Reversal of Changes in Myocardial \( \beta \)-Receptors and Inotropic Responsiveness with Regression of Cardiac Hypertrophy in Renal Hypertensive Rats (RHR)

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SUMMARY. Our previous studies, in vivo and in vitro, have shown reduced inotropic responsiveness to isoproterenol of hypertrophied hearts in renovascular hypertensive rats. In the present study, we have investigated, in the same model, the effects of treatment either by nephrectomy or captopril on the inotropic responsiveness to isoproterenol and on the number and affinity of ventricular \( \beta \)-receptors. Isoproterenol infusion of isolated hearts from renovascular hypertensive rats 12–18 weeks post-clipping produced lower inotropic responses (\( \Delta \) peak \( dP/dt \)) than age-matched sham-operated normotensive rats (\( P < 0.001 \)). Quantitative assessment of \( \beta \)-adrenergic receptors in the same hearts showed a significant decrease in renovascular hypertensive rats ventricular receptor numbers, whether calculated per milligram membrane protein (22.3 ± 2.66 pmol/mg vs. 37.9 ± 4.34, \( P < 0.005 \)) or per gram wet ventricular weight (1.43 ± 0.14 pmol/g vs. 2.2 ± 0.21, \( P < 0.005 \)), with no significant change in \( K_d \). Control of hypertension by either nephrectomy or captopril led to regression of hypertrophy 6 weeks after stabilization of blood pressure (12–18 weeks post-clipping) and returned both the ventricular receptor density and inotropic responsiveness toward normal. The improvement in inotropic responsiveness to isoproterenol in regressed hearts correlated with both the reduction in ventricular weight and the decrease of blood pressure. Regression of hypertrophy did not alter the relationship between inotropic response, receptor density, and ventricular weight. These results indicate that the increase in cardiac mass associated with renovascular hypertension may interfere with adrenergic support to the heart, and that proper control of hypertension and regression of hypertrophy could reverse that impairment and restore its responsiveness to adrenergic stimulation. (Circ Res 54: 125–134, 1984)

THE HEART bears the brunt of arterial hypertension, and hypertrophies in order to compensate for the excessive pressure load. Hypertrophy is viewed in this context as an adaptive mechanism to reduce wall stress (Grossman et al., 1975); it has, however, also been considered as a step to heart failure because of the associated diminution of myocardial contractility (Spann et al., 1967; Capasso et al., 1981), and alteration in myosin ATPase activity (Hoar et al., 1971). Recently, studies have indicated that left ventricular (LV) hypertrophy may interfere with the "contractile reserve" of the heart by reducing its inotropic responsiveness to adrenergic stimulation; this was observed in vivo in two models of hypertension, spontaneously hypertensive rats (SHR) (Pfeffer et al., 1974; Saragoca and Tarazi, 1981a) and renovascular hypertensive rats (RHR) (Saragoca and Tarazi, 1981b). This reduction in contractile reserve of the hypertrophied hearts of RHR (2K-1C Goldblatt) was subsequently confirmed in vitro in isolated hearts perfused in a Langendorff preparation. The reduction in responsiveness was demonstrated only with isoproterenol, but not when other inotropic stimuli—such as calcium ions or cardiac glycosides,—were used; it was related in part to a diminished density of myocardial \( \beta \)-receptors (Ayobe and Tarazi, 1983). A reduction in \( \beta \)-inotropic responsiveness might impair the ability of the hypertrophied heart to sustain stressful stimuli (Braunwald et al., 1976a) and, therefore, the possibility of its reversal by appropriate treatment may be important in restoring a significant aspect of cardiac reserve.

In contrast with the many recent studies demonstrating regression of cardiac hypertrophy with blood pressure control (Sen et al., 1974, Schlant et al., 1977; Devereux et al., 1980; Fouad et al., 1982; Tarazi et al., 1982a), quantitative assessment of the functional performance of hearts after reversal of hypertrophy (Spech et al., 1980; Kuwajima et al., 1982) and of their \( \beta \)-adrenergic characteristics and responsiveness to agonist stimulation is still very limited (Tarazi et al., 1982b). Therefore, the present work designed to determine whether the changes observed in left ventricular hypertrophy, as regards myocardial \( \beta \)-receptors and their responsiveness to isoproterenol, could be reversed by regression of hypertrophy. The latter was obtained, as previously described (Sen et al., 1981), by medical control of renovascular hypertension with captopril or by its surgical cure with nephrectomy of the clipped kidney.
Methods

Experimental Renovascular Hypertension

Male Sprague-Dawley rats weighing 150–174 g (Hilltop Laboratories) were first kept for 1 week before any study, during which time, blood pressure was recorded twice by tail-cuff technique. At 6 weeks of age, the left renal artery in some rats was constricted by a silver clip (0.2 mm internal width) under ether anesthesia, while others underwent sham surgery. Body weight and blood pressure were subsequently recorded twice weekly in both clipped and sham-operated rats. All rats were kept and fed in exactly the same way and handled consistently by the same personnel. Only rats in which systolic blood pressure rose to 160 mm Hg or more within 2 weeks after they had been clipped were considered hypertensive (RHR).

