Impaired inotropic responses to \(\alpha\)-adrenergic stimulation in experimental left ventricular hypertrophy

Fetnat M. Fouad, M.D., Kazumasa Shimamatsu, M.D., Mary M. Hanna, M.D., Philip A. Khairallah, M.D., and Robert C. Tarazi, M.D.

ABSTRACT We have previously reported that left ventricular hypertrophy in two-kidney, one-clip renal hypertensive rats (2K-1C RHRs) was associated with diminished inotropic responsiveness to isoproterenol and glucagon, suggesting an alteration in the receptor-adenylate cyclase cascade. The present study was performed to investigate the hypothesis that in these same hearts, inotropic responses to \(\alpha\)-adrenergic stimuli could be enhanced as a compensatory mechanism. \(\alpha\)-Adrenergic stimulation was achieved by graded phenylephrine infusion (1.02 to 41.2 \(\mu\)M/min) in the presence of propranolol (10^{-7}M). The inotropic response was evaluated in the isovolumetric isolated rat heart (Langendorff preparation) paced at 260 beats/min. Results showed a significantly reduced inotropic response to \(\alpha_1\)-adrenergic stimulation in 2K-1C RHR hearts irrespective of perfusion pressure (50 or 80 mm Hg [PP_{50} or PP_{80}]) (+ 427.5 ± 62.1 vs + 1236 ± 216.4 mm Hg·sec^{-1} at PP_{50}, p < .01 and + 339 ± 97.3 vs + 1440 ± 254 mm Hg·sec^{-1} at PP_{80}, p < .001) even when comparison was made at equivalent myocardial flow rates (RHR hearts perfused at 80 mm Hg vs control hearts perfused at 50 mm Hg). Quantitative assessment of number of \(\alpha_1\)-adrenergic receptors (\(^3\)H-prazosin binding) showed a significant decrease compared with that in age-matched sham-operated normotensive control rats (45 ± 2.5 vs 64 ± 1.7 fmol/mg protein, p < .001). These results indicate that impaired inotropic responsiveness to adrenergic stimuli in left ventricular hypertrophy is not only related to \(\beta\)-agonists or adenylate cyclase activators but also involves \(\alpha\)-adrenergic stimulants, and that the latter may be related to reduced density of left ventricular \(\alpha_1\)-receptors.


WE HAVE PREVIOUSLY REPORTED that left ventricular hypertrophy in two-kidney, one-clip renal hypertensive rats (2K-1C RHRs) was associated with diminished inotropic responsiveness to \(\beta\)-adrenergic stimulation by isoproterenol\(^1\)-\(^3\) and to non-\(\beta\)-receptor-mediated stimulation of the adenyl cyclase system by glucagon\(^4\) or vasoactive intestinal peptide.\(^5\) Thus, a question was raised regarding the compensatory mechanisms that can help the hypertrophied heart maintain cardiac performance under stressful conditions. Since \(\alpha\)-adrenergic stimulation was first shown by Wenzel and Su\(^6\) and later confirmed by others\(^7\)-\(^9\) to have a positive inotropic effect, we investigated the possibility that \(\alpha\)-adrenergic responsiveness might be increased to compensate for the reduced \(\beta\)-adrenergic function. In this respect, Kunos et al.\(^10\) reported that the isoproterenol/phenylephrine potency ratio in isolated atria was significantly lower in spontaneously hypertensive rats (SHRs) than in normotensive controls, while Fujiwara et al.\(^11\) reported no difference in response to phenylephrine between SHRs and Wistar-Kyoto rats. On the other hand, however, was the reported decrease in \(\alpha\)-adrenergic receptor density in the myocardium of RHRs.\(^12\) The present work was therefore designed to determine whether hypertrophied hearts from 2K-1C RHRs with diminished inotropic responsiveness to isoproterenol show a compensatory increase in inotropic \(\alpha\)-adrenergic responsiveness or whether they have impaired responses to both sections of the adrenergic system.

