Cardiomyocyte Functions Couple with Left Ventricular Geometric Patterns in Hypertension

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Previous studies have suggested the prognostic significance of left ventricular (LV) geometric patterns in essential hypertension. However, the relation between cardiomyocyte functional changes and LV geometric patterns has not been clarified. This study was designed to assess the morphological and functional changes in isolated myocytes derived from different LV geometric patterns in hypertension. After 2-3 weeks of a high-salt (8%) diet from the age of 6 weeks, 20 Dahl salt-sensitive (DS) rats were classified into the following three groups on the basis of an echocardiographically determined LV mass index and the relative wall thickness: concentric hypertrophy (11), eccentric hypertrophy (4), and concentric remodeling (5). Ten Dahl salt-resistant (DR) rats served as controls. In vivo LV functions were assessed based on echocardiographic measurements. We examined ventricular myocytes isolated from all groups. To evaluate the force-frequency relation, cardiomyocytes isolated from all groups were paced at stimulation rates of 0.3, 0.5, 1.0, 2.0, and 3.0 Hz. Concentric hypertrophy and eccentric hypertrophy groups exhibited an increase in myocyte width but no changes in the length. Concentric hypertrophy and concentric remodeling groups demonstrated in vivo LV dysfunction. In addition, DS rats, especially those with concentric hypertrophy, demonstrated impaired frequency responses in terms of both myocyte contraction and relaxation compared with DR rats. This impaired force-frequency relationship was especially remarkable at high frequencies. These findings suggest that the structural and functional changes in cardiomyocytes are closely related to the LV geometric pattern and may contribute to a different prognosis according to different geometric patterns. (Hypertens Res 2000; 23: 345-351)

Key Words: hypertension, left ventricular hypertrophy, left ventricular geometry, cardiomyocyte function

Introduction

Left ventricular (LV) hypertrophy predicts a greater risk of cardiovascular events independent of arterial pressure, other risk factors, or the presence of coronary artery disease (1-3). Concentric LV hypertrophy in particular demonstrates the worst prognosis and the greatest degree of extracardiac vascular damage (4-6). In addition, several reports have shown a low level of myocardial function in patients with concentric hypertrophy or eccentric hypertrophy (7, 8). Therefore, the LV geometric pattern may be a determinative factor for cardiac events in hypertension. However, it remains unknown whether cardiomyocyte shape and function are different according to different LV geometric patterns.

The Dahl salt-sensitive (DS) rat is well recognized as an animal model of systemic hypertension (9). The blood pressure of the DS rat depends on the amount of sodium in the diet (10, 11). In DS rats, because the rise in blood pressure is gradual, the LV mass gradually increases in response to the elevation in blood pressure. Thus, the DS rat is a more appropriate model for simulating human hypertension than hypertensive models induced by surgery such as aortic banding. Recently, we examined the gene expression of sarcoplasmic reticulum (SR) pro-
teins in DS rats during the transition from compensatory hypertrophy to heart failure, demonstrating that alterations in the expression of the SR gene are related to changes in systolic and diastolic properties (12). This finding is important because the intracellular SR function is closely related to the cardiomyocyte function.

As shown in our previous studies (13, 14), the assessment of cardiomyocyte function determines the intrinsic LV function in the absence of humoral factors, sympathetic or parasympathetic interactions, the interstitium, and coronary blood flow. Accordingly, the purpose of our study is to clarify whether differences in cardiomyocyte shape and function correspond to the different LV geometric patterns in hypertension. Using the Dahl rat model, we investigated the in vivo LV function and evaluated the morphologic and functional changes in cardiomyocytes isolated from DS rats in the early compensated stage.

