Regional Changes in Creatine Kinase and Myocyte Size in Hypertensive and Nonhypertensive Cardiac Hypertrophy

Shirley H. Smith, Martha F. Kramer, Ilana Reis, Sanford P. Bishop, and Joanne S. Ingwall

Several intracellular enzymes have been shown to have altered total activity or isoenzyme composition in cardiac hypertrophy. This study tests the hypothesis that the accumulation of the fetal-type (BB+MB) creatine kinase (CK) isoenzymes in hypertrophied adult myocardium is related to an increase in blood pressure. Consideration was made for the location, size, and hemodynamic load of the myocytes. By using the two-kidney, one-clip (2K1C) rat model of renal hypertension with and without hydralazine treatment, CK (total and isoenzyme), lactate dehydrogenase, and citrate synthase activities and myocyte size were measured. An increased heart weight/body weight ratio occurred in both untreated 2K1C rats (4.15±0.09) and hydralazine-treated 2K1C rats (4.12±0.13) as compared with control rats (3.25±0.10). Blood pressure was high only in untreated 2K1C rats (196±9 mm Hg), as compared with hydralazine-treated 2K1C rats (142±6 mm Hg) and control rats (135±3 mm Hg). Myocytes were isolated from five ventricular regions: left ventricular epicardial and endocardial free wall, left and right halves of the interventricular septum, and right ventricular free wall. Regional differences in normal and hypertrophied myocardium were demonstrated for morphological and biochemical parameters, with the greatest changes occurring in left ventricular endocardium. The shift in CK isoenzyme expression toward accumulating more BB+MB was greater in “hypertensive hypertrophy” (untreated 2K1C rats) than in “nonhypertensive hypertrophy” (hydralazine-treated 2K1C rats). Calculations incorporating isolated myocyte volume showed that the cellular content of total CK remained the same during the hypertrophic process, accounting for a decrease in the tissue activity. Measurement of lactate dehydrogenase and citrate synthase activities suggests that hypertrophied myocardium has relatively higher glycolytic capacity and that this effect is exacerbated in the presence of high blood pressure. We conclude that increased blood pressure is more closely linked to the fetal CK isoenzyme shift than is hypertrophy alone. (Circulation Research 1990;67:1334–1344)

Cardiac hypertrophy is a common complication of hypertension and is recognized as a risk factor for the development of congestive heart failure, stroke, and myocardial infarction in hypertensive patients. Experimental animal studies have been useful in defining the role of blood pressure in the outcome of disease. For example, high blood pressure in the presence of hypertrophy has been shown to contribute to the susceptibility of the myocardium to ischemic damage. Greater mortality and more rapidly developing, larger infarcts were found in dogs with ischemia induced in the presence of both hypertension and cardiac hypertrophy.

The relation between hypertension and the development of cardiac hypertrophy is complex and not fully understood. For example, attenuation of hypertension did not prevent development of hypertrophy in spontaneously hypertensive rats treated with nerve growth factor antiserum, minoxidil, or hydralazine. On the other hand, angiotensin converting enzyme inhibitors prevented the formation of angiotensin II and prevented the development of both cardiac hypertrophy and hypertension in the spontaneously hypertensive rat. Several reports in both humans and animals have shown a poor correlation between heart weight and blood pressure. These studies and others have led several authors to suggest that factors other than blood pressure alone are important in the

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development of cardiac hypertrophy. Thus, the relative contributions of hemodynamic and nonhemodynamic factors to the development of cardiac hypertrophy and congestive heart failure remain to be defined.

In addition to the enlargement of heart muscle cells that occurs in cardiac hypertrophy,\textsuperscript{10} there are also changes in tissue content of many enzymes that control intermediary metabolism and muscle contraction.\textsuperscript{11} The hypertrophied myocardium contains a greater percentage of the fetal-type isoenzymes (BB+MB) of creatine kinase (CK),\textsuperscript{12} the M-LDH isoenzyme of lactate dehydrogenase (LDH),\textsuperscript{13,14} and the V\textsubscript{3} isof orm of myosin than is seen in normal adult myocardium.\textsuperscript{15} The isoenzymes that accumulate in the hypertrophied myocardium are also predominant in the fetal heart.\textsuperscript{16} The increase in these isoenzymes in adult hypertrophied myocardium has been termed a “fetal” shift. The stimulus linking the morphological and biochemical changes that occur in cardiac hypertrophy remains undefined.

