The hypertrophic heart rat: a new normotensive model of genetic cardiac and cardiomyocyte hypertrophy

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The hypertrophic heart rat: a new normotensive model of genetic cardiac and cardiomyocyte hypertrophy. Physiol Genomics 9: 43–48, 2002. First published February 25, 2002; 10.1152/physiolgenomics.00006.2002.—We describe a new line of rats with inherited cardiomyocyte and ventricular hypertrophy. From a second-generation cross of spontaneously hypertensive and Fischer 344 rats, we selected for low blood pressure and either high or low echocardiographic left ventricular (LV) mass over four generations to establish the hypertrophic heart rat (HHR) and normal heart rat (NHR) lines, respectively. After 13 generations of inbreeding, HHR had significantly greater (P < 0.0001) LV mass-to-body weight ratio (2.68 g/kg, SE 0.14) than NHR matched for age (1.94 g/kg, SE 0.02) or body weight (2.13 g/kg, SE 0.03). The isolated cardiomyocytes of HHR were significantly (P < 0.0001) longer and wider (161 μm, SE 0.83; 35.6 μm, SE 2.9) than NHR (132 μm, SE 1.2; 29.5 μm, SE 0.35). Telemetric 24-h recordings of mean arterial pressure revealed no significant differences between HHR and NHR. The HHR offers a new model of primary cardiomyocyte hypertrophy with normal blood pressure in which to examine genotypic causes and pathogenetic mechanisms of hypertrophy and its complications.

The hypertrophic heart rat: a new normotensive model of genetic cardiac and cardiomyocyte hypertrophy. As in humans, SHR cardiac hypertrophy is associated with reduced contractile function (17), arrhythmias (2), and cardiac fibrosis with diastolic dysfunction (3). Factors other than blood pressure per se also seem to be important in SHR, as increased LV size precedes the development of hypertension (8) and blood pressure reduction does not always lower LV mass (7). The heritability of blood pressure and cardiac size appear to be separate (20), and a genetic locus (Lum-1) is linked with LV mass but not blood pressure in SHR (12).

With the intention of establishing a line of rats with primary cardiac hypertrophy, we selectively bred rats with large hearts and low blood pressure from a second-generation cross of SHR and Fischer 344 (F344) strains (F344 × SHR F2). We report the details of the establishment of this line and its appropriate control and describe the cardiovascular and cardiomyocyte characteristics of these animals.

METHODS

An F344 × SHR F2 population was derived according to methods described previously for a separate genomic analysis (12). Previous reports referred to the F344 as the Donryu (DRY) strain, but their authenticity as F344 has been confirmed recently (10). In an earlier report (20), F344 animals were found to have the second smallest relative heart weights of 20 normotensive strains studied. SHR males were used in the original breeding to capture the SHR Y chromosome in subsequent male offspring.

Phenotype selection. Initial selection and breeding began in 1993. Systolic blood pressure (SBP) was measured in all animals between 6 and 9 wk of age using the tail-cuff plethysmographic method (11). Rats with the lowest average pressures at 7, 8, and 9 wk of age were selected. At 10 wk of age LV dimensions for each rat were measured by echocardiography (13) on two separate occasions by a single observer (E. F. Jones) who was blinded to the blood pressures and identities of the rats. From animals with the lowest SBPs, we selected those with the largest hearts for the hypertrophic heart rat (HHR) line and those with the smallest hearts for the normal heart rat (NHR) line. This selection was applied to each of the first four generations. After this time, animals in the lines were brother-sister mated without further selection. Characterization of the lines was made after 13 generations, when they would be ~95% genetically homozygous.

Cardiovascular phenotypes. We selected 16 male NHR and 19 male HHR for measurements of body weight between ages...
5 and 25 wk and SBP between ages 9 and 25 wk. Mean arterial pressures (MAP) were measured by the direct intraarterial method in conscious animals at the age of 27 wk as described previously (12). Rats were killed by an overdose of pentobarbionate (100 mg/kg, intraperitoneal injection). Ventricular mass was determined after removing the whole heart, carefully excising the atria and dissecting the right ventricular wall from LV and interventricular septum. Ventricles were blotted dry of blood before weighing to the nearest milligram. Because NHR animals were heavier than HHR at the same age, we also studied an additional group of younger male NHR (n = 6) matched for body weight with the HHR.

