Characterization of myocardium, isolated cardiomyocytes, and blood pressure in WKHA and WKY rats

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Deschepper, Christian F., Sylvie Picard, Gaëtan Thibault, Rhian Touyz, and Jean-Lucien Rouleau. Characterization of myocardium, isolated cardiomyocytes, and blood pressure in WKHA and WKY rats. Am J Physiol Heart Circ Physiol 282: H149–H155, 2002. First published September 27, 2001; 10.1152/ajpheart.00672.2001.—We previously reported that the left ventricular (LV) mass of Wistar-Kyoto (WKY)-derived hyperactive (WKHA) rats was higher than that of WKY rats in the absence of a difference in systolic blood pressure. To extend these earlier observations, we conducted a series of functional and morphological investigations on both strains. Analysis of tissue sections revealed that the surface of ventricular tissue from WKHA rats was higher than that of WKY rats, without any enlargement of the cavity area. Analysis of isolated adult cells showed that cell width (as well as cell volume) of ventricular cardiomyocytes was significantly higher in WKHA than WKY rats. However, LV of WKHA rats contained ~33% less cardiomyocytes than those from WKY rats. Mean intracellular free calcium concentration of cardiomyocytes was also higher in WKHA than WKY rats. Hemodynamic measurements revealed that the values of the maximum rates of pressure change (dP/dt) were higher in LV from WKHA rats. However, these differences were reduced (−dP/dt) or abolished (+dP/dt) when the values were normalized for both the number and mean cross-sectional area of ventricular cardiomyocytes. Mean levels of systolic and diastolic blood pressure (corresponding to the 24-h average of measurements obtained continuously in conscious unrestrained animals using radiotelemetric implants) were not different between strains. However, circadian rhythm was more evident in WKY rats, because the difference between morning and night values of systolic and diastolic blood pressure was greater (by 3 mmHg) in WKY rats. Altogether, our data validate the use of WKHA rats as models of predominantly concentric LV hypertrophy developing in the absence of increased mean levels of hemodynamic cardiac load and show that the hypertrophy phenotype is more pronounced in isolated cardiomyocytes than at the level of the whole ventricle.

cell width; intracellular free calcium concentration; left ventricular hypertrophy; cardiac morphology; cardiac function

CARDIOVASCULAR DISEASES are the principal cause of mortality and morbidity in industrialized countries, and several studies have indicated that left ventricular (LV) hypertrophy (LVH) constitutes a powerful independent risk factor for cardiovascular mortality and morbidity (8, 22). Although LVH is commonly associated with hypertension, experimental evidence in both humans and animal models indicates that LVH may develop, at least in part, independently of blood pressure (10, 18, 20, 25, 27). It is therefore important to identify the appropriate models that will make it possible to study the factors that control LV mass independently of blood pressure.

Recently, Sehbi et al. (27) reported that the LV mass of Wistar-Kyoto (WKY) rats was greater than that of F344 rats and used crosses between the two strains to identify a locus on chromosome 3 that was linked to LV mass. These authors measured blood pressure in animals from both strains shortly after the surgical implantation of an arterial catheter and found that both strains were normotensive but that mean blood pressure was slightly higher in F344 rats (the strain with the lower LV mass) than in WKY rats. Likewise, we compared LV mass in WKY-derived hyperactive (WKHA) and WKY rats (23). WKHA are recombinant inbred rats derived from phenotypic selection and repeated inbreeding of the progeny of WKY/spontaneously hypertensive rat (SHR) crosses (19). Genetic tests have established that all alleles found in WKHA rats could be found in the parental WKY or SHR and that WKHA rats showed ~84% genetic relatedness compared with WKY rats (7). We found that the LV mass was higher in WKHA than WKY rats, although systolic blood pressure (as measured by tail plethysmography in conscious animals) was normal and similar in both strains (23). The interest of the latter model is increased by the fact that we (6) recently identified in WKHA/WKY crosses loci linked to LV mass independently of blood pressure. Altogether, these studies indicate that genes may increase LV mass even in the absence of high blood pressure.

