Beta Adrenergic Receptor Response Coupling in Hypertrophied Hearts

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SUMMARY Reports in the literature have indicated that inotropic responsiveness to isoproterenol (ISO) of hypertrophied rat myocardium is decreased. We have studied possible biochemical mechanisms to explain this. Adenylate cyclase activity in myocardial membranes from 13-week-old spontaneously hypertensive rats (SHR) was unchanged compared to enzyme activity in Wystar-Kyoto (WKY) when measured under basal conditions or following NaF, a guanosine triphosphate analog, MnCl₂, or forskolin stimulation. However, ISO-stimulated cyclase activity was decreased as were β-adrenergic receptor density with no change in receptor affinity. On the other hand, hearts from two-kidney, one clip renal hypertensive rats 6 weeks after initiation of hypertension showed decreased basal, ISO, NaF, and GTP analog-stimulated cyclase activity with no change in Mn⁺⁺ or forskolin-stimulated activity. In this model, receptor density increased. When the clipped kidney was removed, these changes returned toward normal as did the blood pressure and heart weight. We interpret these data to indicate that in SHR the biochemical defect leading to decreased inotropic responsiveness of hypertrophied hearts is due to decreased β-adrenergic receptor density, leading to decreased ISO-stimulated cyclase activity. The nucleotide regulatory protein component (N) and the catalytic subunit (C) are not changed. In the renal hypertensive rat, on the other hand, although the C is unchanged, there is a decrease in the activity of N, and this is probably the primary reason why inotropic responsiveness to ISO is decreased. Increases in receptor density seen in this model may possibly be compensatory. (Hypertension 5 (supp 1): I-175-I-183, 1983)

KEY WORDS • β-adrenergic receptor • nucleotide regulatory protein • adenylate cyclase • renal hypertensive rats • spontaneously hypertensive rat • cardiac hypertrophy

It has long been held that cardiac hypertrophy in hypertension is simply due to the increased pressure load imposed on the heart. However, more recently a number of investigators have demonstrated a role for adrenergic factors acting, either directly or indirectly, in the development of cardiac hypertrophy.1-5 Also, a number of observations have been published indicating that cardiac beta receptor characteristics are modified in the myocardium of hypertensive rats.6-14 However, different investigators have reported different results. Thus, beta adrenergic receptor density has been reported to be increased,6 decreased,7-9 or unchanged10-12 in hearts from hypertensive rats, as has the adenylate cyclase activity in these experimental models.12-17 Myocardial catecholamine levels have been reported to be increased, decreased, or unchanged.18 Capasso et al.18 have reported that peak-developed tension was the same in hypertrophied hearts as in sham controls, but the duration of isometric contractions and time-to-peak shortening was longer in the hypertensive heart.

On the other hand, Saragoca and Tarazi20 reported that maximum dp/dt generation following isoproterenol stimulation was decreased in hearts from hypertensive rats. Based on these latter results, we have attempted to study where the biochemical defects lie in the excitation response sequence which lead to decreased responsiveness to isoproterenol. We have recognized that it may be difficult to correlate a physiological response with specific defects in either beta-adrenergic receptor density or adenylate cyclase enzyme activity in isolated cardiac membranes. Thus, measurements have been carried out in two models of experimental hypertension: a renin and angiotensin mediated two-kidney, one clip renal hypertensive rat22 and a neurogenic model, namely, the spontaneously hypertensive rat (SHR).23, 24

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Methods

Animal Models

Six-week-old Sprague-Dawley rats weighing between 170 and 180 g were obtained from Hilltop Laboratories and 12-week-old SHR and WKY weighing approximately 250 g were obtained from Taconic Farms. All animals were kept for 7 to 10 days to recover from shipping and to get adapted to the laboratory before experiments were started. Two-kidney, one clip renal hypertensive rats were prepared together with sham-operated controls according to standard methods in our laboratory.25 Rats were followed for a period of 6 weeks with twice weekly measurements of blood pressure by the tail cuff of Friedman and Freed.26 Six weeks after the application of the clip, animals were sacrificed and the hearts removed. Male SHR and WKY controls were studied at 13 weeks of age so that the renal hypertensive rats and SHR were studied at the same age.

