Regional Myocyte Size in Normotensive and Spontaneously Hypertensive Rats

SANFORD P. BISHOP, D.V.M., PH.D., SUZANNE OPARIL, M.D., REBECCA H. REYNOLDS, M.T. (ASCP), AND JANICE L. DRUMMOND, M.S.

SUMMARY Regional differences in cell size in the hearts of rats with and without cardiac hypertrophy were studied using isolated muscle cells. Isolated cardiac myocytes were prepared from left ventricular free wall inner and outer halves and the right ventricle of six male 12-week-old spontaneously hypertensive (SHR), Wistar-Kyoto (WKY) and Fischer-344 rats. In SHR, blood pressure was increased to 188 ± 4 (SEM) mm Hg versus 143 ± 2 and 133 ± 10 for WKY and Fischer rats, respectively (p < 0.001). Total heart weight was increased to 1103 ± 29 mg in SHR compared to 824 ± 21 in WKY and 951 ± 23 in Fischer rats (p < 0.001).

Isolated cardiac myocytes were prepared by perfusion of isolated hearts with Ca\(^{++}\) free Hanks' solution containing EGTA followed by collagenase-containing media. Mean length, width and volume of 150 cells stained with hematoxylin and eosin from each site were measured with a sonic digitizer. Two nuclei were present in 85 to 87% of isolated cells from all strains and regions. There was no difference among strains in right ventricular cell length, width, or volume, nor between left ventricular inner and outer halves within each strain. Left ventricular cells were larger than right ventricular cells (p < 0.05) in all strains. Left ventricular cells of SHR were larger than left ventricular cells of WKY or Fischer rats in proportion to the increase in total heart weight, indicating that cardiac enlargement in SHR is due to increased cell size rather than increased cell number. (Hypertension 1: 378-383, 1979)

KEY WORDS • spontaneously hypertensive rat • cardiac hypertrophy • regional myocyte size • isolated cardiac myocyte • morphometry • binucleation

During the early neonatal period, heart muscle cells normally stop growing by the hyperplasia characteristic of the fetal period, and further increase in heart size is by cellular hypertrophy. It is during this period that differential pressures and significant differences in the mass of the left and right ventricles develop. Although some investigators have reported no difference in the cross-sectional diameters of right and left ventricular muscle cells of adult mammals, others have found that right ventricular cells are smaller than those in the left ventricle. It is well known that cardiac muscle cells of either ventricle will increase in size when subjected to an increased work load or other appropriate stimulus.

In the normal myocardium, the inner layers of the left ventricular wall are subjected to greater tissue pressure and higher blood flow than the outer or epicardial portions of the wall. During the development of cardiac hypertrophy due to a variety of causes, alterations occur in left ventricular and coronary artery pressure which affect wall tension, pressure, blood flow and possibly work load. If these changes were greater in some regions of the heart than in others, it would be reasonable to expect differences in the degree of hypertrophic response in various regions of the heart. Few studies have attempted to evaluate regional differences in the size of heart muscle cell in normal or hypertrophied hearts. Most reports have been limited to measurement of cell diameter or cross-sectional area and only a few have attempted to determine cell length. Due to the irregular shape of myocytes and...
location of intercalated discs along the lateral aspects of the cell, length determinations of individual cells are difficult in tissue sections. In a study of normal human hearts, Ashley measured the diameter of heart muscle cells at the nucleus and found a greater diameter in cells from the papillary muscles than from the free wall of both right and left ventricle; further, left ventricular fibers were larger than right ventricular fibers. In contrast, Fuster et al. measured cell diameter in normal and hypertrophied human hearts. Cell diameter was greater in the hypertrophied hearts, but there were no regional differences among cells from left ventricular base, apex, subendocardium or subepicardium in either group. In young spontaneously hypertensive rats (SHR), Kawamura et al. found a greater diameter in cells of the left ventricular papillary muscle than of the free wall, but by 21 weeks and older, free wall cells were larger in diameter than papillary muscle cells. Anversa et al. reported a larger cross-sectional area for subendocardial cells than subepicardial cells of normal rats, but in hypertrophy due to renal hypertension, subepicardial cells enlarged to a greater extent than subendocardial cells. These investigators also reported that cell length measured in tissue sections was greater in the subepicardium than the subendocardium in both normal and hypertrophied rat hearts.