Reversal of Hypertrophy

Treatment was undertaken 6 weeks postoperatively, either by nephrectomy of the clipped kidney under ether anesthesia, or by oral treatment with captopril (Sen et al., 1981), starting with a dose of 50 mg/kg per day, which was later increased to 80 mg/kg per day, as needed. A group of sham-operated rats also received the same dose of captopril. All rats were maintained under the same conditions, with free access to regular rat chow. Blood pressure was recorded twice weekly, and rats were weighed to the nearest gram on their final day, just before being killed. Experiments were carried out on treated rats at least 6 weeks after the date of nephrectomy or start of treatment by captopril. Results were compared with those of a separate group of age-matched untreated RHR kept under the same conditions for the same period of time.

Isolated Heart Perfusion

Cardiac responses to isoproterenol were determined in the isolated heart perfused at constant pressure (55 mm Hg) without recirculation by the Langendorff technique, as described previously (Ayobe and Tarazi, 1983). This approach was used to minimize the factors and variations which can occur in vivo studies and which could influence cardiac responses.

Procedure

The rats were given an ip injection of heparin (1000 U) 1 hour before administration of anesthesia. After the injection of pentobarbital (30 mg/kg), and while the rat was artificially ventilated, the heart was rapidly removed with a 0.5-cm-long aorta stump, and placed in ice-cold Krebs-Henseleit bicarbonate buffer. The aorta was slipped onto a grooved perfusion cannula and secured by a silk ligature along the groove; retrograde perfusion was begun from a reservoir 75 cm above the heart. The perfusion medium consisted of a modified Krebs-Henseleit bicarbonate buffer at pH 7.4, equilibrated with O$_2$:CO$_2$ (95:5) at 37°C. The final concentrations of salts in this buffer were (mm): NaCl, 118; KCl, 4.7; CaCl$_2$, 2.5 plus 0.5 to balance EDTA; MgSO$_4$, 1.2; KH$_2$PO$_4$, 1.2; and NaHCO$_3$, 25. Na$_2$EDTA (0.5 mm) was included to chelate any trace quantities of heavy metals in the reagents. Dextrose (11 mm) was added as substrate, and hydroxyethyl starch (HES, American Critical Care) was added in a concentration of 3% to adjust the oncotic pressure of the medium. Adjustment of oncotic pressure was necessary to prevent cardiac tissue edema. In all cases, the heart weight after perfusion was unchanged, compared with preinfusion weight.

Recording of Cardiac Responses

A small needle (21 gauge), directly connected through a larger blunted needle and a steel stopcock to a pressure transducer (Micron MP-15) was used to puncture the left ventricle, 0.5 cm above the apex. The fluid-filled needle transducer system had a natural resonant frequency of 144 Hz and a dampening coefficient of 0.63. The first derivative of LV pressure, dP/dt, was obtained electronically using a differentiator preamplifier with a frequency response of 1–100 Hz (–3 dB). The LV end-diastolic pressure recorded in our experiments averaged 0 ± 1 mm Hg under the conditions described above, both in normotensive and hypertensive hearts. Previous experience in this laboratory showed no significant difference in the pressure-volume curve between normotensive and hypertensive ventricles in the same model of hypertension (Saragoca and Tarazi, 1981b). Since there was no significant difference between the hearts from normotensive and hypertensive rats with regard to LV diastolic pressure, it can be reasonably assumed that the dose-response curves to isoproterenol were derived under the same preload conditions in all the groups investigated.

Isoproterenol Infusion

Once adequate undamped LV pressure recordings were obtained, the heart was left for 20 minutes to stabilize. A baseline recording then was obtained at a paper speed of 200 mm/sec, and isoproterenol (Isuprel, Breos Laboratories) infusions were begun through a catheter (PE-50) opening just above the aortic cannula, using a Harvard infusion pump at sequential rates of 1.38, 2.76, 5.62, 11.24, and 22.5 μmol × 10$^{-5}$/min. Each dose level was continued for 5-minute periods, at the end of which LV pressures were recorded on both the usual and magnified scales at 200 mm/sec paper speed.