Reversal of Hypertension

Reversal of hypertension was produced in the renal hypertensive rats by removal of the clipped kidney 6 weeks after application of the clip. These animals were then followed for 4 to 6 weeks with twice weekly blood pressure measurements and were kept until blood pressure returned toward control levels. Sham-operated controls also had a left nephrectomy and were kept for a corresponding time period.

Beta Adrenergic Receptor Studies

Beta adrenergic receptors were studied according to the method of Williams and Lefkowitz.27 Hearts were removed rapidly, rinsed in ice-cold saline, and the connective tissue, aorta, and atria removed. The ventricles were frozen in dry ice/acetone and kept in a deep freeze at −80°C until used, usually within 3 weeks. On the day of the experiments, ventricles were weighed, minced, and rapidly homogenized at 4°C in nine volumes of 50 mM Tris buffer pH 7.7 containing 10 mM MgCl₂ and 0.25 M sucrose. Homogenization was carried out in a glass to glass homogenizer, the homogenate centrifuged at 1000 × g for 10 minutes, and the supernate re-centrifuged at 40,000 × g for 20 minutes. The pellet was washed with the same buffer and re-centrifuged. The final pellet containing crude myocardial membranes was resuspended in 4 ml of 50 mM Hepes buffer pH 8.0 containing 4 mM MgCl₂, yielding a final protein concentration of 3 to 5 mg/ml. Protein was determined by the Lowry method28 with bovine serum albumin as a standard. On the same day as the membrane fragments were prepared, binding assays were carried out. Membrane suspension (100 μl) was incubated for 20 minutes at 25°C with varying concentrations of [³H]-dihydralpropranolol ranging from 1 to 10 nM in the presence or absence of 10⁻⁵ M propranolol. Total volume of incubation was 150 μl. Incubation was terminated by the addition of 3.5 ml ice-cold 25 mM Hepes buffer pH 8.0 containing 4 mM MgCl₂. This was then followed by rapid filtration through Whatman GF/C, and the filters were then washed with a further 3.5 ml of the above buffer. Membrane-bound radioactivity was retained on the filters, which were then dried and counted by liquid scintillation. Specific binding was determined as the total binding of [³H]-dihydralpropranolol minus the binding in the presence of propranolol and was 50% to 75% of the total counts bound, with higher specific binding at lower ligand concentrations. Experiments were performed in duplicate, which were within 5%, using six concentrations of ligand. Based on the specifically bound radioactivity, Scatchard plots29 were constructed, utilizing linear regression analysis. Correlation coefficients below −0.85 were rejected.

Determination of 5′-Nucleotidase

The enzyme, 5′-nucleotidase, was used as a membrane marker and was determined according to the method of Dixon and Purdom.30 Phosphate liberated was determined by the method of Fiske and Subbarow.31

Calculation of Results

Beta-adrenergic density was expressed in a variety of fashions. Since the membrane yield from the original heart was calculated by measuring both protein concentration and 5′-nucleotidase activity in the original homogenate and in the final centrifuged pellet, receptor densities can be expressed as either fmol/mg membrane protein, fmol/5′-nucleotidase activity measured as inorganic phosphate released, or else as pmol per gram ventricle or per total ventricular weight.