In our previous studies, LVH of WKHA was characterized solely on the basis of the ratio of LV weight...
to body weight and the concentration of biochemical markers of hypertrophy. Furthermore, “spot” measurements of blood pressure may not be sufficient to appropriately evaluate the hemodynamic load of the heart to its full extent. To complete our previous investigations and characterize our animal model in further detail, we performed a systematic functional and morphological comparison of the characteristics of the LV and/or freshly isolated cardiomyocytes from WKHA and WKY rats. Moreover, blood pressure was measured in conscious and unrestrained animals of both strains by continuous monitoring of blood pressure using surgically implanted radiotelemetry devices, because this technique is the one that provides the most precise measurements of arterial blood pressure (4).

METHODS

Animals. All procedures on animals were approved by the Institut de Recherches Cliniques de Montréal (IRCM) Institutional Animal Care Committee and conducted according to guidelines issued by the Canadian Council on Animal Care. The nomenclature of the strains is in compliance with the recommendations of the International Rat Genetic Nomenclature Committee. The WKHA/Cfl rats originated from a colony maintained at the IRCM, as registered with the Institute of Laboratory Animal Resources. WKY/Cfl rats also originated from a colony maintained at the IRCM and were derived from WKY/Cr parents obtained from Charles River (St. Constant, Québec, Canada). All rats were maintained on a 12:12-h light-dark cycle, with lights being switched on at 6:00 AM.

Functional studies. To measure LV hemodynamics, 24-wk-old animals were intubated, mechanically ventilated, and instrumented with an A 2-Fr microtipped pressure transducer catheter (model SPR-407, Millar Instruments) as described previously (2). All variables were recorded on a Gould 2600S recorder. At the end of the dynamic investigations, the heart was stopped in diastole with a saturated potassium chloride solution, removed, and rinsed in saline solution. To construct passive pressure-volume curves, the LV was filled with saline using a catheter connected to a pump delivering at 12.7 μl/s. Pressure was recorded continuously with a calibrated pressure transducer. The procedure was performed twice for each heart.

Histology and morphology. After the pressure-volume curves were constructed, the LV of each diastole-arrested heart was filled with saline to a pressure of 15 mmHg, sealed, and fixed in its distended form in formalin. Two 8-μm-thick cross sections were obtained at 1-mm intervals midway between the base and the apex of the LV. For morphology, tissue sections from nine adult WKHA and nine WKY rats (the same as those used for functional studies) were stained with hematoxylin-eosin, photographed 1:1, digitized with a scanner, and stored as electronic files. Each digital image was then analyzed with Northern Eclipse version 6.0 software (Empix Imaging; Mississauga, Ontario, Canada) to calculate the outer perimeter of the LV as well as the surface occupied by the LV parenchyma and the LV cavity. Additional sections were stained by Sirius red to visualize collagen fibers (see Collagen quantification).

Isolated cardiomyocytes. Cardiomyocytes were isolated from five WKHA and five WKY rats (12 wk of age). Each tissue sample was prepared, and one WKHA and one WKY rat were used concomitantly. The hearts were rapidly removed from anesthetized animals previously injected intraperitoneally with 500 units heparin sulfate, and Ca2+-tolerant cardiomyocytes were isolated by the Langendorff method (cardiac retrograde perfusion) similar to that described previously (30). Cardiomyocytes were separated from noncardiomyocytes by sedimentation on a solution of a 6% bovine serum albumin in medium 199. For morphological studies, isolated cardiomyocytes were fixed for 30 min in 0.08 M phosphate buffer containing 1.5% glutaraldehyde, lightly centrifuged, washed with 0.15 M phosphate buffer, and then maintained in the same buffer at 4°C until analyzed. Both solutions have been shown to preserve the volume of fixed cells compared with unfixed ones (14). Part of these cells were further fixed in osmium tetroxide, lightly centrifuged in microtubes, dehydrated, and Epon embedded. One-micrometer-thick sections were cut and counterstained with toluidine blue.

For intracellular Ca2+ measurements, freshly isolated cells were first allowed to settle in culture dishes for 90 min in culture medium containing 10% fetal bovine serum, switched to serum-free medium, and then finally maintained for another 18 h in a humidified incubator (5% CO2-95% air) at 37°C (see Intracellular calcium measurements).