After cessation of isoproterenol infusion, the heart was perfused with Krebs-Henseleit solution for 30 minutes to wash all traces of isoproterenol. In all cases, the hearts began rapidly to recover from the catecholamine effect and all had returned to pre-infusion levels within 10 minutes. Inotropic responses to isoproterenol were evaluated from changes in maximum dP/dt (Braunwald et al., 1976b) at each dose level; dose-response relationships were determined for each group of rats, and differences among groups were evaluated by an analysis of variance and covariance including repeated measures (BMIDP-2V, UCLA, 1977, a program available on PROPHET).

Assay of Myocardial β-Receptors

The β-receptors were determined in the ventricle of the same heart, as described earlier (Ayobe and Tarazi, 1983); the assay method was based on the technique of Baker and Potter (1980). Two ventricles of each heart were homogenized in 10 ml of ice-cold 10 mm Tris-HCl buffer; pH 8.0 in a 50-ml tube, with a Polytron disruptor at setting number six out of nine, for 15 seconds twice, and left on ice for 30 seconds between homogenizations. Each homogenate was diluted with 3 volumes of 1 M KCl, kept on ice for 10 minutes, filtered through four layers of medical gauze, and then centrifuged at 40,000 g for 30 minutes at 4°C. The supernatant was discarded, and the pellet was gently resuspended in 40 ml of buffer, using
the Polytron at setting number three for 5 seconds, and resedimented at 40,000 g for 30 minutes at 4°C. The supernatant was discarded, the pelleted membrane gently dispersed in incubation buffer (50 mM HEPES plus 4 mM MgCl₂, pH 8.0), and the volume adjusted according to the original wet weight of the ventricles.

Incubations were made in duplicates with six different concentrations of [³H]dihydroalprenolol (DHA) (0.6–15 nm) (New England Nuclear, 45 Ci/mmol) with and without 10⁻⁸ M dl-propranolol and 100-µM aliquot of resuspended pellet containing approximately 0.3 mg of the membrane protein, in a final incubation volume of 150 µl for 20 minutes at 25°C. At the end of incubation, 4 ml of ice-cold washing buffer (25 mM HEPES plus 4 mM MgCl₂, pH 8.0) were rapidly added, vortex mixed, and filtered under reduced pressure through a Whatman GF/C filter, and the tube and filter were rapidly rinsed with three more 4-ml aliquots of washing buffer.

The dried filters were covered with 5 ml scintillation cocktail (Atomlight, Amersham) and counted in a Packard scintillation counter. Specific binding was defined as the difference between total binding in the absence of propranolol and the nonspecific binding in presence of 10⁻⁸ M dl-propranolol. Protein concentration was determined in a 100-µl aliquot of resuspended pellet by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

The number of maximum binding sites (Bmax) and the dissociation constants (Kd) were determined by Scatchard analysis (Scatchard, 1949). The specific binding activity of cardiac membrane, i.e., the concentration of receptor sites per mg membrane protein, was calculated by normalizing for the protein content of the aliquot, and expressed as femtomoles per milligram protein. The density of receptor sites was also calculated per gram original wet tissue weight (specific binding activity x membrane protein yield per gram tissue) and expressed as picomoles per gram ventricle. The total tissue receptors were also calculated as specific binding activity x membrane protein yield per gram tissue x tissue weight in grams, and expressed in picomoles.

The total binding of [³H]DHA amounted to 1–4% of the added ligand. The proportion of specific [³H]DHA binding to its total binding ranged from 80 to 95% at 0.6 nm ligand concentration and from 52 to 64% at 15 nm ligand concentration.

The specific binding to [³H]DHA (displaceable by 10⁻⁵ M dl-propranolol) was found to be stereospecific, with l-propranolol having an IC₅₀ of two orders of magnitude less than d-propranolol (Ayers) (1.47 ± 0.68 M x 10⁻⁵ vs. 1.34 ± 0.34 M x 10⁻⁴, respectively), and also to possess the physiological characteristics of β-receptors, since β-agonists competed with specific [³H]DHA binding in the following order of potency: isoproterenol > epinephrine = norepinephrine; IC₅₀: 0.51 ± 0.16 M x 10⁻⁷, 0.88 ± 0.85 M x 10⁻⁶, and 0.82 ± 0.71 M x 10⁻⁵, respectively.