The availability of isolated heart muscle cells from experimental animals has simplified the measurement of myocyte size. The purpose of this investigation was to determine whether there are differences in size between myocytes from right ventricle and from inner and outer halves of the left ventricle of normotensive rats and spontaneously hypertensive rats with cardiac hypertrophy.

Materials and Methods

Animals used in this study were spontaneously hypertensive rats (SHR) of the strain developed by Okamoto and Aoki and normotensive rats of the Wistar-Kyoto (WKY) and Fischer-344 strains. Fischer-344 rats were obtained from a colony maintained by our laboratory at the University of Alabama in Birmingham; SHR and WKY rats were obtained at 10 weeks of age from Charles River Breeding Laboratory. Animals were maintained in temperature and humidity controlled rooms on a 12-hour light-dark cycle. Animals were housed three or four per cage and fed standard laboratory rat diet and water ad libitum. All animals were studied at 12 weeks of age.

During the 2 weeks prior to preparation of isolated heart muscle cells, systolic arterial blood pressure and heart rate were obtained twice each week using the indirect tail-cuff method without anesthesia. The median value of at least five pressure determinations from each recording session was used and the median of at least three separate sessions taken as the systolic blood pressure. Heart rate was determined from the systemic pressure recording.

The rats were anesthetized with ether, the heart removed and placed in ice-cold saline to wash off blood and stop contractions. The heart was dissected free of adhering lung tissue, blotted dry and weighted to the nearest mg. The aorta was cannulated and the heart mounted on the perfusion apparatus.

Perfusion was done on a modified Langendorff apparatus with a double recirculating system to allow for changing the perfusion medium without interrupting flow through the heart. The perfusion apparatus consisted of two oxygenating flasks, two water-jacketed bubble traps and warming coils, and a water-jacketed heart chamber. The perfusion medium was maintained at 37°C by recirculating water and oxygenated with 95% O₂ and 5% CO₂. Aortic perfusion pressure was monitored with a Micron Instrument MP 15 pressure transducer and recorded throughout the procedure on a Beckman directed writing polygraph. The perfusion medium was pumped by a peristaltic perfusion pump at a rate adjusted to maintain perfusion pressure at less than 100 mm Hg.

The basic perfusion medium consisted of Ca²⁺ and Mg²⁺ free Hanks' balanced salt solution containing 0.5 mM MgSO₄, 0.21 g/100 ml additional NaHCO₃ to give a final concentration of 0.03 M, and 3% fetal calf serum. After the heart was mounted on the perfusion apparatus it was perfused for 5–10 minutes with the basic perfusion medium, plus 2.5 mM EGTA. This was followed by perfusion with the collagenase medium (0.1% Type I collagenase-Sigma — in basic perfusion medium without EGTA) for 15 minutes. The heart was then taken off the cannula and the right ventricular free wall and atria were dissected from the left ventricle and septum. The left ventricular free wall was divided into inner and outer halves under the dissecting microscope using free-hand dissection. Tissues from left ventricular inner and outer halves and the right ventricle were minced in 5 ml of the EGTA medium to disperse the cells. The resulting mixture was filtered through 250-μm nylon mesh to remove clumps of undispersed cells. A portion of each cell suspension was pipetted onto a glass slide and allowed to adhere. The cells were fixed by flooding with 2% glutaraldehyde and stained with hematoxylin and eosin.

Cell size determinations were done on 150 cells from each location (right ventricle, left ventricular inner half and left ventricular outer half) from each of six rats of each strain using a Science Accessories Graf Pen 3 sonic digitizer (resolution 0.1 mm) interfaced with a Hewlett Packard 9825A calculator. The system was calibrated with a ruled microslide. Cells stained with hematoxylin and eosin were projected onto the digitizer work surface and the outline of each myocyte traced with the sonic pen; X and Y coordinates of successive points were transmitted to the calculator for calculation of length, width and volume of each cell. Since the myocytes are usually in the shape of irregular cylinders, volume was calculated as the sum of successive volumes of rotation about the long axis of the cell. This was calculated for both sides of the cell and the average of the two taken as the cell volume. From each preparation, a mean value was obtained for total cells counted, total single nucleated
TABLE 1. Blood Pressure, Heart Rate and Heart Weight in 12-week-old Fischer-344, WKY and SHR rats