Intracellular calcium measurements. Intracellular free Ca2+ concentration ([Ca2+]i) was determined with the use of the fluorescent probe fura 2-AM, as described previously (30, 31). Briefly, cardiomyocytes were loaded with 4 μM fura 2-AM (dissolved in DMSO with 0.02% pluronic F-12) and incubated for 30 min at 37°C in a humidified incubator with 95% air-5% CO2. Cells were then washed three times with modified Hanks’ buffer. Fluorescence was determined with an Axiovert 135 inverted microscope and Attofluor digital fluorescence system (Zeiss) using dual excitatory wavelengths of 343 and 380 nm and a single emission wavelength of 520 nm. Both in situ and in vitro calibrations were made according to our previously described methods (31). [Ca2++]i was determined with the equation of Grynkiewicz et al. (17).

Electron microscopy. Four male WKHA and four male WKY rats (12 wk of age) were used for tissue embedding. After anesthesia, a cannula was placed in the abdominal aorta. The hearts were then arrested in diastole by injection of 1 ml of 2 M KCl into the right ventricle, the right atrium was incised, and the hearts were retrogradely perfused with a heparinized solution of 0.15 M phosphate buffer at a controlled pressure of 80 mmHg. After 5 min of perfusion, the perfusate was switched to a solution of 1.5% glutaraldehyde in 0.08 M phosphate buffer for 15 min. The hearts were then excised, and tissue blocks were collected from both the endocardial and epicardial side of the LV (either free wall or septum) and Epon embedded. Ultrathin sections were counterstained with lead and uranyl acetate, collected on grids, and examined with a Jeol 1200 transmission electron microscope. Each grid was examined at both 4 and 12 K magnifications. Several photographs were taken from each grid, and each negative was scanned and saved as an electronic file for further analysis with Adobe Photoshop 5.0 software. The calculation of the cardiomyocyte fractional volume (CFV) was performed in two steps. First, the percentage of surface occupied by myocardial tissue (excluding vascular and extravascular spaces) was measured on the films of pictures taken at 4 K magnification using Adobe Photoshop 5.0 to calculate the relative surface of the two compartments. In a second step, the films of higher magnification (12 K) pictures were analyzed to calculate the percentage of surface occupied by cardiomyocytes (excluding extracellular spaces and noncardiomyocytes). The final CFV was then calculated by multiplying one fraction by the other. For each strain, nine additional animals (12 wk of age) were killed for the determination of the LV weight and calculation of the number of
cardiomyocytes per LV. The latter calculations were performed as described previously (15) using the equation (CFV/ICV), where CFV was calculated as described above, VTV is the ventricular tissue volume (calculated by dividing the LV mass by 1.06, which corresponds to the tissue-specific gravity of cardiac tissue), and ICV is the isolated cardiomyocyte volume (calculated as described in Quantitative videomicroscopy).

Quantitative videomicroscopy. Isolated and fixed cardiomyocytes (prepared from five animals from each strain) were allowed to settle in petri dishes containing 0.15 M phosphate buffer and examined with a Zeiss Axiosvert microscope connected to a videocamera that allowed capture of the images as electronic files. With the use of Northern Eclipse version 6.0 software, ~100 cells (±5) from each animal were analyzed for determination of cell length and cell surface. On the basis of these measurements, cell width was calculated as the surface-to-length ratio.

For validation of cross-sectional measurements, semithin sections of Epon-embedded cells were also examined at high magnification. We performed measurements on undamaged cardiomyocytes in which cross-sectioned sarcomeres were clearly visible. We calculated that the thickness of the sarcomeres in cross-sectioned cells was 1.89 ± 0.14 (SD) μm for WKY cells and 1.83 ± 0.16 (SD) μm for WKHA cells. In both cases, the maximal deviation from the true sarcomere thickness did not exceed 15%, thus ensuring that the measurements were representative of truly cross-sectioned cardiomyocytes (15). On each ellipsoid cardiomyocyte profile, we used Northern Eclipse version 6.0 software to measure the major diameter, minor diameter, and surface area.