Hemodynamic telemetry. In a separate group of HHR (n = 6) and NHR (n = 6) aged 20–22 wk, we studied 24-h blood pressure profiles in freely moving conscious rats over a continuous period of 7 days by telemetry. Two weeks prior to recording, rats were implanted with blood pressure telemetry transmitters (model TA11PA-C40, Data Sciences International) in the abdominal area according to the method detailed by the supplier. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and MAP were recorded as the reduced mean of a 10-s sampling interval every 10 min using data acquisition and analysis software (Data Sciences International, USA). Pulse pressure (PP) was calculated as SBP minus DBP. Data from the periods 2100 to 0300 h and 0900 to 1500 h were used to represent the awake and sleep periods, respectively.

Cardiac and cardiomyocyte morphology. Prior to the detailed cardiomyocyte analyses, we undertook a preliminary comparison of the macroscopic morphology of HHR and NHR hearts. The animals were aged ~30 wk, and after terminal anesthesia (pentobarbionate 100 mg/kg ip), the hearts were removed, gently washed, fixed, and stored at room temperature in 10% neutral buffered formalin. At the midpoint between the base and apex of the LV, a 2-mm transverse tissue slice was cut, processed, and paraffin embedded. Sections (5 μm) were stained with either Masson's trichrome or by using a modified version of Van Gieson's technique. For estimates of collagen content, Van Gieson's stained sections were viewed at ×10 magnification under bright field, and digital images were acquired by a SPOT camera and software (Diagnostic Instruments). Four standard and adjacent images were sampled at each of three regions: the free LV wall, the interventricular septum, and the free right ventricular wall. Images were analyzed semi-quantitatively using ImageProPlus (version 3.0). Thresholds were applied to color images that were converted to gray scale binary masks and then analyzed densitometrically. The sectional area occupied by stained collagen was computed for each image and expressed as a percentage of total cross-sectional area.

For cardiomyocyte morphology, hearts were excised under anesthesia (pentobarbionate 100 mg/kg ip). Cells were obtained from 12 HHR and 17 NHR aged 20 wk and from 11 HHR aged 14 wk (to match for cardiac weight to the 20-wk-old NHR). To isolate cardiomyocytes, hearts were immediately mounted onto the cannula of a Langendorff column for retrograde perfusion with bicarbonate-buffered Krebs solution at 36°C to wash blood from the coronary vessels. Ventricular myocytes were dissociated using collagenase (0.45 mg/ml), then filtered, washed, and resuspended in HEPES-buffered medium containing trypsin inhibitor as previously described (4). From each heart, 50 rod-shaped and regularly striated myocytes were selected at random for length and width measurement at ×400 magnification using an inverted light microscope and calibrated eye piece as previously described (5). Mean myocyte maximum width and length was calculated for each heart.

**Genotype analyses.** Tests were collected for DNA analysis from 8 SHR, 7 F344, 13 HHR, and 8 NHR. DNA was extracted by standard methods (12). We selected the following polymorphic markers: D1Mit53 in relation to Map-1 on chromosome 1, D2Mit10 in the vicinity of Lum-1 on chromosome 2, and D10Mgh5 for another locus on chromosome 10 that showed suggestive linkage in our original cross to MAP [logarithm of the odds ratio (LOD) score = 3.3] (12). Details of markers were obtained from available rat genetic maps (http://rgd.mcw.edu/, http://www.ncbi.nlm.nih.gov/genome/guide/r.norvegicus.html, and http://ratmap.ims.u-tokyo.ac.jp/menu/Genome.html).

PCR amplification (50 ng DNA) was performed using the ABI-PRISM Catalyst 777 PCR Robotic Workstation. MgCl₂, 1.5 mM, was used in reactions for D2Mit10 and D10Mgh5, and 3.5 mM MgCl₂ was used for reactions for D1Mit53. An annealing temperature of 55°C was used for all reactions. PCR products were sized in a blinded fashion using the ABI-PRISM 377 DNA Sequencer and Genescan software.

**RESULTS**

Selection and breeding. The characteristics of the 9-wk-old animals selected for breeding are shown in Table 1. At each generation we were able to select males and females for the HHR line that had larger LVs than corresponding mating pairs in the NHR line. The differences in LV size remained relatively stable over the first four generations, and in both lines the SBP diminished.