**Methods**

**Animal Models**

Male inbred DS rats and Dahl salt-resistant (DR) rats serving as normal controls developed at the Brookhaven National Laboratory were purchased from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan). According to the protocol of Inoko et al. (10), rats 4 weeks of age were started on a 0.3% NaCl (low-salt) diet until they were 6 weeks old, at which time they were fed an 8% NaCl (high-salt) diet (MF rat diet; Oriental Yeast Industry, Chiba, Japan). All procedures were performed according to the guide for animal experimentation and were approved by the Committee of Animal Experimentation of Ehime University School of Medicine.

According to the methods described by Ganau et al. (15), 20 DS rats 8 or 9 weeks of age were divided into the following three exclusive groups on the basis of the LV mass (LVM) index and the relative wall thickness (RWT) as shown in Fig. 1 (estimated as 2 SD above the mean values in the age-matched 10 DR rats): 11 rats with concentric hypertrophy (55%), 4 with eccentric hypertrophy (20%) and 5 with concentric remodeling (25%).

**Echocardiographic Evaluation**

Transthoracic echocardiographic studies were performed with a 7.5-MHz sector scan probe (model SSH-140A; Toshiba, Tokyo, Japan) when rats were aged 8 or 9 weeks (early compensated stage). After light anesthesia with 15 mg/kg pentobarbital (intraperitoneal), an M-mode echocardiogram was recorded as previously described by Hashida et al. (16), the LV chamber and wall thickness were determined from the mean value of three successive beats according to the method of the American Society of Echocardiography (17). The inner wall shell [half posterior wall and septal thickness at end-diastole (hd) and at end-systole (hs)] was also measured as described previously (18). From these measurements, the LV endocardial fractional shortening (FS) and midwall fractional shortening (mFS) were calculated by using the following formulas:

\[
FS (\%) = \frac{(EDD - ESD)}{EDD} \times 100
\]

\[
mFS (\%) = \frac{(EDD + \frac{1}{2} hd)}{EDD} - \frac{(ESD + \frac{1}{2} hs)}{EDD + \frac{1}{2} hd} \times 100,
\]

where EDD is the LV end-diastolic dimension, and ESD is the LV end-systolic dimension.

LVM and RWT were also determined using the following formulas (19).

\[
LVM (g) = 1.05 \times [(EDD + 2 PWTd)^3 - EDD^3],
\]

\[
RWT = \frac{2 PWTd}{EDD},
\]

where PWTd is the LV posterior wall thickness at end-diastole, and 1.05 is the specific gravity of the myocardium. In addition, the LVM index was calculated as the body weight ratio (10).

**Cardiac Catheterization**

After the echocardiographic survey, a PE-50 catheter (Clay Adams, NJ, USA) filled with heparinized (100 units/ml) saline was inserted into the left ventricle via the right common carotid artery. LV peak-systolic pressure (LVP) and end-diastolic pressure were measured using a multichannel recorder (model WS-682G; Nihon Kohden, Tokyo, Japan). Circumferential end-systolic wall stress (cESS), used as a measure of myocardial afterload, was...
calculated at the midwall from the LV pressure and echocardiography data using the following formula (7):
\[
\text{cESS (10}^3\text{ dyne/cm}^2) = \frac{\text{LVP} \times (\text{ESD}/2)^2 \left[1 + \left(\frac{(\text{ESD}/2 + \text{PWTs})^2}{(\text{ESD}/2 + \text{PWTs})^2 - (\text{ESD}/2)^2}\right)^2\right]}{(\text{ESD}/2 + \text{PWTs})^2 - (\text{ESD}/2)^2}
\]
where PWTs is the LV posterior wall thickness at end-systole. As a useful index of LV performance independent of myocardial afterload (7, 20), the afterload-corrected mFS was calculated using the following formula:
\[
\text{afterload-corrected mFS (%) = mFS} - b \times (\log \text{cESS} - \log \text{cESS}_x),
\]
where \(b\) is the slope of the regression line relating mFS to log cESS, and log cESS\(_x\) is the mean value of log cESS.

