (Tables 2 and 3). β-Block (NE+metoprolol group) did not reduce the DNA concentration when compared with the NE group. However, the significantly elevated DNA concentration in the norfenephrine group was antagonized by concomitant prazosin or verapamil treatment.

Discussion

In this in vivo study it has been shown that stimulation of myocardial β- and α-adrenergic receptor either alone or in combination can result in the development of hypertrophy of the rat heart. Card hypertrophy resulting from β-adrenergic stimulation has been studied extensively in the isoproterenol model in vivo.15-18 However, the hypertrophic response to NE in cultured cardiac myocytes shown to be related only to stimulation of the adrenergic receptor.19,20 This raised the question whether the β-receptor-mediated hypertrophy in vivo could be the result of facilitation of NE release from sympathetic nerve endings induced by stimulation presynaptic β2-receptors and, consequently, we depend on postsynaptic α₁-receptor stimulation. If this mechanism were responsible for the receptor-mediated hypertrophy in vivo, β-adrenergic stimulation in the presence of α-blockade should cause hypertrophy. In our study, however, NE in
sion in the presence of α-blockade caused left ventricular hypertrophy as assessed by a significant increase in the LV weight/body weight and RNA/DNA ratios (Figure 1B). Thus, this hypertrophy model was not likely to be mediated by postsynaptic α1-receptors. It may be argued that simultaneous prazosin administration caused an increase in CO, which might serve as a trigger for cardiac hypertrophy independent of the β-receptor. This possibility could be excluded in our experiments by infusion of prazosin alone, which induced a similar increase in CO but did not evoke an elevation in the LV weight/body weight and RNA/DNA ratios (Table 1). Moreover, additional infusion of metoprolol to the NE+prazosin group entirely prevented the development of cardiac hypertrophy (Figure 1D). Thus, cardiac hypertrophy observed after NE infusion seems to be partially mediated by β-adrenergic stimulation.

Recently, findings were presented that link increased mechanical contractions to an increase in myosin synthesis in association with cell growth. Therefore, it is possible that mechanical alterations such as the enhancement of LVDp/dt max could play an important role in the development of hypertrophy. In the present study, no close correlation between LVDp/dt max and hypertrophy could be detected. Despite significant elevations in LVDp/dt max, the groups treated with prazosin alone and norfenephrine+prazosin did not show left ventricular hypertrophy. Furthermore, groups with similar LVDp/dt max values (NE and NE+prazosin; NE+verapamil and norfenephrine; norfenephrine+prazosin and norfenephrine+verapamil) exhibited significantly different degrees of hypertrophy. Thus, in this model LVDp/dt max seems not to play a major role.

Apart from the involvement of cardiac β-adrenergic receptors, more recently the interest has been focused on cardiac α-receptors. In our present study the effect of α-adrenergic stimulation was tested by addition of the β-blocker metoprolol to the NE infusion. The dose of metoprolol was sufficient to block both β1- and β2-receptors despite the β1-selective properties of the drug. This finding is supported by the data obtained in the NE+propran-
TABLE 3. Effects of 3 Days' Infusion of Norepinephrine (200 µg/kg/hr) or Norfenephrine (2 mg/kg/hr) Alone or in Combination With Prazosin (0.1 mg/kg/hr), Metoprolol, or Verapamil (each 1 mg/kg/hr) on the Development of Left Ventricular Hypertrophy as Assessed by Changes in RNA Concentration, DNA Concentration, RNA/DNA Ratio, and Left Ventricular Weight/Body Weight Ratio