Membrane Preparation for Assay of Adenylate Cyclase Activity

Rats were killed by cervical dislocation, and the heart was removed as rapidly as possible and chilled in ice-cold saline; the connective tissue, aorta, atria, and right ventricular wall were removed. Left ventricles were then frozen in liquid nitrogen and stored in a deep freeze at −80°C until used, usually within 2 weeks. Left ventricles were weighed while in the frozen state just before assay. The assay method for adenylate cyclase was essentially that of Krishna et al.32 The frozen left ventricle was smashed, powdered, and then homogenized twice for 15 seconds in a Polytron at half maximal speed in 20 ml of 4°C 5 mM Tris HCl buffer pH 7.5 containing 0.25 M sucrose. The homogenate was then passed through four layers of gauze, and the enzyme was measured either on the total homogenate or following centrifugation at 12,000 × g for 10 minutes. The resulting pellet was washed twice in the same buffer, and resuspended in the same buffer, giving a protein concentration of 1 to 1.5 mg/ml. An incubation medium was made up in a final volume of 150 μl containing 25 mM Tris HCl pH 7.5 buffer, 5 mM MgCl₂, 6 mM theophylline, 1 mM cAMP, 0.5 mM ³²P-ATP (30-40 cpm/pmol specific activity), a protein sample containing between 50 and 75 μg of protein, and an ATP-regenerating system consisting of 20 mM creatine phosphate and 100 units/ml creatine kinase.
phosphokinase. Loss of cAMP by destruction by cytosolic phosphodiesterase was completely prevented during the assay by the addition of cold cAMP and theophylline. Incubation was carried out at 30°C for 15 minutes in a shaking water bath and stopped by the addition of 100 μl of a solution containing 2% sodium dodecyl sulphate, 40 mM ATP, 1.4 mM cAMP, at pH 7.5. Then 3H-cAMP (approximately 10,000 cpm) was added to calculate the recovery of cAMP, which was between 60% and 75%. A reaction blank was set for each group of determinations by adding the enzyme after the stopping solution. Formed cAMP was purified according to the method Salomon et al. Under these conditions of assay, the rate of formation of cAMP was directly proportional to the time for the 20-minute incubation and to protein concentrations ranging from 10 to 200 μg/50 μl. All measurements were carried out in triplicate. Results were expressed as cAMP formed in pmol/min/mg protein.

Further Studies on Adenylate Cycase

Other than determination of basal adenylate cyclase activity, various enzyme determinations were carried out following the addition of stimulants. In one series of experiments, 10^-9 to 10^-3 M l-isopropenol hydrochloride was added to the membranes to stimulate adenylate cyclase activity. In these experiments, 0.1% (vol/wt) ascorbic acid was added to prevent isoproterenol oxidation. Other experiments had an addition of 8 mM NaF and 10^-4 M guanosine 5'[/3,Y-imido] triphosphate, a guanosine triphosphate analog. Two further studies were performed. In the first, 5 mM MnCl₂ was substituted for MgCl₂, and in the second, 10^-4 M forskolin, an irreversible activator of the catalytic subunit of adenylate cyclase, was added. All of these agents increased activity of adenylate cyclase by varying mechanisms.

Statistics

Statistical analysis on all data was carried out using PROPHET System Computer sponsored by the Biotechnology Resources Program, Division of Research Resources at the NIH. Programs of linear regression analysis, paired and unpaired t tests were primarily used.

Results

Early work indicated that freezing and storing normal hearts for periods up to 6 weeks made no difference in the β-adrenergic receptor number (36.7 ± 3.3 fmol/mg protein, frozen (n = 26) and 36.93 ± 4.74, fresh (n = 8)) and the adenylate cyclase activity (11.2 ± 0.47 pmol/min/mg protein basal activity in fresh hearts vs 10.4 ± 0.67 in frozen hearts and 24.6 ± 2.2 with 10^-4M isoproterenol in fresh hearts vs 22.7 ± 1.08 in frozen hearts). In renal hypertensive rats, cyclase activity was 8.9 ± 0.55 in fresh hearts vs 8.6 ± 0.86 in frozen hearts, and following isoproterenol stimulation 15.4 ± 1.69 in fresh hearts and 14.2 ± 0.87 in frozen hearts. Thus, all the following experiments are reported using frozen hearts.

### Table 1. Systolic Blood Pressure and Cardiac Mass in Hypertensive and Normotensive Rats (Mean ± SEM)

<table>
<thead>
<tr>
<th>Rat group</th>
<th>No.</th>
<th>Systolic BP (mm Hg)</th>
<th>Ventricular weight (g)</th>
<th>Ventricular body weight (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>8</td>
<td>123 ± 1.0</td>
<td>1116 ± 38.7</td>
<td>2.71 ± 0.08</td>
</tr>
<tr>
<td>RHR (6 weeks)</td>
<td>7</td>
<td>204 ± 8.0*</td>
<td>1303 ± 26.0*</td>
<td>3.79 ± 0.142</td>
</tr>
<tr>
<td>WKY</td>
<td>7</td>
<td>130 ± 2.0</td>
<td>717 ± 14.2</td>
<td>2.55 ± 0.04</td>
</tr>
<tr>
<td>SHR</td>
<td>10</td>
<td>198 ± 5.0*</td>
<td>802 ± 20.1*</td>
<td>3.18 ± 0.08*</td>
</tr>
</tbody>
</table>

*p < 0.01.  
**p < 0.005.  
†p < 0.001.