Methodological Aspects of the Receptor Assay

Since the same hearts were used to determine both inotropic responsiveness and myocardial β-receptors, it was important to define whether the infusion of isoproterenol in the Langendorff preparation altered receptor density or affinity. We have, therefore, assessed [³H]DHA-labeled β-receptors in two sets of matched hearts; in one set (n = 9) hearts were perfused in presence of dl-isoproterenol in the same sequence of doses as used in our study followed by a similar period of washout, and in the second set (n = 11), the hearts were perfused in absence of isoproterenol for the same period of time. No difference was found between the two sets either in Bmax (34 ± 2.0 fmol/mg membrane protein vs. 32.2 ± 2.4, NS) or Kd (2.66 ± 0.4 nm vs. 2.80 ± 0.30, NS). It can be concluded, therefore, that in the protocol of our study, neither the duration of isoproterenol infusion (25 minutes for all the five doses together) nor the final concentrations reached (1.5 x 10⁻⁷ to 3 x 10⁻⁸ M) were sufficient to induce changes in [³H]DHA binding. Marsh et al. (1980) also found that incubation of chick embryo ventricles for 30 minutes at 10⁻⁶ M l-isoproterenol had no influence on the number of [³H]DHA binding sites. Also, infusion of l-isoproterenol in vivo in male Sprague-Dawley rats, had no effect on Kd of [³H]DHA; the decrease in Bmax was relatively small, and reached the statistically significant levels only after 7 days of isoproterenol infusion (Chang et al., 1982).

Since a difference in receptor density between normotensive and hypertensive hearts could conceivably be due to differences in membrane recovery, a study of two different enzyme markers was performed in membranes prepared as described above from hearts of normotensive rats (n = 7) and of RHR (n = 6). Na⁺, K⁺-ATPase (EC 3.6.1.3) was determined according to the method of Whitsett and Wallick (1980) in the presence of 1 mM ouabain, using Na₂-ATPase synthetic grade I (Sigma) as substrate. 5'-Nucleotidase (EC 3.1.3.5) was estimated in both homogenate and membrane, using the method of Dixon and Pordum (1954). Results showed no difference in specific activity of either Na⁺, K⁺-ATPase or 5'-nucleotidase enzymes between membranes prepared from normal and hypertrophied hearts (Table 1). We also obtained in our membrane preparations a similar degree of recovery of 5'-nucleotidase activity from homogenates of both types of hearts.

Protocol of Study

Fifty-five rats were used in this study: 33 hypertensives and 22 normotensives. They were allocated as follows: (1) normotensive and hypertensive hearts could conceivably be due to differences in membrane recovery, a study of two different enzyme markers was performed in membranes prepared as described above from hearts of normotensive rats (n = 7) and of RHR (n = 6). Na⁺, K⁺-ATPase (EC 3.6.1.3) was determined according to the method of Whitsett and Wallick (1980) in the presence of 1 mM ouabain, using Na₂-ATPase synthetic grade I (Sigma) as substrate. 5'-Nucleotidase (EC 3.1.3.5) was estimated in both homogenate and membrane, using the method of Dixon and Pordum (1954). Results showed no difference in specific activity of either Na⁺, K⁺-ATPase or 5'-nucleotidase enzymes between membranes prepared from normal and hypertrophied hearts (Table 1). We also obtained in our membrane preparations a similar degree of recovery of 5'-nucleotidase activity from homogenates of both types of hearts.

Protocol of Study

Fifty-five rats were used in this study: 33 hypertensives and 22 normotensives. They were allocated as follows: (1)
untreated sham-operated rats (n = 14), Sh-R, o; (2) untreated hypertensive rats (n = 13), RHR-R, o; (3) nephrectomized hypertensive rats (n = 9), RHR-Nx; (4) captopril-treated sham-operated rats (n = 8), Sh-CEI; and (5) captopril-treated hypertensive rats (n = 11), RHR-CEI.

Statistical Analysis (Zar, 1974)
Results are expressed as mean ± 1 SEM. Regression analyses were performed by standard statistical methods. The statistical significance of differences in body weight, blood pressure, ventricular weight, and ventricular-to-body weight ratio, baseline heart rate, and baseline peak dP/dt, as well as in receptor density, membrane-specific binding, and total ventricular receptors was determined both by Student's t-test for unpaired groups (hypertensive vs. normotensive controls or treated hypertensives) and by analysis of variance (ANOVA). The statistical significance reported thereafter was, however, based on the results obtained by ANOVA because of the number of groups analyzed. If ANOVA revealed a statistically significant difference among the groups, then a Newman-Keuls test was performed to determine the statistical significance of differences among individual groups. Further, in a subsequent step, and because of the multiplicity of interventions performed, we have analyzed the effects of 'treatment' per se (whether nephrectomy or captopril) in normotensive rats and in hypertensive rats; this analysis for differences among the three groups (untreated, nephrectomy, and captopril) within each category, was performed by ANOVA, followed (if a significant difference was found) by Dunnett's test to determine the effect of each form of treatment within each category (normotensive and hypertensive).