The present investigation was undertaken to test the hypothesis that hypertension exacerbates the changes in the isoenzyme composition of CK produced by hypertrophy alone. To test this hypothesis, we used an experimental model that permits assessment of cardiac hypertrophy with and without hypertension. We used the two-kidney, one clip (2K1C) rat model of renal hypertension to produce cardiac hypertrophy in the presence of high blood pressure (“hypertensive hypertrophy”).\textsuperscript{10} Hydralazine treatment of 2K1C rats from the time of renal artery clipping was used to prevent the development of high blood pressure without preventing cardiac hypertrophy (“nonhypertensive hypertrophy”). Since regional variations in myocyte volume are known to occur in normal and hypertrophied myocardium,\textsuperscript{10,17} regional variations in enzyme activity were measured so that the relation between changes in enzyme activity and in cell size could be defined transmurally for the hypertrophied heart with and without hypertension.

Materials and Methods

Experimental Groups

Weanling male Sprague-Dawley rats (50–75 g) were obtained from Charles River Kingston Laboratories, Kingston, R.I., and were randomly placed into four experimental groups: 1) untreated 2K1C rats, with hypertension produced at weaning age (4 weeks) using the 2K1C renal artery clipping procedure, 2) hydralazine-treated 2K1C rats receiving 180 mg/l hydralazine in the drinking water throughout the study, 3) nonoperated, nontreated control rats, and 4) hydralazine-treated nonoperated control rats. The surgical procedure was performed with the rat anesthetized with ketamine plus xylazine (85 mg/kg t.p. ketamine and 13 mg/kg t.p. xylazine). A left flank incision was made, the left renal artery was isolated, and a silver clip (0.20 mm internal diameter) was placed on the vessel.\textsuperscript{10} This size clip is snug around the renal artery at this age and becomes progressively more constricting as the rat increases in weight. The right renal artery was untouched. All rats were housed individually in a room with a 12-hour light-dark cycle for 6 weeks. Rats were fed rodent laboratory chow (No. 5001, Agway, Raleigh, N.C.), and water was changed three times weekly. Weekly tail-cuff systolic pressures were measured using an electrosphygmomanometer (model PE-300, Narco Biosystems, Houston).

Criteria for Selection of 2K1C Rats

Due to the variable response of each rat to the clipping procedure, not all rats with clipped renal arteries develop hypertension.\textsuperscript{18} Since some rats were treated with an antihypertensive drug, it was necessary to apply objective criteria to identify those rats that would have become hypertensive in the absence of antihypertensive treatment. We previously demonstrated that compensatory hypertrophy of the right kidney and slight atrophy but not infarction of the left kidney were consistently associated with high blood pressure.\textsuperscript{18} Rats with a left kidney/right kidney weight ratio of less than 0.40 did not develop high blood pressure; this response was similar to a renal infarction. A ratio greater than 0.90 also failed to increase blood pressure, probably due to insufficient stenosis. Thus, only those 2K1C rats that had left kidney/right kidney weight ratios between 0.40 and 0.90 were used in the study group.

Myocyte Isolation

Myocytes were prepared as previously described.\textsuperscript{10,19} At the time of the study, rats were killed by decapitation, and their hearts were removed, lightly blotted, and weighed. The intact hearts were cannulated via the aortic root and mounted on a modified nonrecirculating Langendorf apparatus. Hearts were perfused for 2 minutes with calcium-free Joklik medium (GIBCO Laboratories, Grand Island, N.Y.) containing 0.5 mM EGTA (Sigma Chemical Co., St. Louis); this pro-

![Diagram of the five myocardial regions studied.](http://circres.ahajournals.org/)