RHR = renal hypertensive rat; WKY = Wistar-Kyoto rat; SHR = spontaneously hypertensive rat.

Studies on Beta Adrenergic Receptors

Six weeks after application of the clip, rats had a mean systolic blood pressure of over 200 mm Hg with evidence of cardiac enlargement. Thirteen-week-old SHR also had both elevated blood pressures and ventricular enlargement compared to their controls (table 1). After the hearts were homogenized and centrifuged, membrane recovery as judged by 5'-nucleotidase activity did not differ between hypertrophied hearts and controls. In the renal hypertensive rats, 5'-nucleotidase activity was 60.8 ± 4.2 as compared to 72.1 ± 7.5 in WKY. The relative specific activity of the 5'-nucleotidase expressed as a ratio of the activity of enzyme in the pellet to its activity in the total homogenate was 2.82 ± 0.2 vs 3.03 ± 0.3 in the sham vs the renal hypertensive rats, and 2.2 ± 0.2 in WKY compared to 2.23 ± 0.2 in SHR. Thus, membrane yield in both hypertensive models was similar when compared to their respective controls. 3H-dihydroalprenol binding in renal hypertensive rats was increased whether expressed as fmol/mg protein in the membrane fraction, fmol/5'-nucleotidase activity, or pmol/total ventricle (table 2). On the other hand, 3H-dihydroalprenol binding in SHR was decreased when expressed as fmol/mg protein. How-

### Table 2. [3H] Dihydroalprenol Binding to Rat Myocardial Membranes in Sham-Operated Controls and in Renal-Hypertensive Rats (Two-Kidney, One Clip, 6 Weeks Duration) (Means ± SEM)

<table>
<thead>
<tr>
<th>Binding activity</th>
<th>Controls</th>
<th>RHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls sham-operated (n = 8)</td>
<td>38.3 ± 2.79</td>
<td>52.07 ± 2.36</td>
</tr>
<tr>
<td>2-kidney, 1 clip Goldblatt (n = 7)</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[3H] Dihydroalprenol binding</th>
<th>fmol/mg Protein</th>
<th>13.13 ± 1.37</th>
<th>17.72 ± 0.73</th>
</tr>
</thead>
<tbody>
<tr>
<td>fmol/5'-Nucleotidase</td>
<td>2.85 ± 0.33</td>
<td>4.79 ± 0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pmol/Ventricle</td>
<td>1.78 ± 0.23</td>
<td>1.71 ± 0.16</td>
<td>n.s.</td>
</tr>
<tr>
<td>Kᵦ (nM)</td>
<td>1.78 ± 0.23</td>
<td>1.71 ± 0.16</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
TABLE 3. $[^3]$H Dihydroalprenolol Binding to Rat Myocardial Membranes in WKY and SHR (13 Weeks) (Means ± SEM)

<table>
<thead>
<tr>
<th>Binding activity</th>
<th>WKY (n = 7)</th>
<th>SHR (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>fmol/mg Protein</td>
<td>47.82±5.39</td>
<td>34.54±3.0</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>fmol/5' Nucleotidase</td>
<td>10.15±1.59</td>
<td>9.67±1.07</td>
<td>n.s.</td>
</tr>
<tr>
<td>pmol/Ventricle</td>
<td>3.44±0.48</td>
<td>2.72±0.42</td>
<td>n.s.</td>
</tr>
<tr>
<td>KD (nM)</td>
<td>4.53±0.77</td>
<td>3.80±0.59</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

ever, when expressed as fmol/5'-nucleotidase or pmol/total ventricle (table 3), receptor number tended to decrease, but this was not statistically significant. In both the renal hypertensive rats and in the SHR the dissociation constant $K_D$ was unchanged from its respective control.