Materials and methods

Hypertensive animal preparation. Thirty-four male Sprague-Dawley rats (Hilltop Lab, Scottsdale, NJ) were stud-
ied. At 6 weeks of age (150 to 174 g body weight) 2K-1C renal hypertension was produced in one group (Goldblatt rats, n = 17), while the others underwent sham operations (n = 17) as previously described.1-3 All rats were then accommodated in a climate-controlled room and fed a regular diet of Rodent Laboratory Chow 5001 with ad lib intake of water. Blood pressure was monitored by the tail-cuff method13 at 2, 3, 6, and 7 weeks after the operation. Only those rats in which blood pressure rose to more than 160 mm Hg within the first 2 weeks of surgery were considered hypertensive and were included in the RHR group. Sham-operated rats remained normotensive. All rats were handled and fed in the same way.

Isolated heart perfusion. The rats were studied 8 weeks after surgery (14 weeks of age) with the use of a modified Langendorff preparation. Under pentobarbital anesthesia (30 mg/kg ip) and adequate ventilation (tracheostomy with positive end-expiratory pressure), the chest of each was opened and the heart was quickly extracted and put in a cold Krebs-Henseleit bicarbonate buffer that had been saturated with oxygen and in which the heart continued to beat spontaneously but very slowly. The heart was then attached securely to the Langendorff apparatus via its aortic stump. Retrograde perfusion was begun immediately through the aorta with an oxygenated (95% O2 and 5% CO2) Krebs-Henseleit bicarbonate buffer solution maintained at 37°C and pH 7.4. The perfuse consisted of (in mM): 117 NaCl, 4.7 KCl, 2.5 CaCl2 plus 0.5 to balance EDTA, 1.2 KH2PO4, 25 NaHCO3, 0.5, Na2EDTA and 8.5 dextrose. Immediately after the start of perfusion, the base of the pulmonary artery was incised to allow efficient drainage of the right ventricle. The left ventricular cavity was drained via PE-50 intramedic polyethylene tubing. This step was followed by the insertion into the left ventricle, via the mitral valve, of a balloon secured around a transducer-tipped catheter. The atrioventricular node was then crushed and the isolated heart was paced at a constant rate of 260 beats/min. This rate was chosen because it was found in preliminary experiments in 26 rats (both normotensive and renal hypertensive) to be the spontaneous beating rate of isolated hearts in our preparation. A bipolar stimulator (Grass S9 stimulator, Grass Instruments, Quincy, MA) was used for pacing; duration of the stimulus was 5 msec at 2 V. The left ventricular balloon was filled with water until end-diastolic pressure reached 0 mm Hg; pressure was determined by a transducer-tipped No. 5F Millar catheter (Millar Instruments, Houston, TX); the frequency response of the system was 200 Hz. The time at which regular rapid spontaneous beating of the heart restarted after mounting was noted at time “zero” of the experiment.

Thirty minutes were allowed for equilibration. During this time, the left ventricular systolic balloon pressure, its first derivative (left ventricular dP/dt), and the left ventricular end-diastolic balloon pressure were continuously monitored and recorded on a Brush recorder (Gould Inc., Cleveland, OH) at a paper speed of 0.05 mm/sec. At the end of the equilibration period, the paper speed was increased to 50 and 200 mm/sec to obtain baseline readings.

Myocardial flow was measured by collecting the perfusate for 5 min periods and myocardial flow rate was calculated in milliliters per gram of left ventricle per minute; left ventricular weight was determined at the end of the experiment with a Mettler PC 440 precision balance (Mettler Instrument Corp., Hightstown, NJ). Initial studies showed that development of tissue edema was minimal during the experiment, averaging less than 5% of the initial left ventricular weight. The accuracy of the measurements of myocardial flow rate was previously ascertained by comparison with rate measured by a T-shaped electromagnetic flowmeter.4

Protocol of the study. Two sets of experiments were performed: (1) in one set 16 hearts from eight control rats and eight RHRs were perfused at 50 mm Hg, and (2) in the other set 18 hearts (nine sham and nine RHRs) were studied in exactly the same way but at a perfusion pressure of 80 mm Hg. These two sets of experiments allowed us to evaluate the effects of both perfusion pressure and myocardial flow rate on cardiac performance in the normal and hypertrophied hearts. All experiments were performed in β-blocked hearts; β-blockade was achieved by infusing 10−7M propranolol to the perfusing buffer. This dose was previously found to completely block the maximum isotropic effect of isoproterenol in our preparation.