<table>
<thead>
<tr>
<th></th>
<th>Fischer-344</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>259 ± 3</td>
<td>230 ± 3</td>
<td>237 ± 5</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>129 ± 7</td>
<td>143 ± 2</td>
<td>188 ± 4</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>423 ± 14</td>
<td>463 ± 10</td>
<td>430 ± 9</td>
</tr>
<tr>
<td>Total heart weight (mg)</td>
<td>951 ± 23</td>
<td>824 ± 21</td>
<td>1103 ± 29</td>
</tr>
<tr>
<td>Heart weight/body weight (mg/g)</td>
<td>3.67 ± 0.11</td>
<td>3.58 ± 0.05</td>
<td>4.66 ± 0.08</td>
</tr>
</tbody>
</table>

*WKY = Wistar-Kyoto rat; SHR = spontaneously hypertensive rat. Values are the mean ± SEM of six animals in each group.
†There was a significant difference from WKY and Fischer-344 rat (p < 0.01).

Results

Table 1 lists the values for systolic blood pressure, heart rate, body weight, heart weight and heart weight/body weight for the three strains of rats. Blood pressure was increased in SHR compared to WKY and Fischer-344 rats, while there was no significant difference in heart rate. The SHR had significantly increased total heart weight and an increased heart weight/body weight ratio when compared to the two control strains.

Light microscopic examination of the myocytes isolated by this procedure revealed that approximately 90% excluded trypan blue and were in an elongated shape without visible surface membrane blebs. Contraction band formation and supercontraction resulting in rounded cells were seen in less than 10–15% of cells in these preparations, and these cells were excluded from the measurement. The presence of EGTA in the isolation medium prevented cells from contracting. In preliminary experiments without added EGTA, spontaneous contractions occurred in cells examined under the microscope. Sarcomere length of the cells in the wet preparation was 1.94 ± 0.02 μm (SEM); after fixation and staining, sarcomere length was 1.62 ± 0.02 μm, corresponding to the physiologically contracted sarcomere length. Isolated myocytes were separated at the intercalated disc region as shown previously. Typical cell morphology is illustrated in figure 1.

Cell measurements are summarized in table 2 and figure 2. The percentage of cells with two nuclei, 84–87%, did not differ among locations or strains. Cell volume of double nucleated cells averaged 1.8 times greater than for single nucleated cells in all three strains.

Statistical evaluation by two-way analysis of variance revealed a significant (p < 0.05) increase in cell volume for left ventricular myocytes from SHR compared to those from either of the two control strains. There was no difference in the size of right ventricular myocytes among the three strains. Double nucleated right ventricular myocytes were significantly smaller than those from the left ventricle in all three strains. Single nucleated myocytes had less...
Table 2. *Length, Width, and Volume of Isolated Cardiac Myocytes from 12-week-old Fischer-344, WKY and SHR Rats*

<table>
<thead>
<tr>
<th></th>
<th>Fischer-344</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV endo</td>
<td>LV epi</td>
<td>RV</td>
</tr>
<tr>
<td>% double nucleated cells</td>
<td>87 ± 2</td>
<td>87 ± 1</td>
<td>87 ± 2</td>
</tr>
<tr>
<td>Combined single and double nucleated cells</td>
<td>Length (µm)</td>
<td>Width (µm)</td>
<td>Volume (µm³)</td>
</tr>
<tr>
<td></td>
<td>98 ± 3</td>
<td>19.5 ± 0.3</td>
<td>30.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>97 ± 3</td>
<td>19.1 ± 0.3</td>
<td>29.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>1.79 ± 0.9</td>
<td>1.74 ± 0.6</td>
<td>1.78 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>1.91 ± 1.0</td>
<td>1.65 ± 0.4</td>
<td>1.63 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>1.84 ± 1.0</td>
<td>1.62 ± 0.4</td>
<td>1.44 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>1.84 ± 1.0</td>
<td>1.76 ± 0.4</td>
<td>1.57 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>1.75 ± 1.0</td>
<td>1.76 ± 0.4</td>
<td>1.71 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>1.61 ± 1.0</td>
<td>1.76 ± 0.4</td>
<td>1.79 ± 0.7</td>
</tr>
</tbody>
</table>

*Values represent mean ± SEM of six animals in each group. WKY = Wistar-Kyoto rat; SHR = spontaneously hypertensive rat; LV endo = left ventricular endocardium; LV epi = left ventricular epicardium; RV = right ventricle.