Adenylate Cyclase Studies

Left ventricular weight in the renal hypertensive groups 6 weeks after clipping was significantly higher than in the sham controls both in absolute terms (1078 ± 32.3 vs 793 ± 19.0 mg, p < 0.001) and relative to body weight (3.34 ± 0.93 vs 2.04 ± 0.32 mg/g, p < 0.001). In the hypertrophied hearts basal adenylate cyclase activity was decreased 6 weeks after application of the clip (fig. 1). Similarly, enzyme activity after the addition of NaF and Gpp(NH)p all were decreased in the hypertrophied hearts when compared to normals. On the other hand, enzyme activity measured in the presence of Mn$^{++}$ or in the presence of $10^{-4}$ M forskolin showed no difference between hypertrophied and normal hearts. At $10^{-3}$ M forskolin stimulated basal cyclase activity threefold and still no difference was seen in the two groups. The addition of isoproterenol stimulated adenylate cyclase activity starting at $10^{-7}$ M and reaching a plateau of $10^{-5}$ M. Total ventricular homogenate showed more enzyme activity than that in a 12,000 x g pellet (fig. 2). However, in both pellet and total homogenate cyclase activity from hypertrophied hearts was less than that from sham control hearts, while the concentration of isoproterenol required for half maximal response did not differ. Since basal cyclase activity was less in hypertrophied hearts, results were also presented as percent increase in enzyme activity above basal levels (fig. 3). Activity in both control and hypertrophied hearts increased to the same extent following isoproterenol in the 12,000 x g pellet, but in the total myocardial homogenates, increase in activity in the hypertrophied hearts was much less than that in the control hearts. Preliminary data indicate that when the supernatant from a 40,000 x g homogenate of normal hearts was added to a 40,000 x g pellet of hypertrophied hearts, isoproterenol-stimulated cyclase activity was restored to the level that measured in homogenates of control hearts. In spontaneously hypertensive rats, no change was detected in basal adenylate cyclase activity in NaF, Gpp(NH)p, or in forskolin-stimulated enzyme activity. However, isoproterenol-stimulated enzyme activity was significantly reduced in SHR as compared to WKY rats (fig. 4).

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Adenylate cyclase activity in left ventricular homogenate of 6-week two-kidney, one clip renal hypertensive rats vs matched sham controls. Means ± SEM are represented. Hypertrophied hearts show significant decrease in enzyme activity except for columns representing 5 mM MnCl$_2$ and $10^{-4}$ M forskolin.
FIGURE 2. Adenylate cyclase activity in myocardium (n = 6) of 6-week two-kidney, one clip renal hypertensive rats, and its response to varying doses of isoproterenol. Values are means ± SEM. The pellet was obtained following centrifugation at 12,000 x g for 10 minutes.

FIGURE 3. Response to isoproterenol of adenylate cyclase in myocardium (n = 6) of two-kidney, one clip renal hypertensive rats expressed as percent increase over basal levels. Values are means ± SEM. The pellet was obtained following centrifugation at 12,000 x g for 10 minutes.

FIGURE 4. Adenylate cyclase activity in left ventricular homogenate of 13-week old male SHR and WKY. Values are means ± SEM. Hypertrophied hearts showed a significant decrease in enzyme activity only following isoproterenol stimulation.
TABLE 4.  Reversal of Ventricular Hypertrophy by Removal of Clipped Kidney (Means ± sem)