After recording baseline data, phenylephrine HCl diluted with Krebs-Henseleit bicarbonate buffer was infused continuously via a Harvard pump into the aorta just above the aortic stump in graded doses (1.02, 2.06, 4.12, 10.2, 20.6, and 41.2 µg/min) until a plateau was reached; this usually occurred within 5 min. This infusion was performed under continuous cover by propranolol (10−7M) to block the β-stimulating effects of phenylephrine.

Assay of myocardial α1-receptors. Ventricular membranes were prepared according to the method of Karliner et al.14 Briefly, the ventricles were frozen in dry ice/acetone and stored at −80°C, and then thawed, minced, and homogenized in 5 mM Tris buffer, pH 7.4 and containing 0.25M sucrose and 1 mM MgCl2, with use of a Polytron (two bursts × 15 sec). The homogenate was centrifuged at 500 g and the resultant supernatant was centrifuged at 50,000 g to precipitate membrane protein. The pellet was suspended in 50 mM Tris buffer, pH 7.5 and containing 10 mM MgCl2. Protein concentration was determined in a 100 µl aliquot of the resuspended pellet by the method of Lowry et al.,15 with the use of bovine serum albumin as a standard.

Incubation of membrane protein (0.8 to 1.0 mg) was carried out in duplicate with six different concentrations of 3H-prazosin ranging from 0.1 to 2 nM for 15 min at 25°C, with or without 10−5M phenolamine. Incubation was stopped by diluting the solution with 4 ml cold Tris buffer and immediately filtering it through Whatman GF/C filters that were then washed with 3 × 4 ml Tris buffer and left to dry overnight. The filter’s radioactivity was then counted.

The specific binding of 3H-prazosin, calculated as that not displaced by phenolamine, was found to average 80% to 90% of the total binding. The calculated number of maximum binding sites and the dissociation constants were determined by Scatchard analysis.16

Statistical analysis.17 Values are reported as mean ± SEM. Findings in the sham-operated and the 2K-1C RHR groups were compared by Student’s t test. The extra sum of squares principle was adopted to compare the dose-response curves in the two groups; results were validated by comparison of maximum responses of the two experimental groups to phenylephrine with the Student t test.

Drugs used. Phenylephrine hydrochloride (Neo-Synephrine, Winthrop Lab, NY), propranolol hydrochloride (Inderal, Ayerst Lab, NY), phenolamine mesylate (Regitine, CIBA Pharmaceutical Co., NJ), and isoproterenol hydrochloride (Isuprel, Breon Lab, Inc., NY) were used. 3H-prazosin was obtained from New England Nuclear and had a specific activity of 19.8 Ci/mM.

Results

Clipping of the renal artery induced hypertension associated with left ventricular hypertrophy. The systolic blood pressure in RHRs was markedly higher than in sham-operated control rats (224 ± 6 vs 124 ± 3
mm Hg [n = 17 in each group], p < .001). Left ventricular weight was increased in RHRs, both in absolute value (1367 ± 62 vs 1065 ± 29 mg, p < .001) and when normalized for body weight (4.17 ± 0.18 vs 2.38 ± 0.05 mg/g, p < .001).