**Figure 1.** Diagram of the five myocardial regions studied. RVFW, right ventricular free wall; RIVS, right half of the interventricular septum; LIVS, left half of the interventricular septum; LENDO, endocardial half of the left ventricular free wall; LEPI, epicardial half of the left ventricular free wall.
procedure was followed by a 15-minute perfusion with calcium-free Joklik medium without EGTA and containing 0.05% collagenase (Sigma). All media were maintained at pH 7.4 and 37°C and were aerated with 95% O₂-5% CO₂. Each heart was then divided into five portions for preparation of isolated myocytes: right ventricular free wall (RVFW), right half of the interventricular septum (RIVS), left half of the interventricular septum (LIVS), epicardial half of the left ventricular free wall (LEPI), and endocardial half of the left ventricular free wall (LEND) (Figure 1). Each portion was minced and passed through a 250-μm nylon mesh, and aliquots of the isolated cells were fixed by addition of an equal volume of isosmolar 2% buffered glutaraldehyde. Glass slide smears of myocytes from each group were stained with hematoxylin and eosin for light microscopic examination. Only those preparations having 80% or more cylindrical myocytes were accepted for study. For cell volume studies, the fixed cells were centrifuged (1,000 rpm) through 5 ml of 6% Ficoll, and the pellet was resuspended in 2% glutaraldehyde. Unfixed cell pellets and remaining minced, partially sieved tissue from each region were immediately frozen in liquid nitrogen, shipped on dry ice, and stored at −70°C for biochemical studies.

Cell Volume

Isolated fixed myocytes were suspended in Isoton II (Coulter Corp., Hialeah, Fla.), and median cell volume was determined by a microprocessor-controlled model ZH Coulter counter with a C-1000 Channelizer system. Median volume was used since distribution curves tended to be skewed to the right. The cells (20,000–40,000 per sample) were passed through an aperture of 140 μm. Adjustment of the shape factor to 1.08 was made, because myocytes are roughly rod-shaped and pass lengthwise through the electric field.

Biochemical Analysis

Samples of collagenase-digested myocardial tissue were weighed (10–20 mg) and homogenized (5 mg/ml) for 10 seconds at 4°C in 0.1 M potassium phosphate buffer, pH 7.4, containing 1 mM EGTA and 1 mM β-mercaptoethanol using a motor-driven scinttered glass pestle. Aliquots were removed for protein analysis. Triton X was then added to the homogenate to a final concentration of 0.1%. All enzyme assays were performed in duplicate. Enzyme activities were expressed as international units per milligram protein. Activity per cell (μIU/cell) was calculated as previously described for each region using the following formula, assuming that the density of tissue is 1.0 μl/mg: μIU/cell=(IU/mg protein)×(mg protein/mg wet weight)×(fmoliters/cell)/1,000.

Total CK activity was measured using a Calbiochem CK-NAC reagent kit (Boehringer Diagnostics, La Jolla, Calif.), which uses a coupled enzyme reaction to measure the rate of NADPH production. The absorbance change that occurs at 340 nm with NADPH production, proportional to the creatine phosphate consumed, was measured using a spectrophotometer (Stasar III, Gilford, Oberlin, Ohio) interfaced with an automatic enzyme analyzer (System 5, Gilford). The relative distribution of the four CK isoenzymes was determined using cellulose acetate strip electrophoresis followed by scanning fluorometry. Stained strips were scanned on a fluorometer (Turner Designs, Sunnyvale, Calif.), and areas of the peaks corresponding to each of the isoenzymes were measured on a digitizer (Hewlett-Packard Co., Palo Alto, Calif.). Since CK isoenzymes have similar specific activities, multiplying the percent distribution by total CK activity yields the tissue activity of each isoenzyme.

Total LDH activity was measured in the direction of lactate production by using a method that employs the colorimetric change of NADH, which is proportional to the consumption of pyruvate substrate.

Citrate synthase activity, used to quantitate mitochondrial mass, was determined by spectrophotometric analysis of mercaptide ion (412 nm), which is the end product proportional to the amount of oxaloacetate substrate consumed in the presence of sample.

Statistical Analysis

Statistically significant differences (p<0.05) among groups and among regions within groups were calculated by analysis of variance and multiple regression analysis (BBN Research Systems, Cambridge, Mass.). The Newman-Keuls multiple-range test was used to test for significant difference among individual groups. Values are reported as mean±SEM.