For analysis of the dose response to graded doses of isoproterenol, the increments in peak dP/dt at each dose level were evaluated by an analysis of variance and covariance including repeated measures (one grouping factor and one trial factor). The trial factor was isoproterenol and the grouping factor consisted of either "hypertrophy" or "treatment." The program takes in consideration that the same heart was used at all doses of the trial factor (cumulative). Results were considered to be statistically significant at P(F) < 0.05 and P(t) < 0.05 in two-tail tables.

Results

Evolution of Hypertension in RHR (2K-1C, Goldblatt) and Its Reversal by Nephrectomy and Captopril
Blood pressure was higher in untreated RHR than in their sham-operated controls (193 mm Hg ± 7.8 vs. 127 ± 2.9, P < 0.001). Treatment with nephrectomy reduced the pressure in clipped rats significantly to 158 ± 8.5 (P < 0.01), although not down to the normotensive levels of sham-operated controls (127 ± 2.9 vs. 158 ± 8.5, P < 0.01) (Table 2). Treatment with captopril reduced the pressure of hypertensive rats effectively (136 ± 3.5 from 193 ± 7.8, P < 0.01), more than nephrectomy (136 ± 3.5 vs. 158 ± 8.5, P < 0.05) (Table 3). The blood pressure of normotensive rats treated with captopril also showed a small but consistent reduction (116 ± 3.1 vs. 127 ± 2.9, P < 0.05).

Development and Regression of Cardiac Hypertrophy (Tables 1 and 2)
Cardiac hypertrophy was evident in all untreated hypertensive rats, whether measured as absolute ventricular weight (1.78 g ± 0.09 vs. 1.43 ± 0.07, P < 0.01) (Table 2) or in relation to body weight (4.09 mg/g ± 0.22 vs. 2.76 ± 0.05, P < 0.001). Ventricular weight was reduced after treatment with either nephrectomy or captopril treatment; the absolute ventricular weight was somewhat lower following captopril, compared with nephrectomy (1.39 ± 0.07 vs. 1.58 ± 0.10), but this difference was not significant (P > 0.05) and did not hold when ventricular weight was normalized for body weight (3.02 ± 0.1 vs. 2.95 ± 0.14; NS) (Table 3).

Baseline Inotropic State and Response to Isoproterenol
There was no significant difference between untreated and untreated RHR hearts with regard to baseline level of LV pressure rise (Tables 1 and 2); however, the magnitude of inotropic responses (Δ dP/dt max) to different levels of isoproterenol was reduced in RHR as compared to control rats (P < 0.001) (Fig. 1). Nephrectomy improved this responsiveness, but not completely, so that the dose-response curve obtained was not statistically different from that of either normotensive rats, at one extreme, or of untreated hypertensive rats, on the other (Fig. 1). In contrast, captopril led to more evident improvement of the inotropic response to isoproterenol which became indistinguishable from the response of normotensive rats and significantly higher than that of untreated RHR (P < 0.001) (Fig. 2).

Myocardial β-Adrenergic Receptors
Tables 2 and 3 show the results of [3H]DHA binding to myocardial membranes. There was no statistically significant difference among all groups in regard to either the total number of ventricular β-receptors or their affinity (Kd). Compared to untreated normotensive sham-operated rats, the untreated RHR ventricles had a reduced β-receptor density per mg membranes protein (22.3 fmol/mg protein ± 2.66 SEM vs. 37.9 ± 4.34) and per g ventricular wet weight (1.40 pmol/g ventricles ± 0.14 vs. 2.24 ± 0.21). This reduction in β-receptor density (whether in fmol/mg protein or in pmol/g ventricle) was statistically significant both by ANOVA analysis, followed by a Newman-Keuls test (P < 0.01), and by the unpaired t-test (P < 0.005).

Nephrectomy of the clipped kidney led to increase in the ventricular β-receptor density of RHR, whether expressed in relation to membrane protein or to ventricular weight (31.0 ± 2.0 fmol/mg membrane protein vs. 22.3 ± 2.66, P < 0.05 and 1.8 ± 0.11 pmol/g ventricles vs. 1.4 ± 0.14, respectively). The changes induced by nephrectomy in RHR showed different degrees of statistical significance
Table 2

Effects of Nephrectomy on Blood Pressure, Ventricular Weight, Baseline dP/dt, and Ventricular Receptors in Renal Hypertension