Collagen quantification. The abundance of collagen fibers was quantified using two different methods. First, sections from seven WKHA and seven WKY adult rats (which had been used previously for the functional studies) were stained with Sirius red and examined under epipolarization with a Zeiss Axiosvert microscope using a ×10 objective. For each ventricular section, 10 separate fields displaying well-stained interfibrillar (and no accumulation of perivascular collagen) were selected. The percentage of the field containing stained fibers was calculated using Northern Eclipse version 6.0 software. In a separate experiment, collagen was measured biochemically in extracts from the LV of five WKHA and five WKY adult rats using a previously described method (5). Briefly, frozen ventricular tissue was pulverized under liquid nitrogen, 100 mg of tissue powder was dissolved in a tube containing 500 μl of concentrated HCl and 500 μl of water, and the tubes were incubated at 108°C for 16 h. After the tubes cooled, 4 ml of 1 N NaOH were added, the tubes were centrifuged, and aliquots of supernatant were used for the assay. Standards were prepared from a stock solution of 1 mg/ml trans-4-hydroxy-L-proline in 10 mM HCl and ranged from 1 to 12 μg/ml. Hydrolysis was as described previously. For the assay, 50-μl aliquots of sample were placed in wells of a 96-well plate. Oxidizing solution (100 μl) was then added to each well and allowed to incubate for 5 min. Ehrlich’s reagent (100 μl) was then added to each well, and the plate was incubated for another 45 min at 60°C. Absorbance was then read at 570 nm using a microplate optical density reader.

Radiotelemetry measurement of blood pressure. Systolic and diastolic blood pressure were continuously and chronically monitored by radiotelemetry as previously described (4, 29). The telemetric transmitters (Datasciences International; St. Paul, MN) were implanted into the abdominal cavity of rats (with the catheter inserted into the distal portion of the descending aorta) at 10 wk of age. After a 2-wk recovery period, averages of 10-s samples obtained every 5 min were used to monitor systolic and diastolic pressures in each rat. Night blood pressure values were defined as the mean of the values recorded between 6 PM and 6 AM. Morning blood pressure values were defined as the mean of the values recorded between 6 AM and 12 PM. Circadian blood pressure differences were defined as the differences between night and morning values (defined as described above).

RESULTS

The morphological characteristics of the LV from 24-wk-old WKHA and WKY rats are summarized in Table 1. LV mass was assessed by calculating the ratio of the LV weight to whole body weight. There was an 11.6% increase of the LV weight-to-body weight ratio of WKHA rats compared with WKY rats (P < 0.007). Analysis of tissue sections prepared from the same hearts revealed that the surface of the ventricular parenchyma was increased in WKHA rats compared with WKY rats (P < 0.016), whereas the outer perimeter of the LV was similar in both strains. There was also a tendency toward a smaller cavity area in WKHA rats compared with WKY rats, although the difference did not reach statistical significance. Collagen abundance was quantified in two different ways. Quantification of Sirius red-stained fibers provided primarily an estimate of the abundance of interstitial collagen, because care was taken not to include perivascular collagen. It revealed that the amount of collagen fibers was ~15% lower in WKHA than WKY rats, the signifi-

<table>
<thead>
<tr>
<th>LV weight/body weight</th>
<th>WKHA Rats</th>
<th>WKY Rats</th>
<th>%Difference (WKHA vs. WKY)</th>
<th>n</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV weight, mg</td>
<td>940 ± 39</td>
<td>1,001 ± 61</td>
<td>6.1%</td>
<td>10</td>
<td>0.12</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>376 ± 6</td>
<td>360 ± 7</td>
<td>4.4%</td>
<td>10</td>
<td>0.22</td>
</tr>
<tr>
<td>Tissue area, mm²</td>
<td>68.95 ± 2</td>
<td>76.15 ± 1.2</td>
<td>10.4%</td>
<td>6</td>
<td>0.016</td>
</tr>
<tr>
<td>Cavity area, mm²</td>
<td>43.9 ± 2.6</td>
<td>40.9 ± 1.5</td>
<td>7.3%</td>
<td>6</td>
<td>0.42</td>
</tr>
<tr>
<td>Outer perimeter, mm</td>
<td>67.65 ± 0.95</td>
<td>66.51 ± 0.64</td>
<td>1.7%</td>
<td>6</td>
<td>0.36</td>
</tr>
<tr>
<td>Collagen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sirius red stained, %area</td>
<td>2.12 ± 0.12</td>
<td>1.81 ± 0.06</td>
<td>−14.7%</td>
<td>6</td>
<td>0.045</td>
</tr>
<tr>
<td>Hydroxyproline, μg/g wet wt</td>
<td>50.9 ± 1.65</td>
<td>55.2 ± 6</td>
<td>−9.2%</td>
<td>5</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Values represent means ± SE; n = no. of animals analyzed in each group. *LV, left ventricle; WKY, Wistar-Kyoto rats; WKHA, WKY-derived hyperactive rats. Significantly different (as determined by Student’s t-test).
WKHA rats are generally wider than those from WKY rats.