Table 1. Blood Pressure and Heart Weight

<table>
<thead>
<tr>
<th></th>
<th>2K1C</th>
<th>2K1C+H</th>
<th>C</th>
<th>C+H</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (8)</td>
<td>11</td>
<td>16</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>196±9*</td>
<td>142±6</td>
<td>135±3</td>
<td>109±5*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>429±14</td>
<td>373±14</td>
<td>398±18</td>
<td>348±5*</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.79±0.08*</td>
<td>1.54±0.08*</td>
<td>1.27±0.03</td>
<td>1.25±0.03</td>
</tr>
<tr>
<td>Heart wt/body wt (g/kg)</td>
<td>4.15±0.09*</td>
<td>4.12±0.13*</td>
<td>3.25±0.10</td>
<td>3.59±0.06</td>
</tr>
</tbody>
</table>

Values are reported as mean±SEM. 2K1C, two-kidney, one-clip renal hypertensive rats; 2K1C+H, hydralazine-treated 2K1C rats; C, control rats; C+H, hydralazine-treated control rats.

*Significantly different from corresponding control value at p<0.05.
than RVFW cells. The size of RVFW cells was not significantly increased by renal hypertension. Myocyte volume in LENDO, LEPI, RIVS, and LIVS regions in 2K1C rats with or without high blood pressure was greater than in respective controls. The extent of increase in cell volume was attenuated by hydralazine treatment in endocardial (LENO and LIVS) regions of 2K1C rats, but not in epicardial (LEPI or RIVS) regions. For example, LENDO increased by 57% in untreated 2K1C rats, but only by 24% in hydralazine-treated 2K1C rats. Thus, antihypertension treatment attenuated the cell volume increase of myocytes in those regions that underwent the greatest degree of hypertrophy, the cells bordering the left ventricular cavity.

Regional Biochemistry

Enzyme activities are calculated in two ways: activity relative to myocyte protein content (in international units per milligram protein) and activity per cell (in micro-international units per cell). Schematic representations illustrate regional variations of enzyme activity per milligram protein (Figure 3, left, and Figure 4, left) and per cell (Figure 3, right, and Figure 4, right) within the control, hydralazine-treated 2K1C, and untreated 2K1C hearts for total CK, the adult isoenzymes of CK (MM and mitochondrial CK), the fetal isoenzymes of CK (BB+MB), citrate synthase, and LDH. Data for myocytes from hydralazine-treated control rats were not different from control rat hearts and are not shown. Tables 2 and 3 give the data from which the schematic diagrams were drawn.

Creatine Kinase

For all four groups of rat hearts, myocardial CK activity relative to total protein content (in international units per milligram protein) was lower in RVFW than in LEPI, LENDO, LIVS, or RIVS regions (Table 2, Figure 3, left). In the 2K1C rats with cardiac hypertrophy, with or without hypertension, total CK activity (in international units per milligram protein) in all regions tended to be lower than for control rats (but did not always reach statistical significance). When CK activity was normalized for cell volume (Table 3, Figure 3, right), the difference between RVFW and the other ventricular regions was accentuated, since RVFW cell volume did not increase. However, despite differences in cell size among regions and groups, there were no regional or group differences in total CK activity per cell. Thus, CK activity per cell was the same, despite the increase in cell size.

MM Creatine Kinase

The activity of the dominant CK isoenzyme (MM CK) (Table 2), expressed as international units per milligram protein (Figure 4, left), did not show any regional variation in any heart, except for an increase in RIVS in the control heart. Hypertrophied myocytes, with and without hypertension, however,
tended to have lower MM CK activity in all regions, reaching significance for LENDO, LEPI, RIVS, and RVFW in untreated 2K1C rats and for LENDO and LEPI in 2K1C rats treated with hydralazine. Thus, differences in MM CK correlated negatively with hypertrophy (cell size), not with hypertension.

The activity of MM CK, expressed as micro–international units per cell (Figure 4, right), was lower in RVFW for all groups but, otherwise, showed neither regional variation nor differences among groups. Thus, accumulation of MM CK in hypertrophied myocytes did not change in proportion to changes in cell size.

**BB+MB Creatine Kinase**

The activity of the B-containing CK isoenzymes (BB+MB), expressed as a percentage of total CK activity, is shown in Figure 5. The relative activity of fetal-type CK isoenzymes is highest in the largest cells, increasing nearly threefold in hypertrophied myocytes in hypertensive rats. The percentage of BB+MB is highest in all regions of hearts with both hypertrophy and hypertension, intermediate in all regions of hearts with hypertrophy but no hypertension, and lowest in control hearts, with or without hydralazine treatment.