**F13 generation characteristics.** Throughout the period from 10 to 25 wk of age (Fig. 1A), the NHR animals were heavier than HHR at any given age (F1.33 = 48.7, P < 0.0001), and the rate of weight gain was slower in HHR (F20.660 = 26.9, P < 0.0001). No significant differences in SBP were observed between the lines over the period from 9 to 25 wk of age (F1.33 = 0.9, P = 0.35) (Fig. 1B). At ~27 wk of age, we found no significant difference in conscious MAP between HHR (113 mmHg, SE = 4) and NHR (123 mmHg, SE = 2) (P = 0.14 by Mann-Whitney U test).

**Telemetric pressures.** Over the entire 7-day recording period, there were no significant differences in 24-h averages of SBP (HHR, 124 mmHg, SD 9; NHR, 122 mmHg, SD 12; P = 0.72), DBP (HHR, 86 mmHg, SD 10; NHR, 88 mmHg, SD 13; P = 0.76), or MAP (HHR, 104 mmHg, SD 9; NHR, 104 mmHg, SD 13; P = 0.99) between the two lines at 20–22 wk of age. There was a slight but significant difference in PP (HHR, 38 mmHg, SD 4; NHR, 33 mmHg, SD 3; P = 0.04) resulting from a higher SBP during sleep (HHR, 123 mmHg, SD 8; NHR, 118 mmHg, SD 12; P = 0.004) and lower DBP when awake (HHR, 88 mmHg, SD 10; NHR, 91 mmHg, SD 13; P = 0.005) in HHR compared with NHR.

**Cardiac weights.** When expressed as a proportion of body weight, all cardiac weights were significantly

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greater in HHR than NHR (Table 2). When HHR were compared with weight-matched NHR, both the absolute and relative weights of the heart and of the left and right ventricles were significantly greater in HHR (Table 2).

**Cardiac and cardiomyocyte morphology.** In Fig. 2, representative midregion transverse sections show that cardiac enlargement in the HHR was associated with reduced chamber lumen and increased wall thickness, indicative of concentric hypertrophy. There was no histological evidence of relative differences in fibrotic tissue between the NHR and HHR hearts, with collagen occupying 4.4–5.5% of the cross-sectional area and no observable regional differences.

Isolated cardiomyocytes from HHR were substantially longer and wider than those from NHR (Fig. 3). The cells from age-matched 20-wk-old HHR (mean cardiac weight = 1.42 g, SD = 0.27) were longer and wider than cells from NHR (mean cardiac weight = 1.30 g, SD = 0.13) (Fig. 4). In addition, the HHR myocyte three-dimensional structure was more complex and branched (Fig. 3). When matched more closely for cardiac weight, cardiomyocytes from 14-wk-old HHR (mean cardiac weight = 1.26 g, SD = 0.18) were significantly longer than 20-wk-old NHR (Fig. 4).

### Table 1. Phenotypic characteristics of animals selected for mating in the first four generations of HHR and NHR lines

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BW</td>
<td>LVM</td>
<td>LVM/BW</td>
<td>SBP</td>
</tr>
<tr>
<td><strong>HHR line</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F0</td>
<td>301</td>
<td>0.692</td>
<td>2.30</td>
<td>154</td>
</tr>
<tr>
<td>F1</td>
<td>236</td>
<td>0.649</td>
<td>2.75</td>
<td>163</td>
</tr>
<tr>
<td>F2</td>
<td>260</td>
<td>0.572</td>
<td>2.20</td>
<td>158</td>
</tr>
<tr>
<td>F3</td>
<td>192</td>
<td>0.720</td>
<td>3.75</td>
<td>125</td>
</tr>
<tr>
<td><strong>NHR line</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F0</td>
<td>258</td>
<td>0.441</td>
<td>1.71</td>
<td>161</td>
</tr>
<tr>
<td>F1</td>
<td>271</td>
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<td>2.00</td>
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</tr>
<tr>
<td>F2</td>
<td>265</td>
<td>0.562</td>
<td>2.12</td>
<td>135</td>
</tr>
<tr>
<td>F3</td>
<td>275</td>
<td>0.484</td>
<td>1.76</td>
<td>125</td>
</tr>
</tbody>
</table>

HHR, hypertrophic heart rat; NHR, normal heart rat; BW, body weight (g); LVM, left ventricular mass (g); LVM/BW, relative LVM (g/kg); SBP, average systolic blood pressure (mmHg) from 7–9 wk of age.