Characteristics of isolated cardiomyocytes

Analysis of cell size was performed on isolated cardiomyocytes, because it has been argued that this method yielded much more reliable data than analysis of tissue sections (12). A representative picture is shown in Fig. 1. Analysis of the cells revealed a 34.8% increase in mean width \((P < 0.0001)\) and a 42% increase of the width-to-length ratio \((P < 0.0001)\), whereas no significant difference was found in cell length (Table 2). To calculate cell volume, we used a technique that allows one to calculate the cross-sectional surface from the cell width. Indeed, it has been reported that the cross section of a cardiomyocyte can be approximated to an ellipse shape and that its surface can be calculated on the basis of the formula \(\pi AB/4\), where \(A\) and \(B\) correspond to the major and minor diameter of the ellipse, respectively (13). To verify that the \(B\)-to-\(A\) ratio was similar in both strains, we first used semithin sections of Epon-embedded cells and performed measurements at high magnification on undamaged cardiomyocytes where cross-sectional sarcomeres were clearly visible. To ensure that the profiles originated from truly cross-sectioned cardiomyocytes, we measured the thickness of sarcomeres within these cells and obtained values of \(1.89 \pm 0.14\) and \(1.83 \pm 0.16\) (SD) \(\mu m\) for WKHA and WKY cells, respectively. These values indicate that, for each strain, the maximal deviation from true sarcomere thickness never exceeded 15\%, thus ensuring that the measurements were representative of truly cross-sectioned cardiomyocytes (15). We then calculated that the \(B\)-to-\(A\) ratio was \(0.59 \pm 0.07\) and \(0.59 \pm 0.11\) (SE) in cardiomyocytes from WKHA and WKY rats, respectively. When using this ratio and the equation described above, we observed that the values of calculated cross-sectional area (CSA) were \(96.2 \pm 20.6\%\) (SD, \(n = 20\)) of the values of directly measured CSA for WKHA rats. Likewise, the values of calculated CSA were \(95.6 \pm 18.7\%\) (SD, \(n = 24\)) of the values of directly measured CSA for WKHA rats. Thus, provided that a sufficiently high number of cells were measured, values calculated on the basis of cell width were comparable with that of directly measured CSA. When these calculations were applied to the greater number of cells (>100 per animal) examined in the petri dishes, we calculated that CSA values and volume of cardiomyocytes from WKHA rats were \(74\%\) \((P < 0.0001)\) and \(54\%\) \((P < 0.0001)\) higher than corresponding values from cardiomyocytes from WKY rats.

Analysis by electron microscopy of sections from hearts fixed by controlled perfusion allowed us to calculate that CFV was \(72.1\%\) for WKHA rats and \(73.7\%\) for WKY rats. With the use of these values, it was possible to calculate that LV from WKHA rats contained \(16.9\) million cardiomyocytes, which corresponded to \(33.6\%\) more cells than the \(11.2\) million cardiomyocytes in LV from WKY rats. Finally, experiments with cells loaded with fura 2-AM indicated that basal \([Ca^{2+}]_i\) in isolated cardiomyocytes was \(54\%\) higher in cells from WKHA rats than in cells from WKY rats \((P < 0.0001)\).