Calculating these results as enzyme activity per milligram protein altered this profile. The sum of the fetal isoenzymes expressed as BB+MB activity per milligram protein remained highest in LENDO in all groups (Figure 4, left). The activity of BB+MB per milligram protein approximately doubled compared with control in each of the five regions of untreated 2K1C hypertensive rats, but for the hydralazine-treated 2K1C rats, activity per milligram protein was not different from control in any region (Figure 4, left, Table 2). Thus, increased accumulation of the fetal CK isoenzymes relative to all other proteins is
related primarily to blood pressure rather than to hypertrophy.

The activity of BB+MB per cell (micro-international units per cell) showed a similar pattern to results expressed as activity per milligram protein. Activity in the LENDO and LEP1 regions was higher than for control rats in untreated 2K1C hypertensive rats but not in hydralazine-treated 2K1C rats, again showing a positive correlation between increased BB+MB activity and blood pressure (Figure 4, right). Normal rats and hydralazine-treated 2K1C rats did not show any significant regional variation in BB+MB per cell, although LENDO had the highest value in each case and RVFW had the lowest value in each case. LENDO did show significantly higher BB+MB activity per cell than all other regions in untreated 2K1C hypertensive rats. Thus, increased accumulation of fetal-type CK isoenzymes occurs in all myocytes of hypertensive rat hearts, and the greatest accumulation occurs in the cells adjacent to the left ventricular chamber.

Mitochondrial Creatine Kinase

Mitochondrial CK (in international units per milligram protein) did not show regional variation within any of the groups studied, although activity tended to be lower in the RVFW (Table 2, Figure 4, left). When each region among experimental groups was compared, mitochondrial CK activity in all regions of hypertrophied hearts, with or without hypertension, tended to be lower than for control (Figure 4, left).

The activity of mitochondrial CK per cell (in micro-international units per cell) was highest in LENDO and lowest in RVFW in normal rats (Figure 4, right), but no other regional variation occurred within any of the groups studied. When each region among experimental groups was compared, LENDO was lower in both untreated 2K1C and hydralazine-treated 2K1C rats than in control rats. Thus, diminished mitochondrial CK activity correlated with hypertrophy rather than blood pressure, a pattern similar to that observed for MM CK.

Citrate Synthase

Activity of citrate synthase, an inner mitochondrial enzyme, expressed as international units per milligram protein, reports the relative amount of mitochondrial protein in the cell. The activity of citrate
synthase in either treated or untreated 2K1C groups did not differ from the activity in control groups in any region, except for a low level in LEPI in hydralazine-treated 2K1C rats (Table 2, Figure 3, left). This small difference disappeared when corrected for cell volume, and citrate synthase per cell did not differ from control in any region studied (Table 3, Figure 3, right). In 2K1C rats, citrate synthase per cell (in micro–international units per cell) in RVFW was lower than in LENDO, LIVS, and RIVS. These variations in citrate synthase were small, especially when compared with the dramatic changes seen in LDH, CK, and cell volume.

**Lactate Dehydrogenase**

For all groups, LDH activity (in international units per milligram protein) was less in RVFW than in LENDO and LEPI and was higher in LENDO than in LEPI regions (Table 2). LDH activity was higher in all regions of the hypertensive hypertrophied myocardium than in control myocardium (Table 2). LDH activity in corresponding regions of myocardium was

<table>
<thead>
<tr>
<th>Table 2. Myocardial Enzyme Concentrations in Hypertensive and Nonhypertensive Cardiac Hypertrophy</th>
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</thead>
<tbody>
<tr>
<td><strong>RVFW</strong></td>
</tr>
<tr>
<td>LDH (IU/mg protein)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>2K1C</td>
</tr>
<tr>
<td>2K1C+H</td>
</tr>
<tr>
<td>CS (IU/mg protein)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>2K1C</td>
</tr>
<tr>
<td>2K1C+H</td>
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<tr>
<td>CK (IU/mg protein)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>2K1C</td>
</tr>
<tr>
<td>2K1C+H</td>
</tr>
</tbody>
</table>

Values are mean±SEM for three groups of rats: control rats; two-kidney, one-clip renal hypertensive rats (2K1C); and hydralazine-treated 2K1C rats (2K1C+H). RVFW, right ventricular free wall; RIVS, right half of the interventricular septum; LIVS, left half of the interventricular septum; LENDO, endocardial half of the left ventricular free wall; LEP1, epicardial half of the left ventricular free wall; LDH, lactate dehydrogenase; CK, creatine kinase; CS, citrate synthase; BB, BB isoenzyme of CK; MB, MB isoenzyme of CK; MM, MM isoenzyme of CK; MITO, mitochondrial isoenzyme of CK.