<table>
<thead>
<tr>
<th></th>
<th>Sham 4 weeks post-nephrectomy</th>
<th>RHR 4 weeks post-nephrectomy</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>116 ± 4</td>
<td>138 ± 6.0</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Ventricular weight (mg/g)</td>
<td>1114 ± 31</td>
<td>1192 ± 44</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ventricular body weight (mg/g)</td>
<td>2.53 ± 0.13</td>
<td>2.8 ± 0.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>[3H] Dihydroalprenolol binding fmol/mg protein</td>
<td>37.74 ± 3.33</td>
<td>45.71 ± 5.26</td>
<td>n.s.</td>
</tr>
<tr>
<td>fmoI/5'-nucleotidase</td>
<td>9.58 ± 1.45</td>
<td>10.67 ± 1.29</td>
<td>n.s.</td>
</tr>
<tr>
<td>pmol/ventricle</td>
<td>3.79 ± 0.49</td>
<td>4.20 ± 0.88</td>
<td>n.s.</td>
</tr>
<tr>
<td>kD (nM)</td>
<td>3.34 ± 0.82</td>
<td>3.80 ± 0.86</td>
<td>n.s.</td>
</tr>
<tr>
<td>Adenylate cyclase activity pmol cAMP formed/min/mg protein basal</td>
<td>8.1 ± 1.02</td>
<td>7.9 ± 2.11</td>
<td>n.s.</td>
</tr>
<tr>
<td>isoproterenol</td>
<td>19.8 ± 2.37</td>
<td>18.5 ± 2.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>NaF</td>
<td>33.9 ± 2.98</td>
<td>31.0 ± 3.15</td>
<td>n.s.</td>
</tr>
<tr>
<td>Gpp (NH)p</td>
<td>33.8 ± 8.56</td>
<td>27.5 ± 3.15</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Beta Receptor Density and Adenylate Cyclase Activity in Reversal of Cardiac Hypertrophy

During reversal of cardiac hypertrophy following removal of the clipped kidney, blood pressure and heart weight decreased significantly and both beta adrenergic receptor density and adenylate cyclase activity returned toward normal values (table 4).

Discussion

A number of investigators have demonstrated the inability of hypertrophied myocardium to respond effectively to adrenergic stimulation, primarily as a reduction in inotropic responsiveness to isoproterenol. This has been reported in the two-kidney, one clip renal hypertensive rat and has been demonstrated both in vivo and in an isolated Langendorff-perfused heart. In the renal hypertensive rat, this decrease in inotropic responsiveness is a transient phenomenon since following reversal of hypertrophy, either by removal of the clipped kidney or by medical treatment with converting enzyme inhibitors, inotropic responsiveness to isoproterenol returns toward normal as both blood pressure and heart weight return towards normal.

During the past few years a number of investigators have studied the multiple regulatory response mechanisms of hormone-sensitive adenylate cyclase system and have been able to separate it into a hormone receptor protein (R), an enzymatic portion of adenylate cyclase (C), and components required for the regulatory effects of guanine nucleotides (N). Since this system in rat myocardium is complex and isolation of relatively pure cell membranes is difficult, most investigators have utilized simpler systems, including catecholamine-sensitive adenylate cyclase activity contained in plasma membranes from a variant clone of S49 lymphoma cells. Solubilizing these membranes by detergents had indicated that the adenylate cyclase activity can be separated into at least two components, a catalytic protein (C), and one or more components required for the display of basal, fluoride, or guanine nucleotide-stimulated enzymatic activity. This latter complex has been called the nucleotide regulatory protein (N). Our results on rat myocardium are discussed on the assumption that measurement of adenylate cyclase activity in the presence of Mn ATP or in the presence of forskolin measures only the catalytic activity (C), while the enzyme measured in the presence of Mg ATP measures the NC complex. Enzyme activity measured in the presence of NaF, GTP or Gpp(NH)p also measures NC, while the addition of isoproterenol measures the total complex of receptor plus NC. Changing responsiveness of the hypertrophied myocardium to catecholamines may involve biochemical changes in any one of the sequence of events between excitation and response. Our investigations have focussed on the receptor (R), the nucleotide regulatory protein complex (N), and the catalytic subunit, adenylate cyclase (C).