### Myocardial α1-adrenergic receptors in RHR

As summarized in Table 1, membrane recovery (mg protein/g tissue) was similar in preparations from normal and hypertrophied hearts. Examination of the density of α1-receptors (expressed either as fmol/mg protein or as pmol/gm ventricle) showed a 30% decrease in RHRs (n = 14) compared with sham-operated controls (n = 20, p < .001); there was no significant difference in affinity between the two groups.

Since the left ventricular weight in hypertensive animals was increased, the number of α1-adrenergic receptors per total ventricle was only slightly decreased (NS).

### Baseline contractility

As shown in Table 2, the 2K-1C RHRs tended to have a lower left ventricular dP/dt than control rats; the difference attained statistical significance when the perfusion pressure was 80 mm Hg (PP0) (2413 ± 173 vs 2971 ± 165 mm Hg·sec−1, p < .05), but not at lower perfusion pressures (50 mm Hg, PP50) (1723 ± 122 vs 2085 ± 156 mm Hg·sec−1, p > .05). The same findings were obtained with regard to left ventricular systolic pressure; it was significantly lower in RHRs at PP50 (85 ± 6.0 vs 107 ± 7 mm Hg, p < .05), but the reduction did not attain statistical significance at PP0 (58 ± 4 vs 68 ± 6 mm Hg, p > .05).

Myocardial flow rate was markedly lower in RHRs than in normal controls at both perfusion pressures: 6.9 ± 0.7 vs 17.7 ± 2.3 ml/g left ventricular weight/min at PP50 (p < .001) and 12.5 ± 1.7 vs 26.0 ± 0.8 ml/g left ventricular weight/min at PP0 (p < .001). The myocardial flow rate in RHRs perfused at 80 mm Hg (12.5 ± 1.7 ml/g left ventricular weight/min) was not statistically different from that in control rats perfused at 50 mm Hg (17.7 ± 2.3 ml/g/min), so that the groups could be compared either at equal perfusion pressures or at equivalent myocardial flow rates.

### Left ventricular response to phenylephrine

Phenylephrine infused in the presence of 10−6 M propranolol significantly increased left ventricular dP/dt without changing left ventricular end-diastolic pressure (which remained at 0 mm Hg); this inotropic effect was completely abolished by the α-adrenoceptor antagonist phentolamine (5 × 10−6 M).

The left ventricular dP/dt response to graded doses of phenylephrine was significantly blunted in RHRs compared with control rats at both PP50 and PP0, whether the results were expressed in absolute terms or as percentages of baseline values (figures 1 and 2). The difference persisted when RHR hearts perfused at 80 mm Hg were compared with those from sham-operated animals perfused at 50 mm Hg (i.e., at equivalent myocardial flow rate) (figure 3). The maximum left ventricular + dP/dt response of RHR hearts to phenylephrine was significantly lower than normal at both PP50 and PP0 (+427.5 ± 62.1 vs +1236 ± 216.4

### TABLE 1

**Myocardial α1-adrenergic receptors in 2K-1C RHRs**

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 20)</th>
<th>RHR (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane yield</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg protein/g ventricle)</td>
<td>21.5 ± 0.2</td>
<td>22.5 ± 0.1</td>
</tr>
<tr>
<td>3H-prazosin binding sites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>receptor density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fmol/mg protein</td>
<td>64 ± 1.7</td>
<td>45 ± 2.5</td>
</tr>
<tr>
<td>pmol/g ventricle</td>
<td>1.4 ± 0.19</td>
<td>0.95 ± 0.04</td>
</tr>
<tr>
<td>Total number</td>
<td>1.5 ± 0.04</td>
<td>1.4 ± 0.06</td>
</tr>
<tr>
<td>Dissociation constant (nM)</td>
<td>0.17 ± 0.004</td>
<td>0.15 ± 0.002</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

