Left ventricular diastolic function of spontaneously hypertensive rats and its relationship to structural components of the left ventricle

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Summary

1. Left ventricular (LV) diastolic function was investigated in three different age groups (15, 28 and 50 weeks) of paired spontaneously hypertensive (SHR) and normotensive (WKY) rats under pentobarbital anaesthesia. A time constant of LV pressure decay, represented by $T$, was used as an index of LV relaxation. We assessed the relationship between haemodynamic parameters and LV structural components as quantified by microspectrophotometry (MSP), using multivariate analysis.

2. $T$ was significantly prolonged in the 28 and 50 week old SHR compared with their normotensive counterparts ($P < 0.05$ and $P < 0.01$, respectively). $T$ was prolonged by volume loading but was not affected with afterload elevation by angiotensin infusion in all age groups of the SHR and WKY.

3. LV wall thickness was greater in the SHR at all ages and was positively correlated with $T$ ($r = 0.42$, $P < 0.05$).

4. A significant correlation was found between the increase in cardiac muscle fibre and collagen, the decrease in elastin and glycoprotein, and $T$ on multivariate analysis ($r = 0.53$, $P < 0.05$).

5. We conclude that LV relaxation of SHR is disturbed from a relatively young age (28 weeks), for which we consider myocardial hypertrophy and LV structural changes found by MSP as being responsible.

Key words: left ventricular hypertrophy, left ventricular relaxation, microspectrophotometry, multivariate analysis, time constant.

Abbreviations: LV, left ventricle; LVEDP, left ventricular end-diastolic pressure; MSP, microspectrophotometry; SHR, spontaneously hypertensive rats; $T$, time constant; WKY, Wistar-Kyoto rats.

Introduction

Left ventricular (LV) hypertrophy is a generalized finding in established arterial hypertension and many controversies exist about LV function with progressive hypertrophy, both in humans and experimental animals. LV pumping ability of the hypertrophied heart has been most intensively investigated in the spontaneously hypertensive rats (SHR) because of the similarities of this model to essential hypertension in humans [1]. The systolic function of mature SHR has been reported as being either depressed [2, 3] or normal [4–6] with respect to normotensive rats.

On the other hand, diastolic function of SHR has received surprisingly little attention. Some investigators [6] have reported that the diastolic function of SHR was not disturbed, using peak negative dp/dt as an index. Peak negative dp/dt, however, appears to be influenced by various factors, and Weiss et al. [7] proposed the time...
constant (T) as a more accurate index of relaxation.

The purpose of this study was to evaluate the LV diastolic function of three different age groups of SHR and age- and sex-matched Wistar-Kyoto rats (WKY) by LV catheterization using a high fidelity micromanometer while the animals were breathing spontaneously. We also assessed the effects of volume loading with normal saline and acute pressure elevation with angiotensin on T.

Few attempts have been made to study the relationship between the functional abnormalities and quantitative changes of LV architectural components. We thus additionally investigated the relationship between haemodynamic parameters and LV structural changes as quantified by microspectrophotometry (MSP), using multivariate analysis.

Methods

Rats

Experiments were performed on male SHR of 15, 28 and 50 weeks of age (n = 12, 7 and 5, respectively) and WKY (n = 15, 6 and 7, respectively). The rats, obtained from our colony of inbred SHR and WKY, were housed four to five in plastic cages under conditions of identical temperature (22°C) and 12 h light-dark cycles. The animals were provided with a standard rat chow (Funahashi Farm) and tap water ad libitum until they were studied.

Experimental procedures and recordings

After the induction of pentobarbital anaesthesia (35 mg/kg intraperitoneally) the rats were placed on their backs with limbs gently extended and taped in position; they allowed to breathe spontaneously. A 5 cm catheter (PE 50) connected to a micromanometer (model PC-350, Millar Instruments) was inserted by way of the right carotid artery into the ascending aorta, and then advanced to the LV for measurements of LV pressure. Zero level was calibrated electrically with a transducer control unit (model TCB-100, Millar Instruments) before insertion of the catheter, and zero reference was fixed at the mid-chest level. The system had a natural resonant frequency of 116 Hz and a damping coefficient of 0.63. The first derivative of LV pressure (dp/dt) was obtained by electrical differentiation of the pressure signal. A venous catheter (PE 50), connected to a fluid-filled system (P23ID, Statham), was inserted via the right jugular vein near the level of the right atrium for measurement of central venous pressure.