Our results in two models of cardiac hypertrophy lead us to propose that the biochemical mechanisms involved in decreased inotropic responsiveness differ in the two models. In the 13-week-old SHR, there is no change in the forskolin or Mn**+-stimulated adenylate cyclase activity, indicating no change in the catalytic subunit. There is also no change in the NaF- or in the Gpp(NH)p-stimulated enzyme activity, indicating no change in the nucleotide regulatory protein catalytic subunit complex (NC). There is, however, a decrease in isoproterenol-stimulated adenylate cyclase activity in SHR when compared to WKY. Since the nucleotide regulatory protein and the catalytic subunit per se are not changed, this decrease in isoproterenol-stimulated activity must indicate a decrease in the number or affinity of the receptor or else a defect in coupling between R and N. Measuring receptor density from these hearts indicates a significant decrease in receptor density in SHR when expressed as fmol/mg protein. Since 5' nucleotidase recovery from SHR was less than from WKY, receptor density per 5' nucleotidase did not differ significantly in both hearts (table 3). Most investigators studying either adenylate cyclase activity, receptor density, or inotropic responsiveness of SHR compared to the WKY have utilized 20 to 26-week-old rats and have reported decreased activity. Thus, our data in 13-week-old rats indicate some decrease in receptor density with no change in affinity, and this may become more prominent in 20- to 26-week-old rats. Our results on adenylate cyclase activity are similar to those of Blumenthal et al., who have demonstrated in the 100- to 125-day-old SHR that...
there is no change in basal and Gpp(NH)p stimulated adenyly cyclase activity, while there is a reduction in isoproterenol-stimulated activity. However, our results differ from theirs in measurements of adrenergic receptor density, since they report no change in density in the 100- to 125-day-old SHR compared to WKY. They do, however, show changes in density that are age-dependent, with the number declining with age in both SHR and WKY. No studies were done, however, on the 20- to 26-week-old rats. Our results also confirm those of Amer et al., and of Chatelain et al., who reported unchanged basal and NaF-stimulated adenyly cyclase with a decreased isoproterenol-activated cyclase activity, and of Robberecht et al., who reported decreased \( \beta \)-adrenergic receptor number. On the other hand, Bhalla et al. reported decreases in basal, isoproterenol, NaF, and Gpp(NH)p-stimulated adenyly cyclase. Thus, from our results and others, except Bhalla, we would like to suggest that the decreased inotropic responsiveness of the enlarged hearts from SHR is due to decreased \( \beta \)-adrenergic receptor density, with no change in the regulatory protein complex or the catalytic subunit, and that these events are age-dependent and become more manifest as the rats grow older and cardiac hypertrophy more prolonged.

In contradistinction to the findings above, results with hearts from two-kidney, one clip renal hypertensive rats indicate that the major defect leading to decreased responsiveness lies in the adenyly cyclase complex. Our results indicate no change in adenyly cyclase activity when stimulated with MnCl\(_2\) or forskolin, thus indicating no change in the catalytic subunit. However, NaF and Gpp(NH)p-stimulated activity is decreased in hearts from hypertrophied animals when compared to sham-operated controls, indicating a defect in the nucleotide regulatory protein complex (N). Basal and isoproterenol stimulated activity is also decreased in hypertrophied hearts. Since these responses, however, require the total complex RNC, the defect in the nucleotide regulatory protein (N) should also lead to decreased isoproterenol-stimulated activity. Receptor numbers could be increased, decreased, or unchanged, and yet isoproterenol-stimulated adenyly cyclase would be decreased because of the defect in coupling between the receptor and the catalytic subunit. This defect in N could be due to a decrease of a cytosolic factor in hypertrophied hearts that stimulates the nucleotide regulatory protein complex. Preliminary evidence in our laboratory indicates the possibility for the existence of such a factor. This defect in adenyly cyclase activity and the nucleotide regulatory protein complex is a transient phenomenon, since following reversal of hypertrophy by removing the clipped kidney, these biochemical changes return towards normal.

Measurement of the \( \beta \)-adrenergic receptors in the two-kidney, one clip renal hypertensive rats utilizing the method of Williams and Lefkowitz, indicates an increased number of receptors when expressed either as fmol/mg protein, fmol/5'-nucleotidase, or pmol/ventricle. In a review, Hoffman and Lefkowitz have postulated that myocardial \( \beta \)-adrenergic receptors may be modulated by ventricular catecholamines. Tarazi et al. have reported that myocardial catecholamine concentration is increased in spontaneously hypertensive rats, as have other investigators, and these animals have decreased number of receptors, while animals treated with guanethidine or 6-hydroxy dopamine, associated with a depletion of myocardial catecholamines, have an increased number of \( \beta \)-adrenergic receptors. In a two-kidney, one clip renal hypertensive rat, as demonstrated by Tarazi et al., there is decreased myocardial catecholamine concentration. Thus, if the postulate of Hoffman and Lefkowitz is correct, then one would expect increased \( \beta \)-adrenergic receptor density in the two-kidney, one clip renal hypertensive hearts, with decreased \( \beta \)-adrenergic density in spontaneously hypertensive rats.