<table>
<thead>
<tr>
<th></th>
<th>RNA (mg/g)</th>
<th>DNA (mg/g)</th>
<th>RNA/DNA</th>
<th>LVW(mg)/BW(g)</th>
<th>BW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=23)</td>
<td>4.07±0.06</td>
<td>2.25±0.04</td>
<td>1.83±0.03</td>
<td>2.35±0.02</td>
<td>232±2</td>
</tr>
<tr>
<td>NE (n=13)</td>
<td>6.35±0.18*</td>
<td>2.40±0.07</td>
<td>2.66±0.07*</td>
<td>3.26±0.09*</td>
<td>217±4</td>
</tr>
<tr>
<td>NE+P (n=8)</td>
<td>5.08±0.13*</td>
<td>2.23±0.06</td>
<td>2.28±0.04**</td>
<td>2.67±0.08*</td>
<td>236±3</td>
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<tr>
<td>NE+M (n=8)</td>
<td>5.25±0.15*</td>
<td>2.37±0.06</td>
<td>2.22±0.06*</td>
<td>2.71±0.07*</td>
<td>216±6</td>
</tr>
<tr>
<td>NE+P+M (n=10)</td>
<td>4.20±0.14*</td>
<td>2.09±0.07†</td>
<td>2.01±0.04†</td>
<td>2.44±0.04†</td>
<td>227±4</td>
</tr>
<tr>
<td>NE+V (n=8)</td>
<td>5.63±0.17*</td>
<td>2.27±0.06</td>
<td>2.49±0.07*</td>
<td>3.10±0.10*</td>
<td>213±5</td>
</tr>
<tr>
<td>NORF (n=8)</td>
<td>6.22±0.45*</td>
<td>2.64±0.16*</td>
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<td>2.72±0.10*</td>
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<tr>
<td>NORF+P (n=8)</td>
<td>4.32±0.07</td>
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<td>2.02±0.06*</td>
<td>2.38±0.08</td>
<td>232±6</td>
</tr>
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<td>NORF+V (n=8)</td>
<td>4.78±0.14*</td>
<td>2.13±0.04†</td>
<td>2.25±0.07*</td>
<td>2.69±0.03</td>
<td>226±5</td>
</tr>
</tbody>
</table>

Values are mean±SEM; number of experiments in parentheses. LVW/BW, left ventricular weight/body weight; BW, mean body weight; NE, norepinephrine; P, prazosin; M, metoprolol; V, verapamil; NORF, norfenephrine.

*p<0.05 vs. control.
†p<0.05 vs. NE.
‡p<0.05 vs. norfenephrine.

olol group, which were in close correlation with the NE+metoprolol group. During β-blockade, the LV weight/body weight ratio turned out to be elevated to the same extent as in the NE+prazosin group. However, the NE+metoprolol group was characterized by an increase in TPR, most probably due to activation of vascular α-receptors. One may argue that this alteration in TPR could have served as a trigger for the remaining hypertrophy. In this context the results of the NE+verapamil group are important. Verapamil did reduce TPR and MAP to values slightly below the control level. HR was also reduced but remained elevated (Table 2, Figure 1E). Despite these reductions, the LV weight/body weight and RNA/DNA ratios were not significantly affected as compared with the NE group. In con-

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Percentage changes in functional parameters (HR, MAP, CO, TPR) and in RNA/DNA and LVW/BW ratios in norfenephrine experiments as compared with control (C) values (C=100%) (see Tables 2 and 3). Panel 2A, norfenephrine (NORF); 2B, norfenephrine+prazosin (NORF/P); 2C, norfenephrine+verapamil (NORF/V). HR, heart rate; MAP, mean aortic pressure; CO, cardiac output; TPR, total peripheral resistance; LVW/BW, left ventricular weight/body weight. Significant changes are indicated by asterisks (p<0.05 vs. control).
by stimulation of \( \beta \)-receptors. These results suggest that pressure overload elicits an increase of \( \alpha \)-adrenergic binding sites already in the prehypertrophic period. \( \alpha \)-Blockade attenuated the hypertrophic response.