*Significantly different from corresponding region in control group at p<0.05.
TABLE 3. Myocardial Enzyme Concentrations

<table>
<thead>
<tr>
<th></th>
<th>RVFW</th>
<th>RIVS</th>
<th>LIVS</th>
<th>LENDO</th>
<th>LEPI</th>
</tr>
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<tr>
<td><strong>LDH (µIU/cell)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>1.85±0.47</td>
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<td>4.76±0.60</td>
<td>5.25±0.69</td>
<td>4.09±0.70</td>
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<td>2K1C</td>
<td>4.80±0.81*</td>
<td>10.69±1.50*</td>
<td>11.59±2.13*</td>
<td>12.59±2.04*</td>
<td>8.17±0.86*</td>
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<tr>
<td>2K1C+H</td>
<td>4.07±0.61*</td>
<td>8.55±1.42</td>
<td>8.05±1.14</td>
<td>8.23±0.80*</td>
<td>4.82±0.70</td>
</tr>
<tr>
<td><strong>CS (µIU/cell)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>2.12±0.16</td>
<td>3.19±0.35</td>
<td>2.75±0.26</td>
<td>3.08±0.18</td>
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<td>2K1C</td>
<td>2.18±0.24</td>
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<td>3.93±0.50</td>
<td>3.88±0.44</td>
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<td>2K1C+H</td>
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<td>4.27±0.58</td>
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<td>3.07±0.37</td>
<td>2.62±0.32</td>
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<td><strong>CK (µIU/cell)</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>11.38±1.45</td>
<td>20.46±2.09</td>
<td>19.22±1.93</td>
<td>21.82±1.85</td>
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<td>2K1C</td>
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<td>19.46±3.01</td>
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<td>2K1C+H</td>
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<td>22.15±4.79</td>
<td>21.82±3.62</td>
<td>17.23±1.95</td>
<td>14.19±1.20</td>
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<tr>
<td><strong>BB+MB (µIU/cell)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>0.77±0.23</td>
<td>1.04±0.30</td>
<td>1.08±0.11</td>
<td>2.21±0.51</td>
<td>1.60±0.36</td>
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<tr>
<td>2K1C</td>
<td>1.44±0.31</td>
<td>3.85±0.76</td>
<td>3.64±0.82</td>
<td>7.19±1.44*</td>
<td>3.12±0.77*</td>
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<tr>
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<td>1.04±0.22</td>
<td>2.21±0.60</td>
<td>2.21±0.42</td>
<td>2.31±0.39</td>
<td>1.50±0.20</td>
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<td><strong>MM (µIU/cell)</strong></td>
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<tr>
<td>Control</td>
<td>6.01±0.84</td>
<td>13.32±1.71</td>
<td>11.41±0.96</td>
<td>11.36±0.96</td>
<td>9.21±1.06</td>
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<tr>
<td>2K1C</td>
<td>4.34±0.93</td>
<td>9.26±1.45</td>
<td>9.00±1.81</td>
<td>12.17±1.91</td>
<td>7.74±1.34</td>
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<tr>
<td>2K1C+H</td>
<td>4.60±1.42</td>
<td>14.58±3.28</td>
<td>13.76±2.66</td>
<td>10.00±1.30</td>
<td>7.68±0.63</td>
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<td><strong>MITO (µIU/cell)</strong></td>
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<tr>
<td>Control</td>
<td>4.40±0.40</td>
<td>6.10±0.53</td>
<td>6.72±0.94</td>
<td>8.24±0.60</td>
<td>6.30±0.59</td>
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<td>2K1C</td>
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<td>6.33±1.23</td>
<td>5.58±0.80</td>
<td>5.94±1.05*</td>
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<tr>
<td>2K1C+H</td>
<td>3.36±0.80</td>
<td>5.72±1.12</td>
<td>5.90±0.90</td>
<td>4.98±0.68*</td>
<td>4.98±0.50</td>
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</tbody>
</table>