**Cardiomyocyte Function Evaluation**

**Myocyte Isolation**

Ventricular myocytes were enzymatically dissociated using a previously described technique (21). The heart was perfused via a Langendorff apparatus with a 100% oxygenated, calcium-free HEPES buffer containing (in mmol/l): NaCl 110.0, KCl 5.4, MgSO\(_4\) 1.2, KH\(_2\)PO\(_4\) 1.2, HEPES 10.0, mannitol 45.0, and glucose 15.0. The perfusion solution was then switched to a 100% oxygenated recirculated HEPES-collagenase solution with 35 \(\mu\)mol/l CaCl\(_2\), 10 mg of collagenase, type II (0.05%, w/v) (228 U/mg, Worthington, Freehold, NJ, USA), and 30 mg of bovine serum albumin (0.1%, w/v) (Fraction V, Sigma, St Louis, MO, USA) for a total of 30 min. Next, the left ventricle was minced into cubes and transferred to a centrifuge tube containing 10 ml of the HEPES-collagenase solution. The supernatant was removed, and fresh HEPES-collagenase solution was added and incubated. The Ca\(^{2+}\) concentration of the HEPES buffer was increased in a stepwise fashion. The final pellet was suspended in the modified collagenase-free HEPES buffer (the study buffer), which consisted of (mmol/l): NaCl 137.0, KCl 5.4, MgSO\(_4\) 1.2, KH\(_2\)PO\(_4\) 1.2, HEPES 10.0, CaCl\(_2\) 1.2, and glucose 15.0.

**Measurement of Cardiomyocyte Function**

The isolated myocytes were placed in a cell dish continuously superfused with the oxygenated study buffer at 22°C. The myocytes were imaged with an inverted microscope using a \(\times40\) phase-contrast objective, and we selected myocytes with a rod-shaped appearance, which consistently included more than 70-80% of the preparation, for the measurement of myocyte size. Then, myocyte width and length were measured in 20 rod-shaped cells from each sample. The rod-shaped cells from 16 DS rats and 6 DR rats were electrically stimulated by an electronic stimulator (model 6002 Stimulator; Harvard Apparatus Inc., South Natick, MA, USA). To evaluate the force-frequency relation, the myocytes were then continuously paced at stimulation rates of 0.3, 0.5, 1.0, 2.0, and 3.0 Hz. An analysis of the beating activity of the myocytes was carried out using the edge detection method of Okayama et al. (14). The peak velocities of myocyte shortening and relengthening were determined, and the velocity was then normalized by dividing each by the resting length. The percent of shortening (% shortening) was determined as the percent difference between the maximum and minimum myocyte lengths of each contraction. Measurements were carried out with 10 rod-shaped cells per isolation.

**Table 1. Echocardiographic and Hemodynamic Characteristics**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DR ((n=10))</th>
<th>DS Concentric remodeling ((n=5))</th>
<th>Concentric hypertrophy ((n=11))</th>
<th>Eccentric hypertrophy ((n=4))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>440 ± 12</td>
<td>481 ± 22</td>
<td>474 ± 32</td>
<td>469 ± 20</td>
</tr>
<tr>
<td>LV end-diastolic dimension (mm)</td>
<td>5.9 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>5.7 ± 0.4</td>
<td>6.4 ± 0.3* (\ddagger)</td>
</tr>
<tr>
<td>LV mass index (mg/g)</td>
<td>2.4 ± 0.2</td>
<td>2.6 ± 0.1</td>
<td>3.5 ± 0.4* (\ddagger)</td>
<td>3.2 ± 0.3**</td>
</tr>
<tr>
<td>Relative wall thickness</td>
<td>0.57 ± 0.03</td>
<td>0.74 ± 0.04* (\ddagger)</td>
<td>0.79 ± 0.08* (\ddagger)</td>
<td>0.58 ± 0.04</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>49.7 ± 3.9</td>
<td>61.2 ± 3.3*</td>
<td>58.5 ± 4.9**</td>
<td>53.9 ± 5.4</td>
</tr>
<tr>
<td>Midwall fractional shortening (%)</td>
<td>41.9 ± 1.8</td>
<td>34.6 ± 2.6* (\ddagger)</td>
<td>37.7 ± 4.1** (\ddagger)</td>
<td>42.0 ± 3.2</td>
</tr>
<tr>
<td>LV peak-systolic pressure (mmHg)</td>
<td>136 ± 11</td>
<td>205 ± 9*</td>
<td>192 ± 12*</td>
<td>201 ± 8*</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mmHg)</td>
<td>9.5 ± 3.3</td>
<td>27.2 ± 3.8*</td>
<td>26.5 ± 4.3*</td>
<td>30.0 ± 5.2*</td>
</tr>
<tr>
<td>Circumferential end-systolic wall stress (10(^3) dynes/cm(^2))</td>
<td>48.4 ± 6.7</td>
<td>76.8 ± 14.7</td>
<td>87.4 ± 9.6</td>
<td>91.0 ± 20.1**</td>
</tr>
<tr>
<td>Afterload-corrected midwall fractional shortening (%)</td>
<td>41.7 ± 2.1</td>
<td>33.8 ± 2.7* (\ddagger)</td>
<td>37.3 ± 3.3** (\ddagger)</td>
<td>41.3 ± 3.8</td>
</tr>
</tbody>
</table>