Our results are in agreement with clinical observations in patients with hypertensive cardiac hypertrophy. The reversal of left ventricular hypertrophy seemed to be determined not only by blood pressure control, but may also depend on other factors such as the activity of the adrenergic system. Treatment of hypertensive patients with diuretics revealed no regression of cardiac hypertrophy in spite of significant reduction of blood pressure. In addition, muscle mass was reduced overproportionally in relation to blood pressure reduction after treatment with clonidine. In contrast with diuretics, clonidine diminished the adrenergic drive. Finally, significant left ventricular hypertrophy was observed in dogs treated with subhypertensive doses of NE. These findings illustrate that altered plasma or tissue levels of catecholamines can be important mediators of cardiac hypertrophy independent of the work load.

In contrast with the myocytes, the adaptive changes in nonmyocardial cells seemed to be dependent on the stress induced by the increase in TPR rather than direct activation by catecholamines. An increase in TPR turned out to be significantly correlated with an increase in DNA concentration (Figure 3). On the other hand, concomitant \( \beta \)-blockade did not reduce the DNA concentration compared with the NE group, and the elevated DNA concentration in the norfenephrine group was antagonized by verapamil or prazosin treatment. Since only about 25% of the nuclei in rat left ventricle are muscle nuclei and since cardiac myocytes lose their mitotic capacity shortly after birth, an increase in the DNA concentration reflects mainly proliferation of connective tissue and other nonmyocardial cells. Myocardial fibrosis is a typical sign of abrupt pressure overload. Thus, our data demonstrate that cardiac hypertrophy can occur with and without an increase in DNA concentration. Furthermore, it is evident that verapamil is effective in reducing the work load of the heart without affecting the NE- or norfenephrine-induced cardiac hypertrophy. Finally, the proliferation of connective tissue in this model seems not to be due to direct activation mediated by \( \alpha \)- or \( \beta \)-receptors.

In conclusion, our data show that in the model of NE-induced hypertrophy myocardial \( \alpha \)- and \( \beta \)-receptors may primarily contribute to the development of cardiac hypertrophy in vivo. However, we want to emphasize that this mechanism probably is limited to pathophysiological circumstances with

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**Figure 3.** Correlation between DNA tissue concentrations and total peripheral resistance (TPR). Alterations in TPR were significantly correlated (correlation coefficient \( r=0.71 \)) with changes in DNA concentration. 

- **•**, control; 
- **○**, norepinephrine; 
- **●**, NE+metoprolol; 
- **○**, NE+prazosin; 
- **△**, NE+prazosin+metoprolol; 
- **‡**, NE+verapamil; 
- **●**, norfenephrine; 
- **○**, norfenephrine+prazosin; 
- **△**, norfenephrine+verapamil.

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trast, simultaneous \( \alpha \)- and \( \beta \)-blockade prevented cardiac hypertrophy. Thus, the NE-induced hypertrophy in our model seems to depend primarily on the direct activation of myocardial \( \alpha \)- and \( \beta \)-receptors, while the hemodynamic changes may play only a minor role.

This concept is confirmed by the results of our norfenephrine experiments. Similar to the NE+metoprolol group, norfenephrine evoked an elevation of the LV weight/body weight and RNA/DNA ratios (Table 3). Likewise, TPR was increased (Table 2). The norfenephrine-induced hypertrophy was abolished by prazosin; however, a slight increase of the RNA/DNA ratio remained (Table 3). This increase and the small enhancement of HR and LVdP/dt\text{max} may be related to a mild \( \beta \)-adrenergic stimulation by norfenephrine. In contrast with prazosin, verapamil did not affect the development of hypertrophy, although it normalized MAP, HR, and TPR and substantially reduced LVdP/dt\text{max}. Thus, this hypertrophy model is due to stimulation of myocardial \( \alpha \)-receptors and seems not to be the response of the myocardium to the circulatory changes.