<table>
<thead>
<tr>
<th>Variable</th>
<th>SH-R,o (n = 14)</th>
<th>RHR-R,o (n = 13)</th>
<th>RHR-N, (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>127 ± 2.9</td>
<td>193 ± 7.8†</td>
<td>158 ± 8.5‡</td>
</tr>
<tr>
<td>Ventricular wt (mg/g body wt)</td>
<td>2.76 ± 0.05</td>
<td>4.09 ± 0.22†</td>
<td>2.95 ± 0.14‡</td>
</tr>
<tr>
<td>Peak dP/dt (mm Hg/sec)</td>
<td>1734 ± 82</td>
<td>1601 ± 70</td>
<td>1535 ± 74</td>
</tr>
<tr>
<td>Receptor density (pmol/g)</td>
<td>2.24 ± 0.21</td>
<td>1.40 ± 0.14*</td>
<td>1.80 ± 0.11</td>
</tr>
<tr>
<td>Membrane specific activity (fmol/mg membrane protein)</td>
<td>37.9 ± 4.34</td>
<td>22.3 ± 2.66*</td>
<td>31.0 ± 2.0</td>
</tr>
<tr>
<td>Total ventricular receptors (pmol/2 ventricles)</td>
<td>3.44 ± 0.45</td>
<td>2.61 ± 0.31</td>
<td>2.9 ± 0.23</td>
</tr>
<tr>
<td>Dissociation constants (Kd) (nM)</td>
<td>3.52 ± 0.31</td>
<td>3.66 ± 0.49</td>
<td>2.74 ± 0.47</td>
</tr>
</tbody>
</table>

Values are mean ± 1 se. Abbreviations: SH-R,o = untreated sham-operated rats; RHR-R,o = untreated hypertensive rats; RHR-N, = nephrectomized hypertensive rats.

Statistical analysis by ANOVA and Newman-Keuls: *P < 0.05 and †P < 0.01 from SHR-R,o; ‡P < 0.01 from SHR-R,o.

according to the test applied: (1) they were significant (P < 0.01) by Student’s t-test, (2) the increase in density (fmol/mg protein) was also significant by ANOVA and Dunnett’s test for the effect of treatment on RHR (P < 0.05), but (3) the changes did not attain statistical significance when tested by ANOVA and Newman-Keuls test for differences among all groups of rats in this study. Thus, in contrast to the results in untreated RHR which were consistently different by all methods of analysis from normotensive controls, the RHR treated by nephrectomy had values that were not significantly different from either normotensive rats or from untreated hypertensive rats. The results were interpreted as a partial reversal by nephrectomy of the significant reduction of ventricular β-receptor density found in RHR. Nephrectomy per se in normotensive rats did not alter the density of β-receptors (2.367 ± 0.25 pmol/g vs. 2.324 ± 0.21), the membrane-specific binding activity (33.57 ± 2.8 fmol/mg protein vs. 37.98 ± 4.3) or the total receptor recovery (3.164 ± 0.34 pmol vs. 3.429 ± 0.46) (P > 0.1 for all). It did however, reduce Kd slightly (2.54 ± 0.28 nM vs. 3.51 ± 0.31); but this reduction was not significant by either the Newman-Keuls or Dunnett’s tests.

The receptor density in captopril-treated shams, as well as their membrane specific activity, was

Table 3

Effect of Captopril on Blood Pressure, Ventricular Weight, Baseline Peak dP/dt, and Ventricular Receptors in Renal Hypertension

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sh-CEI (n = 8)</th>
<th>RHR-CEI (n = 11)</th>
<th>RHR-R,o (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>116 ± 3.1</td>
<td>136 ± 3.5</td>
<td>193 ± 7.8</td>
</tr>
<tr>
<td>Ventricular wt (mg/g body wt)</td>
<td>2.64 ± 0.15</td>
<td>3.04 ± 0.1</td>
<td>4.09 ± 0.22</td>
</tr>
<tr>
<td>Peak dP/dt (mm Hg/sec)</td>
<td>1572 ± 194</td>
<td>1867 ± 60</td>
<td>1601 ± 70</td>
</tr>
<tr>
<td>Receptor density (pmol/g)</td>
<td>1.85 ± 0.2</td>
<td>1.8 ± 0.22</td>
<td>1.40 ± 0.14</td>
</tr>
<tr>
<td>Membrane specific activity (fmol/mg membrane protein)</td>
<td>30.9 ± 3.2</td>
<td>30.8 ± 3.8</td>
<td>22.3 ± 2.66</td>
</tr>
<tr>
<td>Total ventricular receptors (pmol/2 ventricles)</td>
<td>2.6 ± 0.36</td>
<td>2.46 ± 0.29</td>
<td>2.61 ± 0.31</td>
</tr>
<tr>
<td>Dissociation constant (Kd) (nM)</td>
<td>4.64 ± 0.78</td>
<td>3.74 ± 1.0</td>
<td>3.66 ± 0.49</td>
</tr>
</tbody>
</table>

Values are mean ± 1 se. Abbreviations: RHR-R,o = untreated hypertensive rats; Sh-CEI = captopril-treated sham-operated rats; RHR-CEI = captopril-treated hypertensive rats.