Discussion

It has been recognized for many years that heart muscle cells undergo a dramatic increase in size during normal growth and that further increase in size occurs with work load induced hypertrophy. Most measurements have been limited to cross-sectional diameter or area of myocytes, however, due to the difficulty of obtaining accurate length measurements in tissue sections. Few studies have made comparisons of cell size in different regions of the left ventricle. The development of the isolated heart muscle cell preparation has provided the opportunity to accurately measure cell length, and when combined with width measurements, to calculate cell volume. In the present study, cell size measurements of isolated cells from endocardial and epicardial regions were not significantly different within any of the three strains. Left ventricular cells from the hypertrophied hearts of SHR were larger than from the normotensive control strains. Right ventricular cells were smaller than left ventricular cells and were not different in size among the three strains, consistent with the absence of right ventricular hypertrophy in the 12-week-old SHR.27 Isolated heart muscle cells were used by Korecky and Rakusan21 and by Katzberg et al.14 to measure cell length and width, from which they calculated volume in growing rats21 and in rats with left ventricular hypertrophy due to banding of the aorta.21 These investigators did not measure regional differences in cell size, however. They found a positive correlation between body weight and cell volume. In the study by Korecky and Rakusan,21 heart weight in 465-g rats increased by approximately 20%, from 1143 to 1381 mg after aortic banding. Calculated cell volume increased from $48 \times 10^3 \mu m^3$ to $67 \times 10^3 \mu m^3$. In our study, total heart weight of the hypertensive SHR was approximately 30% greater than for normotensive controls (table 1). Calculated left ventricular cell volume.
was greater in the 250 g, 12-week-old SHR (40 × 10^4 μm^2) than in age- and weight-matched WKY (30 × 10^4 μm^2). The difference in calculated cell volume reported by Korecky and Rakusan and by us may be accounted for by the difference in body weight at the time of study. Actual increase in left ventricular weight could not be determined in this study due to the technique employed, which requires enzyme perfusion of the whole heart. However, it has been shown that in the 12-week-old SHR, heart weight increase is restricted to the left ventricle.

Left ventricular myocytes were larger than those of the right ventricle in 12-week-old rats of both normotensive and hypertensive strains. This is consistent with most other studies in which both left and right ventricular cell diameters have been measured. In some studies, however, no difference in size between left and right ventricular myocytes have been found. The failure to find such a difference may be due to use of paraffin embedding of tissue, which produces considerable shrinkage artifact.

The size differential between right and left ventricular myocytes appears to develop during postnatal growth. At birth the right and left ventricles weigh nearly the same since during the fetal period they have been subjected to similar work loads. During the fetal and early neonatal periods, cardiac myocytes grow by hyperplasia and presumably the cells in both ventricles are of similar size. Right and left ventricular pressures change dramatically after birth due to closure of the ductus arteriosus, a fall in pulmonary vascular resistance, and an increase in systemic resistance. Previous studies in the dog have shown that the left ventricle increases linearly in mass from birth, while the right ventricular mass remains stable for approximately 10 days. In the rat this stable period appears to last about 5 days (unpublished studies). At the end of this period, the heart muscle cells rapidly convert to a hypertrophic mode of growth with parallel increases in left and right ventricular weights. Tritiated thymidine studies in the dog from our laboratory (unpublished studies) indicate that during this early neonatal period there is decreased mitotic activity in myocytes of the right ventricle, while muscle cell proliferation continues in the left ventricle. Therefore, the smaller mass of the right ventricle compared to the left ventricle appears to be due both to the smaller number of cells determined during the early neonatal period and to the smaller cell size, which may be related to intracavitary pressure or afterload.