### TABLE 2

**Inotropic response to phenylephrine in isolated rat hearts: baseline data**

<table>
<thead>
<tr>
<th>Groups (n)</th>
<th>LV wt (mg/g BW)</th>
<th>MFR (ml/g LV wt/min)</th>
<th>LV + dP/dt (mm Hg/sec)</th>
<th>LV sys pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (8)</td>
<td>2.44 ± 0.08</td>
<td>17.7 ± 2.3</td>
<td>2085 ± 156</td>
<td>68 ± 6</td>
</tr>
<tr>
<td>RHR (8)</td>
<td>4.35 ± 0.35</td>
<td>6.9 ± 0.7</td>
<td>1723 ± 122</td>
<td>58 ± 4</td>
</tr>
<tr>
<td>PP50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (9)</td>
<td>2.32 ± 0.07</td>
<td>26.0 ± 0.8</td>
<td>2971 ± 165</td>
<td>107 ± 7</td>
</tr>
<tr>
<td>RHR (9)</td>
<td>4.38 ± 0.38</td>
<td>12.5 ± 1.7</td>
<td>2413 ± 173</td>
<td>85 ± 6</td>
</tr>
</tbody>
</table>

Values and mean ± SE. The difference in MFR between control rats perfused at 50 mm Hg and RHRs perfused at 80 mm Hg was not significant.

LV = left ventricular; wt = weight; BW = body weight; MFR = myocardial flow rate; sys = systolic.

Significantly different from controls.
mm Hg·sec\(^{-1}\) at PP\(_{50}\), \(p < .01\) and \(+339 \pm 98.3\) vs \(+1440 \pm 254\) mm Hg·sec\(^{-1}\) at PP\(_{80}\), \(p < .001\); similar findings were obtained when results were expressed as percentages of control values (25.5 \(\pm 4.2\)% vs 64.2 \(\pm 14.3\)% at PP\(_{50}\), \(p < .05\) and 14.1 \(\pm 3.8\)% vs 49.4 \(\pm 8.4\)% at PP\(_{80}\), \(p < .01\)).

A significant inverse correlation was found between left ventricular weight and the maximum left ventricular dP/dt response to phenylephrine \((r = .66, p < .0001, n = 34)\) (figure 4).

### Discussion

Adrenergic support is a major cardiac compensatory mechanism, particularly in cases of myocardial dysfunction.\(^\text{18}\) However, we\(^\text{1-5}\) and others\(^\text{10,19}\) have previously documented a significant reduction in inotropic responsiveness of the hypertrophied left ventricle to \(\beta\)-adrenergic agonists as well as to other stimuli of the adenylate cyclase system.\(^\text{4}\) Under these conditions, the adrenergic nervous system might become increas-
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mechanism to override the blunting of end-organ responsiveness; this overactivity is particularly evident in advanced dysfunction and cardiac failure. 20

Until recently most studies of cardioadrenergic support dealt with the better known \( \beta \)-adrenergic system. However, after the development of an assay for \( \alpha \)-receptors, 14 a large body of evidence has been obtained demonstrating the presence of \( \alpha \)-adrenergic receptors in cardiac muscle. The balance of \( \alpha \) - and \( \beta \) -receptors in the myocardium was shown to be altered by temperature 21 and thyroid hormone, 22 as well as in different types of hypertension. 11, 23 Since \( \alpha \)-adrenergic stimulation was also shown to have a definite cardiac inotropic effect, 5-8, 24 it is possible to hypothesize that in the earlier stages of compensated left ventricular hypertrophy, the balance between the \( \beta \) and \( \alpha \) components of the adrenergic nervous system is readjusted by increasing responsiveness to \( \alpha \)-stimulation that develops to compensate for diminished \( \beta \) responses. Extending the work of Woodcock and Johnston 11 in 1K-1C RHRs, our results in the 2K-1C preparation confirmed the reduction of myocardial \( \alpha \)-adrenoceptors. However, very little is known regarding the functional consequences of these changes in cardiac \( \alpha \)-adrenoceptors; extrapolation from changes in number or affinity to parallel alterations in responsiveness would be hazardous at best and sometimes erroneous. A single report by Kunos et al. 9 described a reduction in response of the left atrium of the SHR to phenylephrine (in terms of maximal increase in force), but no change in \( EC_{50} \) (effective concentration for 50% of the group) values; they ascribed their results to reduced sensitivity of the SHR to \( \beta \)-stimulation. The data obtained in our study are the first demonstration of a significant reduction in ventricular inotropic response to \( \alpha \)-stimulation. This reduction was highly significant under all conditions of the study, irrespective of the perfusion pressure or of myocardial flow rate at which the inotropic response was determined (figures 1 to 3).