Another venous line (PE 50) was inserted into the left femoral vein to be used for infusions. Once all the haemodynamic variables (arterial and venous pressures) had stabilized after surgical preparation, they were recorded at a paper speed of 100 mm/s on a multi-channel recorder (Rectigraph-8K, San-ei Ins, Japan). Left ventricular end-diastolic pressure (LVEDP) was displayed at a high sensitivity on an additional channel.

Angiotensin II [Hypertensine, Ciba, 0.625 mg in 500 ml of normal saline (154 mmol/l NaCl)] was infused into the femoral vein to elevate the arterial systolic pressure by about 30 mmHg and haemodynamic variables were recorded. After the arterial pressure returned to the pre-infusion level, physiological saline was infused at a rate of 2 ml/100 g of body weight for 1 min. The greatest increases in LVEDP and venous pressure occurred almost simultaneously, and were recorded along with the heart rate.

Five consecutive beats were averaged for determinations of pressures, dp/dt, LVEDP and heart rate. Body temperature was maintained by an over-head lamp and room temperature was kept constant (22 ± 2°C) with an air-conditioner. All the procedures were complete in 30–40 min. To avoid any circadian variation in the rats all the experiments were performed at the same time of day (from 14.00 hours to 15.00 hours).

Time constant

T was obtained by plotting LV pressures beginning at the time of peak negative dp/dt at 5 ms intervals up to the level of the LVEDP of the subsequent beat on a logarithmic scale (Fig. 1), according to the method of Weiss et al. [7]. We averaged the values of five consecutive beats. LV pressure fall during isovolumic relaxation period is exponential, and the following relationship is obtained: 

\[ P = e^{At+B}, \]

where \( P \) is pressure, \( A \) is the slope of exponential pressure fall, \( t \) is the time after peak negative dp/dt, and \( B \) is the intercept. At \( t = 0 \), \( dp/dt = \text{peak negative} \) dp/dt = \( A e^B = APO \), where \( PO \) is the LV pressure at peak negative dp/dt. The time constant \( T \), which is the negative reciprocal of \( A \), is therefore obtained as: 

\[ T = 1/A = PO/\text{peak negative dp/dt}. \]

Left ventricular geometry

At the conclusion of the haemodynamic study, the heart was arrested in diastole with an intra-ventricular infusion of 5% potassium citrate [8]. The heart was then excised, blotted dry, and the right and left ventricles plus septum weight was determined. LV wall thickness and LV internal
Diastolic function of SHR

\[ \ln = At + B \]
\[ A = -0.075 \text{ ms}^{-1} \]
\[ T = 1/-A = 13.3 \text{ ms} \]

\[ \ln = At + B \]
\[ A = -0.050 \text{ ms}^{-1} \]
\[ T = 1/-A = 20.0 \text{ ms} \]

FIG. 1. LV pressure beginning at the time of peak negative \( dp/dt \) from a representative beat was plotted at 5 ms intervals up to the level of the LVEDP on a logarithmic scale. Note the exponentiality.

\[ E = kcl, \]
\[ k \] is extinction coefficient, \( c \) is concentration and \( l \) is thickness. Since the thicknesses of the sections are precisely the same, extinction is proportional to concentration. The absolute content of each component was obtained by multiplying wall thickness by \( E \).

Multivariate analysis

To determine the factors that contribute to alterations of diastolic properties, principal component analysis, one of the types of multivariate analysis [10, 11], was carried out. The main idea behind this procedure is that the first few principal components (two principal components in this study) may well account for most of the variability in the original data, and for many purposes it may be reasonable to discard the remainder of them and so reduce the number of variables (from six to four variables in this study) that it is necessary to consider. In the present study two principal components were extracted from six variables (i.e. cardiac muscle fibre, elastin, collagen, acid mucopolysaccharides, glycoprotein and age) and each principal score was calculated with a microcomputer. With these principal scores the relationship between the haemodynamic data and LV structural components was analysed statistically.

Statistical analysis

All the variables are expressed as means ± standard error of the mean. The unpaired Student’s t-test was used to determine differences between strains of rats within a given age group. To evaluate the effects of interventions on a given variable within the same rat, the paired t-test was used. Analysis of variance was used to determine whether statistically significant changes occurred within a given strain over the three age groups of rats.