Our study demonstrated no difference in cell size between inner and outer halves of the left ventricle in SHR or two strains of normotensive rats at 12 weeks of age. Kawamura et al. reported that cross-sectional cell diameter was increased in papillary muscles at 5 and 9 weeks of age in SHR compared to normotensive WKY, while the lateral wall cell diameter was not different. However, by 11 and 14 weeks of age, the difference between left ventricular regions was no longer apparent. Loud et al. and Anversa et al. reported a slightly larger mean cross-sectional area of cells in the subendocardium than in the subepicardium of normotensive Wistar Kyoto rats. However, following induction of renal hypertension, the cross-sectional area of subepicardial cells increased at a faster rate than for subendocardial cells so that with the development of hypertrophy, the cells became nearly equal in cross-sectional area in the two left ventricular regions. In their study, calculated cell length increased more in the subepicardium than in the subendocardium, however, resulting in a greater increase in calculated cell volume for subepicardial cells than for subendocardial cells. Cell volume calculated by these investigators was slightly less than half the volume we calculated for myocytes from the same age and strain animals. This discrepancy appears to be due to the assumption in their calculations that cardiac myocytes are mononucleated, rather than 85% binucleated as shown by the present study and by others.

Calculations used for cell volume in our study may overestimate cell volume if the isolated myocytes deviated markedly from a round cross-sectional area. Flattening of cells could be accentuated by the process of attachment to glass slides, resulting in an increased cell width and overestimation of cell volume. In addition, with increasing age and cell size, or development of hypertrophy from work overload, there is increasing complexity of the cell surface that could result in further deviation from a round cross-sectional area. It is our impression from scanning electron micrographs of similarly prepared cardiac myocytes that cells from 12-week-old and younger rats maintain an irregular, but generally round cross-sectional shape, similar to that found in transverse sections of myocardial tissues studied by light or transmission electron microscopy. Further evidence that the cells in our study were not excessively flattened during preparation resulting in erroneously large width measurements comes from published values for cell diameter obtained from myocardium embedded with epoxy resin. Kawamura et al. measured the shortest transverse diameter through the nuclei of left ventricular myocytes in 11-week-old WKY rats and reported a mean diameter of 18.2 μm. This value for minimum diameter at the nucleus is not greatly different from our value of 19.1 μm in 12-week-old WKY rats, which represents the calculated mean cell diameter over the entire cell length.

Regional differences in the size of heart muscle cells may have an important influence on the susceptibility of different regions of myocardium to degenerative changes leading to decreased functional performance or cell death. The predilection of the inner half of the left ventricular wall to ischemic cell death in severe coronary artery disease and in conditions associated with severe left ventricular pressure overload such as aortic valve stenosis is well known. Whether the size of the individual heart muscle cells plays a determining role in the development of mechanical dysfunction...
or ischemic cell death is unknown, but it has been suggested that increasing cell diameter and therefore diffusion distance from the capillaries would increase the probability of ischemic cell damage.27 In the 12-week-old SHR the degree of left ventricular hypertrophy is modest, (approximately 30%), there is no difference in cell size between inner and outer wall layers, and there is little evidence of myocardial dysfunction.27,28 Further studies are required to determine if regional differences in cell size are present in severely hypertrophied hearts with myocardial dysfunction.

References

2. Turek Z, Grünthner M, Kreuger F: Cardiac hypertrophy, capillary and muscle fiber density, muscle fiber diameter, capillary radius and diffusion distance in the myocardium of growing rats adapted to a simulated altitude of 3500 m. Pfugers Arch 335: 19, 1972
17. Powell T, Twist VW: A rapid technique for the isolation and purification of adult cardiac muscle cells having respiratory control and a tolerance to calcium. Biochem Biophys Res Commun 72: 327, 1976
26. Bishop SP, Drummond JI: Scanning electron microscopy of the developing intercalated disc in rat isolated cardiac myocytes. Scan Elec Micro 2: 95, 1978
30. Bishop SP, Drummond JI: Surface morphology and cell size measurement of isolated rat cardiac myocytes. J Mol Cell Cardiol. In press
Regional myocyte size in normotensive and spontaneously hypertensive rats.
S P Bishop, S Oparil, R H Reynolds and J L Drummond

_Hypertension_. 1979;1:378-383
doi: 10.1161/01.HYP.1.4.378

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1979 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/1/4/378

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org/subscriptions/