Functional evaluations were performed on ten 24-wk-old animals of each strain to measure the maximum rate of positive and negative pressure change \((+dP/dt\) and \(-dP/dt\), respectively; Table 3). In WKHA rats, the values of \(LV + dP/dt\) \((P = 0.03)\) and \(LV -dP/dt\) \((P = 0.004)\) were significantly higher than those of WKY rats. Of note, the differences became more significant when the values were normalized for the numbers of cardiomyocytes in the LV from either WKHA or

Table 2. Characteristics of isolated cardiomyocytes

<table>
<thead>
<tr>
<th></th>
<th>WKHA Rats</th>
<th>WKY Rats</th>
<th>%Difference (WKHA Vs. WKY Rats)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, (\mu m)</td>
<td>131.7 \pm 1.3</td>
<td>128.8 \pm 1.14</td>
<td>↓ 2.20% (P = 0.20)</td>
<td></td>
</tr>
<tr>
<td>Width, (\mu m)</td>
<td>22.7 \pm 0.3</td>
<td>28.8 \pm 0.5</td>
<td>↑ 26.9% (P &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td>Width/length</td>
<td>0.176 \pm 0.03</td>
<td>0.250 \pm 0.013*</td>
<td>↑ 42% (P &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td>Calculated CSA, (\mu m^2)</td>
<td>250.5 \pm 6</td>
<td>435.9 \pm 26*</td>
<td>↑ 74.4% (P &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td>Calculated volume, (\mu m^3)</td>
<td>32,985 \pm 2,132</td>
<td>50,771 \pm 1,819*</td>
<td>↑ 53.9% (P &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td>Cardiomyocytes/LV, in millions</td>
<td>16.92 \pm 0.39</td>
<td>11.24 \pm 0.19*</td>
<td>↓ 33.6% (P &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td>(Ca^{2+}), nmol/l</td>
<td>96.6 \pm 4.5</td>
<td>148.6 \pm 5.2*</td>
<td>↑ 53.8% (P &lt; 0.0001)</td>
<td></td>
</tr>
</tbody>
</table>

Values represent means \(\pm SE\); \(n\) = no. of animals analyzed in each group. For morphometric measurements on isolated cardiomyocytes, \(n = 5\) (5 animals for each strain, each value representing the mean of measurements performed on \(\pm 100\) cells). For counts of cardiomyocytes/LV, \(n = 9\) for each strain. For \(Ca^{2+}\) values, \(n = 13\) for WKY rats (13 microscopy fields comprising a total of 31 cells), and \(n = 8\) for WKHA rats (8 microscopy fields comprising a total of 47 cells). CSA, cross-sectional area. *Values are significantly different.
WKHA rats. However, the differences were either abolished (for \( +dP/dt \)) or greatly reduced (\(-dP/dt\)) when the normalized values were further divided by the average CSA. There was no significant difference in the LV end-diastolic pressure (LVEDP). Examination of the passive diastolic properties of the ventricles from each strain (Fig. 2).

Figure 3 shows a graphic representation of the mean values of systolic and diastolic blood pressures as recorded by telemetry for four continuous nyctemers in five 12-wk-old animals from each strain. When averaged over 4 days, the mean values of systolic and diastolic blood pressure (130.2 ± 4.1 and 92.4 ± 0.9 mmHg for WKHA rats compared with 129.5 ± 3.5 and 90.5 ± 4.9 mmHg for WKY rats, respectively) were not significantly different. However, examination of the profile of blood pressure variations revealed that circadian differences were more apparent in WKY than WKHA rats. Accordingly, WKHA rats exhibited a night-morning circadian difference of systolic and diastolic pressure that amounted to \(~3.5\) mmHg. That value was significantly lower \((P < 0.05)\) than the night-morning circadian difference of systolic and diastolic pressure of 6.5 mmHg observed in WKY rats.

### DISCUSSION

The main finding of our study is that many features of LVH exist in WKHA rats despite mean levels of arterial blood pressure that are not different from WKY rats. From these features, it is also possible to characterize the type of hypertrophy present in WKHA hearts. Classically, LVH has been categorized into two basic types (16): concentric hypertrophy (where wall thickness increases without chamber enlargement) and eccentric hypertrophy (where chamber volume enlarges without a relative increase in wall thickness). According to these criteria, analysis of cross-sectional tissue sections indicated that LVH in WKHA rats is predominantly of the concentric type. Furthermore, our analysis of isolated cells revealed that the width of cardiomyocytes from WKHA rats was larger than that from WKY rats. In addition to confirming the role of cardiomyocytes in the general enlargement of the ventricles, the fact that the enlargement was caused by the larger width of cardiomyocytes (in contrast to absence of difference in length) is also consistent with what is expected when concentric hypertrophy is present (11, 16). Interestingly, despite the LVH in WKHA rats, there was no increased fibrosis, as indicated by the quantification of collagen and by the absence of shift of the passive pressure-volume relationship of the WKHA LV compared with that of the WKY LV.