Results

Body weight of the youngest SHR was comparable with that of the age-matched WKY, but the older SHR weighed significantly less than their normal counterparts (Table 1). In contrast, heart weight increased more in the SHR than in WKY, so that the heart weight relative to body weight was...
TABLE 1. Body and heart weights
Results are expressed as means ± SEM. Significant difference from age-matched WKY: *P < 0.05, **P < 0.01.

<table>
<thead>
<tr>
<th>Weeks of age</th>
<th>15</th>
<th>28</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>300 ± 14</td>
<td>361 ± 20</td>
<td>442 ± 23</td>
</tr>
<tr>
<td>SHR</td>
<td>296 ± 11</td>
<td>338 ± 15*</td>
<td>377 ± 17*</td>
</tr>
<tr>
<td><strong>Heart weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>0.93 ± 0.03</td>
<td>1.18 ± 0.06</td>
<td>1.28 ± 0.06</td>
</tr>
<tr>
<td>SHR</td>
<td>1.05 ± 0.05</td>
<td>1.30 ± 0.06*</td>
<td>1.48 ± 0.11*</td>
</tr>
<tr>
<td><strong>Heart weight/body weight (mg/g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>3.1 ± 0.05</td>
<td>3.3 ± 0.08*</td>
<td>2.9 ± 0.09</td>
</tr>
<tr>
<td>SHR</td>
<td>3.5 ± 0.11</td>
<td>3.8 ± 0.08**</td>
<td>3.9 ± 0.09**</td>
</tr>
</tbody>
</table>

TABLE 2. LV wall thickness and internal radius
Results are expressed as means ± SEM. Significant difference from the age-matched WKY: *P < 0.02, **P < 0.01.

<table>
<thead>
<tr>
<th>Weeks of age</th>
<th>15</th>
<th>28</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LV wall thickness (mm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>2.9 ± 0.1</td>
<td>3.0 ± 0.2</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>SHR</td>
<td>3.2 ± 0.1</td>
<td>3.5 ± 0.2*</td>
<td>3.6 ± 0.1**</td>
</tr>
<tr>
<td><strong>LV internal radius (mm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>3.5 ± 0.6</td>
<td>3.4 ± 1.1</td>
<td>3.8 ± 1.1</td>
</tr>
<tr>
<td>SHR</td>
<td>3.4 ± 0.6</td>
<td>3.6 ± 1.1</td>
<td>3.9 ± 1.3</td>
</tr>
</tbody>
</table>

significantly greater in the older groups of SHR than in the age-matched WKY (Table 1).

LV wall thickness of the SHR was significantly increased compared with those of WKY at all ages (Table 2). Moreover, while little change was seen in wall thickness with age in the WKY, the SHR showed significant increases with advancing age. On the other hand, LV internal radius did not differ between the SHR and WKY in each age group and it did not change significantly with ageing (Table 2).

Table 3 summarizes the results of control haemodynamics. Mean arterial pressure of the SHR was significantly higher than that of the WKY at all ages. LVEDP showed no difference even between the oldest SHR and WKY. Peak positive dp/dt was significantly increased in the youngest SHR.

Peak negative dp/dt was not decreased even in the older groups of SHR as compared with the age-matched WKY. In sharp contrast, T was significantly prolonged in the older groups of SHR in comparison with the corresponding WKY. Fig. 2 illustrates a weak but statistically significant correlation between T and LV wall thickness in the SHR (r = 0.42, P < 0.05), while no correlation was found in the WKY.

After volume loading with physiological saline, heart rate was decreased, and end-diastolic and venous pressures were consistently elevated in all the rats. T was significantly prolonged with volume loading both in the SHR and WKY (P < 0.01), but the degree of prolongation (24 ± 0.78% vs 22 ± 0.56%) did not differ between them.

An acute elevation of LV systolic pressure by about 30 mmHg was associated with no significant changes in venous pressure or in LVEDP. In contrast to volume loading, T was not affected by the acute afterload elevation with angiotensin.

Amongst all the haemodynamic parameters, apart from mean arterial pressure, only T showed significant differences in the older SHR and WKY.
TABLE 3. Control haemodynamics

Results are expressed as means ± SEM. Significant difference from the age-matched WKY: *P < 0.05, **P < 0.01.