The impaired inotropic response to \( \alpha \)-stimulation by phenylephrine in left ventricular hypertrophy of RHRs is similar to previous findings of reduced left ventricular responses to \( \beta \)-stimulation in both SHRs and RHRs. 1-3 In both preparations of hypertensive left ventricular hypertrophy, the dP/dt response to either \( \alpha \)- or \( \beta \)-adrenergic stimulation was inversely related to left ventricular weight; the heavier the left ventricle, the lesser its inotropic response (figure 4). These findings could be theoretically related to a number of factors, such as differences in myocardial perfusion between normal and hypertensive hearts or impaired response of the contractile proteins in left ventricular hypertrophy. These possibilities, however, are not likely for the following reasons: (1) the impairment in inotropic response was still evident when perfusion pressure was raised in the RHR group (PP50) to obtain a myocardial flow rate equivalent to that in normal control rats perfused at lower pressure (PP50); (2) under similar situations exactly the same preparation of left ventricular hypertrophy, the inotropic response to extracellular calcium and to scillaren remained normal, 2 suggesting that impairment in response to \( \beta \)-stimulation pointed to a defect in the early pathway of excitation-contraction coupling. 25 It therefore seems reasonable to conclude that the reduction in inotropic response of \( \beta \)-blocked left ventricular hypertrophy to phenylephrine may be related, at least in part, to the significant diminution in \( \alpha \)-ventricular receptor density (table 1). In addition, alterations in the affinity state of the receptors could influence any effect of the reduction in density; however, complete analysis of our prazosin binding data and early results obtained with phenylephrine competitive curves have not given any evidence for more than one affinity state, as previously reported for the specific ligands \( H^3 \)-prazosin 14 and \( H^3 \)-WB-4101. 26, 27 Alterations in ventricular \( \alpha \)-receptors might not be the sole explanation for the reduction in inotropic response and their presence certainly does not exclude the possibility of some other defects in the biochemical steps consequent to \( \alpha \)-receptor stimulation.

The contribution of \( \alpha \)-adrenergic stimulation to myocardial inotropy might not be inconsequential when cardioadrenergic support is needed. The maximum response of left ventricular dP/dt to phenylephrine (in the presence of propranolol) averaged 46.7 ± 16.5% of the maximum response to isoproterenol in hearts from control rats. In 2K-1C RHRs, the maximum response to \( \alpha \)-stimulation averaged 37.7 ± 5.2% of the maximum inotropic response to isoproterenol. A reduction in responsiveness to \( \alpha \)-stimulation may therefore aggravate the effect of impaired inotropic response of left ventricular hypertrophy to \( \beta \)-stimulation. Diminished effectiveness of cardioadrenergic support due to hypertrophy, hypertension, or age has been suggested as a factor in progression of cardiac dysfunction. 1, 28, 29

In conclusion, hypertrophied hearts from RHRs have a diminished inotropic response to \( \alpha \)-adrenergic agonists in addition to their reduced inotropic responsiveness to \( \beta \)-adrenergic stimuli. These results suggest that under stress, the hypertrophied heart may have to depend on other compensatory mechanisms (such as
the Frank-Starling mechanism) to maintain cardiac performance. Alternatively, the sympathetic nervous system may be activated even at rest to compensate for the blunted responsiveness of the end organ; this increased adrenergic activity may initiate further injury to the myocardial cell membranes. A vicious cycle could thus be created that might eventually result in cardiac decompensation.1, 2

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