### Table 2. Cardiac phenotypes in HHR and NHR matched for age or weight

<table>
<thead>
<tr>
<th></th>
<th>HHR</th>
<th>Matched for Age</th>
<th>Matched for Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Age, wk</td>
<td>26.9(0.4)</td>
<td>27.9(0.4)</td>
<td>12.7(0.2)*</td>
</tr>
<tr>
<td>BW, g</td>
<td>346(10)</td>
<td>444(9)*</td>
<td>318(7)</td>
</tr>
<tr>
<td>CW, mg</td>
<td>1,213(55)</td>
<td>1,148(22)</td>
<td>862(17)*</td>
</tr>
<tr>
<td>CW/BW, g/kg</td>
<td>3.56(0.20)*</td>
<td>2.59(0.04)</td>
<td>2.72(0.05)</td>
</tr>
<tr>
<td>LVM, mg</td>
<td>913(40)</td>
<td>858(18)</td>
<td>677(13)*</td>
</tr>
<tr>
<td>LVM/BW, g/kg</td>
<td>2.68(0.14)*</td>
<td>1.94(0.02)</td>
<td>2.13(0.03)</td>
</tr>
<tr>
<td>RVM, mg</td>
<td>265(20)†</td>
<td>229(10)</td>
<td>165(3)</td>
</tr>
<tr>
<td>RVM/BW, g/kg</td>
<td>0.78(0.07)†</td>
<td>0.52(0.02)</td>
<td>0.52(0.01)</td>
</tr>
</tbody>
</table>

Values are means, with SE in parentheses. CW, cardiac weight; RVM, right ventricular mass. Tukey’s HSD test; *P < 0.05 compared with the other two groups; †P < 0.05 compared with NHR matched for weight.

Fig. 1. Longitudinal measurements of body weight between 5 and 25 wk of age (A) and systolic blood pressure between 9 and 25 wk of age (B) in normal heart rats (NHR, open squares) and hypertrophic heart rats (HHR, solid squares) at the F13 generation. Data are means ± SE.

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**Genotypes.** For the D1Mit3 marker linked with MAP all HHR rats and all NHR rats were homozygous for the F344 allele. For D10Mgh5 marker linked with MAP, all HHR were homozygous for the F344 allele and all NHR were homozygous for the SHR allele. For the marker D2Mit10 linked with relative LV mass, all NHR rats were homozygous for the F344 alleles, while 8 of the 13 HHR rats were either homozygous or heterozygous for the SHR allele.

**DISCUSSION**

The HHR is a novel genetic model of cardiac and cardiomyocyte hypertrophy without hypertension. The availability of such a line has potential to extend the understanding of the genetic and phenotypic correlates of cardiac hypertrophy. The HHR has the advantage that analyses of etiological mechanisms of cardiac hypertrophy should not be confounded by secondary effects of the usual experimental hypertrophic stimuli such as pressure or volume overload. HHR may also be of particular relevance to variation in human LV size and the associated complications such as heart failure and dysrhythmia. The SHR, from which the HHR was derived, shows many similarities in terms of the development and complications of LV hypertrophy in humans (2, 3).

The success of the selective breeding program is reflected by the fact that after 13 generations we have achieved clear separation of the relative LV masses of the HHR and NHR lines. Indeed, this was achieved after only four generations of selection. As a relevant comparison, at the same age, the relative LV mass of the HHR (2.68 g/kg) is only slightly less than SHR (2.83 g/kg; see Ref. 20) and that of the NHR (1.94 g/kg) only slightly greater than the progenitor F344 (1.82 g/kg). These similarities hold for comparisons with other independent analyses. For example, the relative cardiac masses for HHR (3.56 g/kg) and NHR (2.59 g/kg) are close to those reported for SHR (3.42 g/kg) and F344 (2.42 g/kg), respectively, in a previous detailed study of heart size (12).