<table>
<thead>
<tr>
<th></th>
<th>Weeks of age</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>28</td>
<td>50</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>349±9.8</td>
<td>345±7.9</td>
<td>342±9.4</td>
</tr>
<tr>
<td>SHR</td>
<td>390±9.9</td>
<td>369±7.5</td>
<td>360±8.5</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>118±3.1</td>
<td>115±6.5</td>
<td>107±6.8</td>
</tr>
<tr>
<td>SHR</td>
<td>149±5.4**</td>
<td>146±11**</td>
<td>144±11**</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>2.8±0.6</td>
<td>2.3±1.0</td>
<td>2.8±0.9</td>
</tr>
<tr>
<td>SHR</td>
<td>3.1±0.8</td>
<td>3.0±0.8</td>
<td>3.3±1.2</td>
</tr>
<tr>
<td>Peak positive dp/dt (mmHg/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>3172±133</td>
<td>3600±295</td>
<td>3502±246</td>
</tr>
<tr>
<td>SHR</td>
<td>3859±232*</td>
<td>3721±207</td>
<td>3794±324</td>
</tr>
<tr>
<td>Peak negative dp/dt (mmHg/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>2721±62</td>
<td>2955±284</td>
<td>3040±342</td>
</tr>
<tr>
<td>SHR</td>
<td>3191±188*</td>
<td>2862±190</td>
<td>2958±301</td>
</tr>
<tr>
<td>T (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>13.7±0.56</td>
<td>13.7±0.93</td>
<td>14.9±0.91</td>
</tr>
<tr>
<td>SHR</td>
<td>15.1±0.89</td>
<td>16.1±0.87*</td>
<td>18.5±1.21*</td>
</tr>
</tbody>
</table>

FIG. 2. Relationship between LV wall thickness and T, demonstrating a positive correlation in SHR. ○, WKY; ●, SHR.

We therefore investigated the relationship between T and quantitative changes of LV structural components, using multivariate analysis.

First, a univariate correlation between the LV structural components and wall thickness was assessed, because wall thickness was significantly increased in the SHR at all ages. Table 4 shows that the increase in cardiac muscle fibre and collagen, and the decrease in elastin, acid mucopolysaccharides and glycoprotein, are related to wall thickness.

Then principal component analysis was employed to evaluate the relationship between T and these five LV structural components as well as age (six variables in all). Table 5 illustrates the principal score of the six variables calculated by principal component analysis. Two principal components were extracted from the six variables by
TABLE 4. A univariate correlation between wall thickness and LV structural components

<table>
<thead>
<tr>
<th></th>
<th>SM</th>
<th>EL</th>
<th>CL</th>
<th>AMPS</th>
<th>GP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wall thickness</td>
<td>0.03</td>
<td>-0.43</td>
<td>0.54*</td>
<td>-0.05</td>
<td>-0.27</td>
</tr>
</tbody>
</table>

Abbreviations: SM, cardiac muscle fibre; EL, elastin; CL, collagen; AMPS, acid mucopolysaccharides; GP, glycoprotein. Statistical significance: *P < 0.05.

TABLE 5. Principal score of each variable

<table>
<thead>
<tr>
<th></th>
<th>First principal component</th>
<th>Second principal component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.2275</td>
<td>0.5832</td>
</tr>
<tr>
<td>Cardiac muscle fibre</td>
<td>0.7067</td>
<td>0.3143</td>
</tr>
<tr>
<td>Elastin</td>
<td>-0.6465</td>
<td>0.2468</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.5159</td>
<td>0.5070</td>
</tr>
<tr>
<td>Acid mucopolysaccharides</td>
<td>0.1109</td>
<td>0.9439</td>
</tr>
<tr>
<td>Glycoprotein</td>
<td>-0.7763</td>
<td>0.3816</td>
</tr>
<tr>
<td>Contribution rate</td>
<td>30.84%</td>
<td>29.89%</td>
</tr>
<tr>
<td>Cumulative rate</td>
<td></td>
<td>60.73%</td>
</tr>
</tbody>
</table>

Fig. 3. Relationship between $T$ and the first principal component (the increase in cardiac muscle fibre and collagen, and the decrease in elastin and glycoprotein), demonstrating a positive correlation. ○, WKY; •, SHR.

Discussion

Myocardial relaxation has received increasing attention over the past decade. It is of particular interest to discover how myocardial hypertrophy would affect LV relaxation. Contrary to earlier beliefs [12] that relaxation was a passive process requiring no energy, abundant evidence now available [13, 14] concludes that it is a complex, energy-dependent process, consuming as much as 15% of the total energy of the cardiac cycle. During this period, calcium released from troponin is sequestered in the sarcoplasmic reticulum.