None of the differences between sham-operated rats given captopril (Sh-CEI) and RHR given captopril (RHR-CEI) reached statistical significance except for systolic blood pressure (P < 0.05); see text for full details of statistical analysis.
lower than in untreated controls, but the difference did not reach statistical significance. Both the membrane-specific binding activity and receptor density in captopril-treated RHR were indistinguishable from that of captopril-treated controls (30.8 ± 3.8 fmol/mg membrane protein vs. 30.9 ± 3.2, and 1.8 ± 0.22 pmol/g ventricles vs. 1.85 ± 0.2, respectively, both NS). Analysis of variance of all the groups investigated revealed that results in the captopril-treated RHR were not significantly different from those of either untreated RHR or untreated normotensive controls.

Since captopril therapy involved the use of a drug, the rats treated with captopril were randomly separated into two groups. In some captopril-treated rats, experiments were performed while they were still consuming the drug; in others, however, the drug was removed from the drinking water 48 hours before the experiments were performed, allowing a significant washout because of the short half-life of the drug. Because of the short period involved, blood pressure remained well-controlled, despite cessation of the drug (Dustan et al., 1968). The data obtained were similar in both groups (Table 4).

In summary, untreated RHR were consistently found, whatever the statistical test applied, to have a statistically significant reduction in ventricular β-receptor density compared with sham-operated, normotensive controls. Treatment of sham-operated rats by either method led to no statistically signifi-


current change in β-receptors, whether the statistical analysis (ANOVA) included all the groups of rats or was restricted to the sham-operated group alone. Treatment of RHR led to increase in ventricular β-receptor density; this reversal toward normal was only partial, so that the treated RHR were not significantly different from either the untreated RHR or from the normotensive controls. Further, analysis of the hypertensive groups only for the effects of "treatment" showed a statistically significant increase in ventricular β-receptors following nephrectomy of the clipped kidney (P < 0.05 by ANOVA and Dunnett's test).

**Discussion**

Reduced inotropic responsiveness to isoproterenol has often been documented in hypertensive rats, both SHR (Fujitava et al., 1972; Pfeffer et al., 1974; Saragoca and Tarazi, 1981a) and in 2K-1C Goldblatt hypertensive rats (Saragocona and Tarazi, 1981b). Similarly, reduction in ventricular β-adrenergic receptors has been reported in different models of hypertension by many investigators (Limas and Limas, 1978; Woodcock et al., 1979; Yamada et al., 1980), although not by all (Giacheti et al., 1979; Limas, 1979). We have determined in a previous study, both ventricular β-adrenergic receptors and inotropic response to isoproterenol in the same heart of RHR (2K-1C), and reported that left ventricular hypertrophy in this model was associated with a concomitant reduction in both variables; both alterations were significantly related to LV mass in untreated hypertensive rats (Ayobe et al., 1982). In this study, we have further documented that control of hypertension and regression of hypertrophy by nephrectomy or by captopril, increased ventricular inotropic responsiveness (Figs. 1 and 2) as well as ventricular β-receptor density in the same hearts.

The improvement in functional response of regressed hearts to isoproterenol correlated with the reduction in ventricular weight so that the relationship of inotropic response to ventricular weight (Saragoca and Tarazi, 1981b; Ayobe et al., 1982) was not altered; all data from regressed hearts fell within the 95% prediction limits of the line of regression constructed from data of normotensive and untreated hypertensive animals (Fig. 3). Similarly, the reversal of hypertrophy did not alter the basic correlation found between receptor density and ventricular weight and that between inotropic response and ventricular β-receptor density established on the basis of data from the untreated hypertensive rats (Figs. 4 and 5).

However, these correlations between number of β-receptors and inotropic responsiveness to isoproterenol could represent merely an associative, rather than a causal, connection. The reduction of inotropic responses of the hypertrophied hearts could conceivably be related to ischemia of the preparation or to a defect in capacity of maximal energy development by the myocardium instead of a specifically disturbed adrenoceptor mediation. That possibility, however, was not substantiated in our previous studies of the same model of hypertensive cardiac hypertrophy: inotropic responsiveness of hypertrophied hearts to scillaren as well as to changing concentrations of calcium in the perfusion medium was not different from that of normal hearts (Ayobe and Tarazi, 1983).