Despite the genetic relatedness and gross phenotypic similarity, it is unlikely that the HHR hearts are absolutely identical to those of SHR. One reason to expect differences is the fact that SHR hearts are exposed to chronically increased afterload. Also, if SHR heart size is indeed determined by polygenes (12), then it is likely that some but not all of the relevant SHR quantitative trait loci (QTLs) were passed to HHR (see below). However, the absence of increased afterload in HHR provides an opportunity to study the underlying cardiomyocyte growth processes that form at least part of hypertrophy in the SHR. The nature of these processes will emerge from detailed comparisons of HHR and SHR. Moreover, the relative responses of the HHR and NHR hearts to hemodynamic stress will help define the predisposition to pathological cardiac hypertrophy.

The macroscopic picture (Fig. 2) and microscopic analyses indicated increased cardiomyocyte mass with
no difference in the proportion of fibrous tissue in HHR and NHR hearts. The dimensions of cardiomyocytes indicate that cellular hypertrophy is prominent. For the same average cardiac mass, it is possible to estimate (19) that the cell volumes of 14-wk-old HHR (35,635 μm³) are ~20% greater than 20-wk-old NHR (29,555 μm³).

In the absence of any overall differences in SBP, DBP, and MAP, the small difference in PP between HHR and NHR is not likely to be relevant as a cause of the LV hypertrophy. The presence of a significant increase in relative right ventricular hypertrophy also indicates load-independent hypertrophy in HHR hearts. Nevertheless, the HHR offers new opportunities to examine the interaction between genetic predisposition to LV hypertrophy and increased hemodynamic loads. For example, it would be particularly interesting to determine whether hypertension associated with activation (renal artery stenosis) or suppression (mineralocorticoid treatment with salt-loading and uninephrectomy) of the renin-angiotensin system has a differential effect on the adaptive LV hypertrophic response in HHR.

Our genotypic analyses were largely consistent with the phenotypic patterns observed. The HHR were homozygous for the F344 alleles associated with lower blood pressure at loci on chromosomes 1 (D1Mit3) and 10 (D10Mgh5) (12). In relation to heart size genetic loci, we found that none of the NHR had inherited the SHR allele associated with increased relative LV mass at the Lvm-1 locus (D2Mit10) on chromosome 2 (12). However, the analysis in the HHR animals was less conclusive. At D2Mit10, 8 of the 13 rats tested were either homozygous or heterozygous for the SHR allele. It should be noted that these preliminary experiments were designed to seek genotype-phenotype parallels and not to identify the genetic loci responsible for the phenotypes. The presence of genetic heterogeneity (at one of the three loci tested in the two rat lines) is not unexpected given the brother-sister mating for only 13 generations. These observations might or might not indicate that the Lvm-1 locus has been transferred from SHR to HHR. For example, even if Lvm-1 were the explanation for hypertrophy in HHR, the D2Mit10 marker might be just on the margins of linkage disequilibrium and not reliable in these circumstances. Detailed linkage and association analyses in inbred animals (after generation F21) will be required to address this question.

The cardiac hypertrophy of HHR is most obvious when expressed as a proportion of body weight because HHR are smaller than NHR. Although this indicates an unusually big heart for a given body size, it needs to be considered whether the primary abnormality in HHR is a small body rather than a large heart. However, when matched for body size, the hearts of HHR are significantly larger than NHR. Therefore, the HHR presents an interesting contrast of cardiomyocyte and cardiac hypertrophy on one hand and reduced body growth on the other. This disparity is qualitatively different from the pattern seen with nutritional restriction in the neonatal period, in which body and particularly cardiac growth are limited, resulting in a lower LV/body weight ratio (6). Therefore, the pattern in HHR of small bodies, high LV/body weight ratio, and enlarged cardiomyocytes may reflect the special genetically programmed growth effects that are unique to the HHR line.

In summary, these experiments confirm the success of our breeding strategy to create a line of animals with primary genetic cardiac hypertrophy. Our findings indicate that both the left and right ventricles were involved and point to cardiomyocyte hypertrophy as the underlying cellular abnormality. Further studies are required over the lifespan of HHR to establish when hypertrophy is first evident and how it affects life expectancy. Once the HHR and NHR strains are established after the 21st generation, they will offer a potentially important resource with which to study postulated mechanisms of cardiomyocyte hypertrophy. They will also facilitate investigation of the factors that
REFERENCES