Multiple physiological and pathophysiological factors have been advocated as influencing relaxation of cardiac muscle. Prolonged relaxation has been reported after hypoxia and ischaemia [15, 16] and in hypothyroidism [17]. Shortened relaxation has been suggested to occur in hyperthyroidism [17] and to be produced by catecholamines [18].

Recent investigations [18–20] have indicated that contraction and relaxation are loosely coupled processes that may be separately in-
fluenced by certain stimuli or manipulations. Studies by Spann et al. [21] suggest that relaxation can be altered separately from contractility.

Maximal rate of LV pressure fall, or peak negative $dp/dt$, has often been used as an index of LV relaxation and some investigators [6] reported that the diastolic function of SHR was not depressed. Peak negative $dp/dt$, however, has many shortcomings, because it is influenced by multiple factors such as heart rate, aortic systolic pressure, stroke volume and end-systolic volume [22, 23]. Weiss et al. [7] reported that the time-course of isovolumetric pressure fall subsequent to peak negative $dp/dt$ was exponential, and was therefore characterized by a time constant, $T$. They found that $T$ was independent of systolic stress and end-systolic fibre length, and only minimally dependent on heart rate, but sensitive to factors thought to influence the process of relaxation. Since then $T$ has been widely utilized as a more accurate index of LV relaxation.

Although we demonstrated that peak negative $dp/dt$ was not decreased in any age of SHR, $T$ was abnormal in the older SHR, indicating impaired relaxation. A possible explanation for the disparity lies in the fact that peak negative $dp/dt$, which is influenced by peak systolic pressure, reflects the rate of decline of pressure at only a single instant near aortic valve closure, while $T$ is derived from multiple pressure measurements throughout the entire period of isovolumetric relaxation and does not appear to be directly influenced by events at or near the time of aortic valve closure [7].

Many investigators [3, 5, 6] utilized thoracotomy and artificial ventilation to study cardiac function of SHR, but these insults would considerably affect the haemodynamic states of anaesthetized animals [24, 25]. In this study we paid particular attention to minimizing the insults as much as possible, allowing the animals to breathe spontaneously.

As revealed by the present study, acute volume loading prolonged $T$ significantly both in SHR and WKY but no species difference was found. Gaasch et al. [26] and Raff & Glantz [27] also reported that $T$ increased significantly with volume loading in dogs. On the other hand, $T$ was not affected by increased afterload with angiotensin, suggesting that hypertension per se does not explain the prolonged $T$ in the SHR. Our finding of $T$ being independent of LV systolic pressure is in agreement with that of an earlier report [28].

The mechanisms responsible for impaired relaxation have been subjected to much debate. LV hypertrophy per se has been suggested to cause lower calcium binding and disturbed relaxation [29, 30]. We demonstrated that wall thickness, which was significantly increased in each age group of SHR, was positively correlated with $T$. Grossman et al. [31] reported that wall thickness was an important determinant of LV diastolic stiffness. It is easily conceivable that as wall thickness increases the ventricle becomes 'stiffer'. Mirsky et al. [32] found increased myocardial stiffness in 18 month old SHR. Interestingly, Hess et al. [33] reported that myocardial stiffness of the human hypertrophied heart was more influenced by LV interstitial fibrosis than by LV muscle mass or by muscle fibre size.

Several groups of investigators [5, 34, 35], including ourselves [36], have observed that fibrosis was increased in the myocardium of mature SHR. We have postulated that fibrosis is one of the causes of impaired relaxation.

Multivariate analysis is usually utilized to evaluate a phenomenon influenced by multiple factors, such as risk factors for coronary heart disease [37, 38]. To establish to what extent the histochemical factors were involved in the relaxation abnormality, we employed MSP and further evaluated the relationship between these factors and $T$. To do so we used multivariate analysis because many factors are involved in the relaxation abnormality and these factors could be variably influenced by each other. Thus we found that collagen played a significant role in the first principal component which showed a significant correlation with $T$.

It is widely accepted that collagen is increased both in concentration and in content in hypertrophied hearts [30, 39, 40] and is postulated [41] to cause decreased LV compliance. Thiedemann et al. [42] found a marked increase in collagen in 80 week old SHR, whose passive elastic properties in isolated LV papillary muscle were disturbed.

Another interesting aspect of this study was that elastin was negatively correlated with wall thickness, and it was also one of the major factors comprising the first principal component.

The increase in collagen and the decrease in elastin, in addition to the increase in cardiac muscle fibre, thus all seem to be responsible for the impairment of LV relaxation.

References