Data shown are mean values ± SD and numbers of rats. DR, Dahl salt-resistant rat; DS, Dahl salt-sensitive rat; LV, left ventricular; \(*p < .005\), \(**p < .05 vs. DR, \(\ddagger p < .005\) vs. concentric remodeling. \(\ddagger p < .005\) vs. concentric hypertrophy. \(\ddagger p < .05\), \(\ddagger p < .05\) vs. eccentric hypertrophy.
Statistical Analysis

All values are presented as mean ± SD. An ANOVA analysis was performed by which multiple means were compared. Bonferonni’s post-hoc test was used to detect differences among the subgroups. The slope and y intercept of the relation between mFS and log cESS were calculated using a linear regression analysis. P values of less than 0.05 were considered statistically significant.

Results

Hemodynamic Data and in Vivo LV Functions

Table 1 shows the hemodynamic data and in vivo LV functions measured at 8 or 9 weeks of age. There was no significant difference in heart rate between the groups of DS and DR rats. The LV peak-systolic and end-diastolic pressures were significantly higher in all groups of DS rats than in those of DR rats. However, there were no significant differences in the LV peak-systolic and end-diastolic pressures among the three groups of DS rats. mFS was significantly lower in the concentric remodeling and concentric hypertrophy groups than in the eccentric hypertrophy group and in DR rats, whereas FS was significantly greater in the same two groups than in the DR rats. cESS was significantly higher only in the eccentric hypertrophy group. The afterload-corrected mFS was significantly lower in the concentric remodeling and concentric hypertrophy groups than in the DR rats.

Myocyte Morphology

Table 2 shows myocyte morphometric data from four different geometric pattern groups. There was no significant difference in length among the four groups. In contrast, myocyte widths of concentric hypertrophy and eccentric hypertrophy groups were significantly greater (13% and 15% increase, respectively) than those of DR rats.

Table 2. Myocyte Morphometric Data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DR (n=10)</th>
<th>Concentric remodeling (n=5)</th>
<th>Concentric hypertrophy (n=11)</th>
<th>Eccentric hypertrophy (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell length (μm)</td>
<td>134.8±3.8</td>
<td>1314.3±3.9</td>
<td>136.4±6.2</td>
<td>133.2±20</td>
</tr>
<tr>
<td>Cell width (μm)</td>
<td>28.6±1.5</td>
<td>30.1±1.6</td>
<td>32.5±3.8*</td>
<td>32.8±0.9*</td>
</tr>
</tbody>
</table>

Data shown are mean values ± SD and numbers of rats. DR, Dahl salt-resistant rat; DS, Dahl salt-sensitive rat; *p < .005 vs. DR.