Values are mean±SEM for three groups of rats: control rats; two-kidney, one-clip renal hypertensive rats (2K1C); and hydralazine-treated 2K1C rats (2K1C+H). RVFW, right ventricular free wall; RIVS, right half of the interventricular septum; LIVS, left half of the interventricular septum; LENDO, endocardial half of the left ventricular free wall; LEPI, epicardial half of the left ventricular free wall; LDH, lactate dehydrogenase; CK, creatine kinase; CS, citrate synthase; BB, BB isoenzyme of CK; MB, MB isoenzyme of CK; MM, MM isoenzyme of CK; MITO, mitochondrial isoenzyme of CK.

*Significantly different from corresponding region in control group at p<0.05.

**FIGURE 5.** Bar graph showing percent of total creatine kinase activity represented by the fetal isoenzymes (BB+MB). Values are given as mean±SEM. RVFW, right ventricular free wall; RIVS, right half of the interventricular septum; LIVS, left half of the interventricular septum; LENDO, endocardial half of the left ventricular free wall; LEPI, epicardial half of the left ventricular free wall; C, control rats; C+H, hydralazine-treated control rats; 2K1C, two-kidney, one-clip renal hypertensive rats; 2K1C+H, hydralazine-treated 2K1C rats. *Statistically different from C at p<0.05. **Statistically different from 2K1C at p<0.05.

dium hypertrophied without hypertension had intermediate activity (Figure 3, left). When LDH concentration was calculated as micro–international units per cell, these regional distributions were maintained (Figure 3, right). Multiple regression analysis showed that the accumulation of LDH increased the most in the largest cells. Thus, hypertrophic myocytes subjected to renal hypertension preferentially accumulate LDH, and the accumulation occurs primarily in the cells adjacent to the left ventricular chamber.

**Discussion**

The results of this study demonstrate the multiplicity of factors that influence cardiac hypertrophy and the diversity of mechanisms by which individual cells can adapt to a new environment. In this study, we established normal values for enzyme concentration calculated relative to total cell protein (in international units per milligram protein) and also as cell content (in micro–international units per cell). In this way, biochemical differences between hypertensive and nonhypertensive hypertrophy in each region could be analyzed. To interpret these data, consideration must be given to the following characteristics: 1) morphology of the individual myocyte, 2) location
of the myocyte within the myocardium, 3) hemodynamic or other external forces upon the myocyte, 4) metabolic state of the myocyte, and 5) capacity of myocytes to adapt to changes in environment.

Morphology of the Myocyte

The data reported here are consistent with our previous studies demonstrating that hypertension produced by clipping one renal artery is a pressure-overload model in which the left ventricular myocytes are primarily affected. We previously demonstrated that the increase in myocyte size is due to an increase in cross-sectional area, rather than length of the cell. In this study, we found that some enzymes increased in proportion to the increase in cell size while others did not. In spite of large differences in cell size, cell content of total CK and citrate synthase activities did not change. Thus, our results show that, compared with other cardiac proteins, CK and citrate synthase activities are relatively diminished. On the other hand, cell content of total LDH activity in myocytes from hypertensive and nonhypertensive hypertrophied hearts increased disproportionately beyond the degree of increase of cell size. Importantly, we observed that the relations between CK isoenzyme activity and cell size were not the same: MM and mitochondrial CK activities per cell were unchanged, whereas BB + MB activity per cell increased in the largest cells found in hypertensive, hypertrophied hearts. Therefore, the size of the cell and its change in morphology in the pathological situation are important in the analysis of these biochemical data. Although regional variations in the isoenzymes of other proteins such as acetylcholinesterase and myosin have been reported, these changes have not been analyzed in relation to cell morphology.

Location of the Myocyte

Location of the myocyte influences the extent of hypertrophy that occurs during pressure overload produced in the 2K1C rat model. In the study presented here, the endocardial (LIVS and LENDO) portions of the left ventricle underwent the greatest increase in myocyte volume in 2K1C hypertension, and the RIVS and LEPI regions acted in concert with one another exhibiting a lesser degree of hypertrophy. In the present study, we show that regional variations in enzyme content occur in both normal and hypertrophied myocardium. We found that total CK and LDH activities were highest in the endocardial portion of the normal and hypertrophied left ventricle (LENO and LIVS) and lowest in the RVFW whether expressed as per milligram protein or as cell content. In hypertensive cardiac hypertrophy, all five regions of the heart had higher LDH activity than in control hearts, but the relative regional distribution was maintained with the highest level of LDH activity in LENDO. A shift of CK isoenzymes was also most pronounced in LENDO, showing an increase in the sum of BB + MB activity but a decrease in MM and mitochondrial CK activities.