Another important finding was that alterations were much more important at the level of isolated cardiomyocytes than at the macroscopic level (as determined by calculation of the LV weight-to-body weight ratio). Indeed, we calculated that the volume of cardiomyocytes was increased by \(~54\%\) in WKHA rats compared with WKY rats. In light of such large differences at the cellular level, one must postulate the existence of other factors to explain why the LV mass was only 10% larger. Interestingly, we also calculated that the total number of cardiomyocytes was \(~33\%\) lower in LV from WKHA rats than the LV from WKY rats, a fact that might explain in part why the difference in LV mass between WKHA and WKY rats was less pronounced.
than the differences in volume of isolated cardiomyocytes.

Functionally, we observed that the dP/dt values obtained in WKHA hearts were significantly higher than those in WKY hearts, similar to what has been previously reported in papillary muscles from SHR compared with WKY rats (3). However, the differences became more significant when the values were normalized for the numbers of cardiomyocytes in LV from either WKHA and WKY rats but were then either abolished (+dP/dt) or greatly reduced (−dP/dt) when the values normalized for cell number were further divided by average CSA. This most likely indicates that changes in contractility observed at the level of whole ventricles most likely reflect changes in the number and size of cardiomyocytes and presumably by changes in the number of contractile units in parallel (3). Another important parameter is that of diastolic function, which has previously been shown to be impaired early in the course of LVH (9). However, comparison of the passive diastolic pressure-volume curves revealed no difference in compliance of the LV of both strains. In vivo, the LVEDP was slightly more elevated at 24 wk of age in WKHA than WKY rats, but the difference was not significant. Finally, we observed that basal [Ca\(^{2+}\)] was 54% higher in isolated cardiomyocytes of WKHA rats than in those of WKY rats, but the difference was not significant. In WKHA LV, the basal [Ca\(^{2+}\)] and Ca\(^{2+}\) handling were affected in several models of experimental and/or genetic LVH.

Taken together, our observations concur to indicate that LVH in WKHA rats is predominantly concentric in nature and that the cardiomyocytes from WKHA LV are both increased in size and reduced in number compared with cardiomyocytes from WKY LV. In light of these findings, it is important to know whether LV from both strains are exposed to the same level of hemodynamic load. To have the most accurate measurements of blood pressure, we recorded blood pressure continuously in unrestrained conscious animals using radiotelemetric implants (4). Mean levels of systolic and diastolic blood pressure (as averaged over 4 days) were identical in both strains. However, the plot of mean levels of blood pressure over time revealed that there might be more subtle differences, because circadian variation was more obvious in WKY than WKHA rats. Calculation of the differences of blood pressure between morning and night values revealed that systolic and diastolic morning pressure decreased to lower levels in WKY than in WKHA rats. This observation raises the question as to whether differences in circadian variability may have an impact (beyond that of mean levels of blood pressure) on LV mass. In humans, differences in circadian variability have been associated with an increase in cardiovascular mortality (24). More specifically, some studies (26, 32) have found that a blunting of circadian differences was associated with an increase in LV mass. However, such an association has not been found consistently across studies (1). Furthermore, circadian differences were much more pronounced in humans than rats, because the circadian differences of blood pressure ranged from 15 to 20 mmHg in “dippers” and from 3 to 6 mmHg in “nondippers” (24, 32). The difference in circadian rhythm between WKHA and WKY rats was significant but amounted to only 3 mmHg in blood pressure. It remains to be determined whether such a small difference may have any impact on LV mass. Nonetheless, given what has been reported in humans, the association between higher LV mass and blunted circadian rhythm in WKHA rats is intriguing and constitutes the first instance where it has been reported in an animal model.

In summary, our data validate the use of WKHA rats as models of a predominantly concentric LVH developing in the absence of significant changes in mean levels of hemodynamic cardiac load and show that the hypertrophy phenotype is more pronounced in isolated cardiomyocytes than at the level of the whole ventricle. These data should prove particularly useful when trying to elucidate which features of LVH are linked to particular genes or loci in the course of forthcoming linkage studies performed with crosses between these two strains.
REFERENCES