Fig. 2. Plots of changes in the percent of shortening (% shortening) in response to increasing stimulation frequency in myocytes isolated from five rats with concentric remodeling ( ), four with eccentric hypertrophy ( ■ ), seven with concentric hypertrophy ( ● ), and six Dahl salt-resistant (DR) rats ( ○ ). *p < 0.05 vs. DR rats, #p < 0.05 vs. the value at 1.0 Hz in DR rats.

Frequency Responses of Myocytes

Figure 2 shows the changes in % shortening associated with the change in stimulation frequency in the four groups. There was no significant difference in % shortening among the four groups at stimulation frequencies of 0.3, 0.5, and 1.0 Hz. In addition, at these frequencies the % shortening decreased linearly according to the increase in frequency. At high stimulation frequencies, there was a disparate response of % shortening between DS rats and DR rats. In DS rats, the % shortening at frequencies of 2.0 and 3.0 Hz was greater than that at 1.0 Hz (increase of 17.2% at 2.0 Hz and of 18.0% at 3.0 Hz). In contrast, in the DS rats, the % shortening at frequencies of 2.0 and 3.0 Hz remained unchanged or decreased. In particular, the % shortening in the concentric hypertrophy group was significantly lower than that in the DR rats.
Figure 3 shows the changes in the normalized peak velocities of myocyte shortening and relengthening in response to increasing stimulation frequency. The changing patterns in the normalized peak velocities of shortening and relengthening associated with the changes in stimulation frequencies were almost identical in all four groups, but the extent of these changes was different between the DS and DR rats. The velocity of shortening and relengthening was the lowest at a stimulation frequency of 1.0 Hz in all four groups. The highest values were at a stimulation frequency of 3.0 Hz in the DR rats, whereas in the DS rats, clear highest points were not determined. In addition, in the concentric hypertrophy group, the velocities of shortening and relengthening were significantly lower than in the DR rats at all frequencies.

Discussion

The hypertensive model in this study displayed different geometric patterns regardless of blood pressure on the basis of the echocardiographically calculated LVM index and RWT. In the present study, we clarified for the first time that the cardiomyocyte itself undergoes structural and functional changes according to the LV geometric changes observed in hypertension. Cardiomyocytes isolated from DS rats with an increased LVM index demonstrated a significant increase in width but no change in length. In addition, cardiomyocytes isolated from DS rats, especially those with concentric hypertrophy, showed impaired stimulation frequency responses of contraction and relaxation compared with those from DR rats. These findings indicate that impaired cardiomyocyte function is responsible for in vivo LV dysfunction and, thus, may contribute to a different prognosis according to the different LV geometric patterns.

Myocyte morphologic changes have been reported in several animal models (22-24). Briefly, pressure overload leads to enhanced wall thickness (concentric hypertrophy), which is reflected as an increase in the myocyte cross-sectional area at the cellular level. On the other hand, volume overload leads to a proportional increase in chamber diameter and wall thickness (eccentric hypertrophy) because of a proportional increase in myocyte length and cross-sectional area. A recent study using the Dahl rat model showed a significant increase in myocyte width but no change in length at the compensatory stages (25). In our study, DS rats with concentric hypertrophy and eccentric hypertrophy exhibited an increase in myocyte width but no change in length. This discrepancy represents the inconsistency between myocyte shape and echocardiographic LV geometry, which might be attributable to the following. New intercellular junctions are generated between cardiomyocytes at multiple stages during the development of compensated hypertrophy. Previous study has shown that the number of cardiomyocytes with lateral connections is significantly decreased in human hearts with eccentric hypertrophy compared with the number in control hearts (26). Cardiomyocytes with new intercellular junctions might have disadvantages in relation to geometric remodeling and mechanical perfor-
In vivo LV functions associated with hypertension have been well studied. Among systolic functions, FS is widely used to evaluate the condition of the left ventricle. However, FS is known to be less sensitive than mFS (7). In our study, mFS could sensitively differentiate DR rats from DS rats, especially in those with concentric remodeling and concentric hypertrophy. We have previously indicated that mFS is found less frequently in hypertensive patients with concentric hypertrophy than in those with eccentric hypertrophy, despite the LVM indices of these patients being the same (27). In view of this finding, it is reasonable to conclude that RWT is a significant predictor of mFS in hypertension.