Our findings indicate that different models of cardiac hypertrophy, all of which show decreased inotropic responsiveness to isoproterenol, may have different biochemical pathways leading to this. Whether these differences are associated with the renin-angiotensin system, which is predominant in the two-kidney, one clip renal hypertensive rat, and with catecholamines, which are predominant in SHR, or whether other factors are involved is still not clear. It is also unknown whether still other changes in the protein kinase cascade and in the myosin light chain kinase enzymes contribute to the decreased responsiveness of the myocardium. Based on our results, however, one can propose that in SHR, a decreased inotropic responsiveness to isoproterenol is likely due to decreased \( \beta \)-adrenergic receptor density with no change in the subsequent cascade coupling receptors to response, while in the two-kidney, one clip renal hypertensive rats a primary defect lies in the nucleotide regulatory protein complex (N). Thus, whether there is an increase or decrease in the adrenergic receptor number becomes a moot question, since the block occurs post receptor.

**Methodological Problems**

Bristow et al. have recently reported decreased adrenergic receptor density in failing human hearts. In their studies, adrenergic density was measured by two different methods. In the first, membranes were prepared according to the method of Williams and Lefkowitz by a density separation technique, while the second method was that first proposed by Baker and Potter in which the myocardium was homogenized and then the contractile proteins solubilized by addition of KCl to a final concentration of 0.75 M. Both methods consistently give different results. With the Baker method, more protein is extracted from the myocardium and the beta receptor density expressed as fmol/mg protein or pmol/g wet weight is much greater than that expressed by the method of Williams and Lefkowitz. Our results using the method of Williams and Lefkowitz have consistently indicated increased \( \beta \)-adrenergic receptor density when expressed as fmol per mg membrane protein, when expressed as fmol bound per 5'-nucleotidase activity, or when expressed
per total ventricle. With the same methods, decreases in β-adrenergic receptor density were seen in hearts from SHR. In our renal hypertensive rats following nephrectomy and return of the blood pressure toward normal, receptor density returned toward normal. Other investigators, however, have reported that in the same model of two-kidney, one clip renal hypertensive rats 6 weeks after application of the clip, there is decreased beta adrenergic receptor density when utilizing the method of Baker and Potter.48 On the average, reported receptor density expressed as fmol/mg protein is somewhat higher using the method of Baker et al. than the method of Williams and Lefkowitz, as also reported by Bristow et al.,47 and when expressed per gram heart wet weight the average is 4 to 7 times greater. By using either method, it is not clear what fraction of the total membrane β-adrenergic receptors are measured nor what the effect of different extractable proteins from the myocardium may have on binding of [3H]dihydroalprenolol. Thus, it is not surprising that consistency in results, both qualitative and quantitative, does not exist among varying investigators and varying laboratories.

Another variable in quantitating β-adrenergic receptor density may be due to the labelled ligand used to measure receptors. Since we have attempted to study both receptor density and cyclase activity, the use of a labeled agonist ligand instead of an antagonist would have been preferable. However, the available agonist ligands preferentially measure β₁ receptors which are not present in hearts. Also, agonist binding is known to preferentially measure only high affinity receptor, while antagonist binding measures both high and low affinity sites. Thus, antagonist binding will measure all available receptor sites, while agonist binding will measure high affinity “active” receptors. Since measurement of adenylate cyclase requires the high affinity state, agonist displacement studies on myocardium have to be performed before conclusions can be stated correlating receptor density and physiological responses. Wessels et al.49 have reported that in frog erythrocyte membrane the rate of loss of adenylate cyclase responses correlated well with the loss of binding sites for an agonist ligand [3H]-hydroxybenzyl isoproterenol, but did not correlate well with binding sites determined with an antagonist ligand [3H]-dihydroalprenolol.

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