\( \alpha \)-Receptors have been shown to exist on cardiac myocytes; their biological relevance, however, is still a matter of debate. The putative second messenger system linked to myocardial \( \alpha \)-receptors is the hydrolysis of phosphatidylinositol 4,5-bisphosphate, leading to formation of inositol triphosphate (IP\text{3}) and diacylglycerol. Both reaction products are probably involved in the generation of the positive inotropic effect seen after activation of \( \alpha \)-receptors. However, this \( \alpha \)-receptor-mediated increase in contraction force is relatively small compared with the effect mediated by stimulation of \( \beta \)-receptors. The anabolic effect of \( \alpha \)-receptor stimulation in cultured myocytes is possibly mediated by activation of protein kinase C by diacylglycerol, since direct activation of protein kinase C by tumor-promoting phorbol esters also results in cellular hypertrophy.}

Recently, findings were presented that support a role of myocardial \( \alpha \)-receptors in the development of cardiac hypertrophy due to aortic banding in guinea pigs. These results suggest that pressure overload elicits an increase of \( \alpha \)-adrenergic binding sites already in the prehypertrophic period. Blockade attenuated the hypertrophic response.

Our results are in agreement with clinical observations in patients with hypertensive cardiac hypertrophy. The reversal of left ventricular hypertrophy seemed to be determined not only by blood pressure control, but may also depend on other factors such as the activity of the adrenergic system. Treatment of hypertensive patients with diuretics revealed no regression of cardiac hypertrophy in spite of significant reduction of blood pressure. In addition, muscle mass was reduced overproportionally in relation to blood pressure reduction after treatment with clonidine. In contrast with diuretics, clonidine diminished the adrenergic drive. Finally, significant left ventricular hypertrophy was observed in dogs treated with subhypertensive doses of NE. These findings illustrate that altered plasma or tissue levels of catecholamines can be important mediators of cardiac hypertrophy independent of the work load.

In contrast with the myocytes, the adaptive changes in nonmyocardial cells seemed to be dependent on the stress induced by the increase in TPR rather than direct activation by catecholamines. An increase in TPR turned out to be significantly correlated with an increase in DNA concentration (Figure 3). On the other hand, concomitant \( \beta \)-blockade did not reduce the DNA concentration compared with the NE group, and the elevated DNA concentration in the norfenephrine group was antagonized by verapamil or prazosin treatment. Since only about 25% of the nuclei in rat left ventricle are muscle nuclei and since cardiac myocytes lose their mitotic capacity shortly after birth, an increase in the DNA concentration reflects mainly proliferation of connective tissue and other nonmyocardial cells. Myocardial fibrosis is a typical sign of abrupt pressure overload. Thus, our data demonstrate that cardiac hypertrophy can occur with and without an increase in DNA concentration. Furthermore, it is evident that verapamil is effective in reducing the work load of the heart without affecting the NE- or norfenephrine-induced cardiac hypertrophy. Finally, the proliferation of connective tissue in this model seems not to be due to direct activation mediated by \( \alpha \)- or \( \beta \)-receptors.

In conclusion, our data show that in the model of NE-induced hypertrophy myocardial \( \alpha \)- and \( \beta \)-receptors may primarily contribute to the development of cardiac hypertrophy in vivo. However, we want to emphasize that this mechanism probably is limited to pathophysiological circumstances with
increased levels of catecholamines, since in other models cardiac hypertrophy seems to occur independent of adrenergic mechanisms. 1, 5, 6, 9, 10 Moreover, our model is limited to a relatively short period. If the model were expanded to longer time intervals, other mechanisms may replace the mediation by adrenoceptors and continue to serve as a trigger for hypertrophy. This could help to explain the difference in the results of several reports on the role of adrenergic mechanisms in the development of cardiac hypertrophy. 1, 5, 6, 9, 10 Given these limitations, our results demonstrate that direct activation of myocardial α- or β-adrenoceptors may elicit cardiac hypertrophy in vivo. The weight gain of the left ventricle is at least additive when both receptors are stimulated. Furthermore, the changes in peripheral circulation caused by NE seem not to play a major role within the observed period of 3 days.

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