The most striking finding in our study is that myocytes from the DS rats, especially those with concentric hypertrophy, demonstrated a markedly impaired contraction and relaxation responses at higher stimulation frequencies. This finding indicates that myocyte loss and abnormalities of the matrix are not solely responsible for the LV dysfunction associated with hypertension. Clinically, it is well recognized that LV dysfunction becomes apparent at higher stimulation rates (28, 29). Several investigators have assessed the cardiomyocyte functions in hypertrophied hearts (22) and their evaluations, including the force-frequency relation, are controversial.

Schouten et al. (30) have indicated that the force-frequency relation obtained when using thin ventricular trabeculae from the normal rat heart shows a biphasic pattern, an initial negative slope, and a subsequent positive slope. This pattern is identical to that observed in myocytes isolated from the DR rats in our study. Schouten et al. have also determined that the increase in force at low frequencies is reversed by drugs such as caffeine or theophylline, both of which inhibit SR Ca\(^{2+}\) uptake, but not by calcium antagonists, which block the inward Ca\(^{2+}\) current. In contrast, the increase in force at high frequencies is eliminated by calcium antagonists, but not by caffeine or theophylline. These findings suggest that myocyte function at lower frequencies might depend on SR function, and that at high frequencies it might be related to an Na\(^{+}\)/Ca\(^{2+}\) exchange mechanism.

Some studies have demonstrated abnormal Ca\(^{2+}\) handling induced by an altered SR function in pressure-overloaded hypertrophy (31). However, in our previous study (12), Ca\(^{2+}\)-ATPase messenger RNA levels in DS rats with compensated hypertrophy were not significantly different from those in DR rats. In the present study, the cardiomyocyte function in DS rats was preserved at lower frequencies, suggesting that the SR function of cardiomyocytes from DS rats in the compensated stage is preserved. In contrast, in concentric hypertrophy, cardiomyocyte function is extremely impaired, especially at high frequencies, compared with that in DS rats with other geometric patterns, whereas the myocyte shape is quite similar to that in eccentric hypertrophy. The impaired myocyte function is responsible for in vivo LV dysfunction. The difference in cardiomyocyte function corresponding to LV geometric patterns might be caused by changes in the Na\(^{+}\)/Ca\(^{2+}\) exchange mechanism. However, the mechanism for the disturbed Na\(^{+}\)/Ca\(^{2+}\) exchange is unknown. In addition, some investigators have reported a change in myofilament Ca\(^{2+}\) sensitivity and a shift in myosin heavy chain isoforms in pressure-overloaded hypertrophy (31, 32). These findings may be related to the myocyte dysfunction at high frequencies.

Our study has a few limitations. We used the cube-function formula to estimate LVM from the echocardiographic measurements, but the LV shape in rats does not assume a perfectly elliptical shape. Thus, a possible limit to this approach might be the instability of the parameter used to estimate the LV long axis in the formula. According to the study by Inoko et al. (10), a comparison of the LVM calculated by the cube-function formula from the echocardiographic measurements with those observed at autopsy has shown a linear correlation with narrow deviations in every stage of DS and DR rats. This finding may support our echocardiographically determined data.

In conclusion, we clarified in this study that the remodeling of myocyte shape occurs according to the LV geometric changes associated with hypertension in DS rats. In addition, depending on the suppression of in vivo LV function, the cardiomyocyte function in DS rats, especially in those with concentric hypertrophy, is markedly suppressed at higher frequencies. These findings indicate that the structural and functional changes in myocytes are closely related to the LV geometric pattern.

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