Hemodynamic Factors

In the present study, we observed an almost threefold (154%) increase in percent (BB + MB) CK isoenzymes in hypertensive cardiac hypertrophy but only a 32% increase in nonhypertensive cardiac hypertrophy. Since the extent of hypertrophy was the same in these two models, this difference in the extent of CK isoenzyme shift between hypertensive and nonhypertensive hypertrophy suggests that the mechanical signal of increased blood pressure is instrumental in producing the CK isoenzyme shift observed in hypertensive hypertrophy in this model of pressure overload. The differences in hemodynamic load across the wall of the left ventricle in these two models of hypertrophy probably account for the accentuated response of the endocardial regions. Studies on the effect of load on individual myocytes in culture have demonstrated that cell growth can be induced by cell deformation. In the present study, the enlargement of endocardial myocytes in nonhypertensive hypertrophy without an accompanying shift in B-containing CK isoenzyme expression supports the hypothesis that cardiac hypertrophy and hypertension are separate, though interrelated, processes. These results are also consistent with the hypothesis that humoral factors contribute to increased cardiac mass as well as to changes in gene expression for specific proteins, since total CK decreased and total LDH increased in RVFW as well as in left ventricular regions in nonhypertensive cardiac hypertrophy.

Metabolic State of Myocyte

As shown in a previous study, relative glycolytic capacity, assessed by comparing activities of glycolytic and mitochondrial enzymes, was increased in hypertensive hypertrophy. In the present study, the change toward an increased glycolytic capacity was also seen in nonhypertensive hypertrophy. Total LDH activity was increased, whereas activity of two mitochondrial proteins, mitochondrial CK and citrate synthase, were decreased in both forms of hypertrophy. As a result of this change in relative composition of glycolytic and mitochondrial proteins, hypertrophied myocytes become more like fetal myocardium. The increase in B-containing CK isoenzyme that we observed is also characteristic of fetal-like myocardium.

Adaptation to Stress

The results of this study strongly suggest that the physiological factor of increased blood pressure can alter the expression of the genome in an adult organ. Experimental studies of several pathological states have shown alterations in mRNA production for several proteins, including c-myc, myosin heavy chain, and G protein. These are only a few examples demonstrating the ability of the adult myocyte to adapt to its environment by changes at several levels, including transcription.
The regional variation in the CK isoenzyme composition observed here demonstrates that neighboring myocytes differed from one another in protein composition. The regulation of gene expression is known to be tissue specific and is both developmentally regulated and subject to hormonal control.36 The results of this study show that regulation of gene expression also varies among neighboring cells within the same organ. Further, in spite of large differences in cell size, we observed that M-containing CK isoenzyme activity per cell did not change while B-containing CK isoenzyme activity did change. This demonstrates differences in the control of gene expression of the M- and B-CK polypeptides within the same myocyte. This observation suggests that synthesis of M- and B-CK monomeric chains is under separate control.

The multifactorial nature of pathogenetic mechanisms in cardiac hypertrophy is apparent from the results of this study. The ability of individual cells to respond to hemodynamic changes31 may be counterbalanced by the more global effects due to hormonal and humoral factors. Changing the relative glycolytic and oxidative capacities may affect myocardial oxygen consumption, which in turn will also affect the ability of the myocardium to generate force.37

In summary, the results of this study show that glycolytic metabolism increases in hypertrophied myocardium, with or without accompanying high blood pressure. The shift in the production of CK to its fetal isoenzymes, however, occurs primarily in cardiac hypertrophy in the presence of the added stress of high blood pressure. The ability of the heart to adapt to stress by the synthesis (or diminished degradation) of fetal CK isoenzymes may be a favorable adaptation, ensuring that ATP resynthesis via the CK reaction remains high.

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Smith et al

Creatine Kinase in Hypertrophy and Hypertension 1343


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