These observations still do not exclude, however, the possibility that mechanisms other than just the number or density of ventricular β-receptors may also be interfering with inotropic responses of the

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A (n = 5)</th>
<th>Group B (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure</td>
<td>133 ± 5</td>
<td>139 ± 7</td>
</tr>
<tr>
<td>Ventricular wt/body wt</td>
<td>2.90 ± 0.06</td>
<td>3.2 ± 0.16</td>
</tr>
<tr>
<td>Baseline peak dP/dt</td>
<td>1772 ± 92</td>
<td>1946 ± 71</td>
</tr>
<tr>
<td>Δ dP/dtΔt</td>
<td>1548 ± 104</td>
<td>1509 ± 179</td>
</tr>
<tr>
<td>Receptor density</td>
<td>1.98 ± 0.28</td>
<td>1.68 ± 0.153</td>
</tr>
<tr>
<td>Kd (nM)</td>
<td>4.44 ± 1.62*</td>
<td>2.77 ± 0.31</td>
</tr>
</tbody>
</table>

Values are mean ± 1 se.
* The mean ± se were high (although nonsignificant) because of the high Kd of one heart which we did not exclude.
VENTRICULAR BETA ADRENERGIC RECEPTORS
AND REGRESSION OF CARDIAC HYPERTROPHY

**Figure 4.** See legend for figure 3. Regression equation for untreated RHR and their controls: $Y = 3.314 - 0.24X$ ($r = -0.45; P = 0.017$).

Hypertrophied heart to adrenergic stimuli. Other factors distal to the receptors may be involved in the impairment of inotropic responses to adrenergic stimuli. As a matter of fact, membranes prepared from hypertrophied hearts of hypertensive animals have demonstrated lower isoproterenol-stimulated adenylcyclase activity than control (Amer et al., 1974; Woodcock et al., 1979). In another study, hearts from the same hypertensive model (2K-1C RHR) showed reduced isoproterenol-stimulated adenylcyclase activity, together with evidence for $\beta$-receptor-adenylcyclase uncoupling (Kumano et al., 1983). Whatever the exact extent of involvement of different components of the $\beta$-adrenergic system, it is evident that hypertensive cardiac hypertrophy was associated in many studies with a reduction in inotropic response to adrenergic stimuli and in ventricular $\beta$-receptor density, and these alterations were reversible by control of hypertension and regression of LV hypertrophy.

Our results cannot, however, differentiate between the variables of "hypertension" and of "hypertrophy." In this model of hypertension, blood pressure levels and left ventricle mass are very closely correlated as documented both in this study ($r = 0.80, P < 0.001$) and in previous reports (Hall et al., 1953; Sen et al., 1981; Saragoca and Tarazi, 1981b; Wicker and Tarazi, 1982). The answer to whether the hypertensive process or the increase in cardiac weight plays the more important role depends on the dissociation in experimental models of blood pressure levels from cardiac mass. In this regard, Tse et al. (1979) have reported a reduction in ventricular $\beta$-receptors in isoproterenol-induced cardiac hypertrophy; however, it is still not clear whether this reduction was related to the hypertrophied process or to down-regulation of $\beta$-receptors by their agonist (Davis and Leftkowitz, 1981).

**Functional Implications**

Relatively little is known regarding the quantitative assessment of ventricular function following reversal of hypertrophy; the few reports available were concerned mainly with the responses of the heart to volume overload (Spech et al., 1980, Kuwajima et al., 1982). However, cardiac performance cannot be assessed adequately from the response to only one type of stress (Saragoca and Tarazi, 1981b). The results of the present study suggest that inotropic responsiveness to adrenergic stimuli is definitely reduced in hypertensive animals; this responsiveness is restored to normal as blood pressure is controlled, and ventricular hypertrophy regressed with normalization of ventricular $\beta$-receptor density.

To the extent that cardioadrenergic support is a major adaptive mechanism of the heart (Bugge-Asperheim and Kiil, 1973; Braunwald et al., 1976a; Tarazi and Levy, 1982), the variation in ventricular $\beta$-receptors and responsiveness to isoproterenol can play an important role in the evolution of hypertensive heart disease. Bristow et al. (1982) have recently reported a marked decrease of ventricular $\beta$-receptors in hypertrophied failing human hearts. The
results of the present study suggest that effectiveness of adrenergic support to the heart may improve with reversal of ventricular hypertrophy.

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INDEX TERMS: Myocardial \(\beta\)-receptors • Isoproterenol • Contractile reserve • Regression of cardiac hypertrophy • Hypertension
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