In Vivo Evaluation of Left Ventricular Function in Aged SHR by Volume Loading and the Positive Inotropic Effect of Denopamine

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Abstract—The positive inotropic effect of denopamine on the hypertrophied heart was studied in 12-months spontaneously hypertensive rats (12-month-SHR) with and without volume loading. Firstly, the influence of volume loading on cardiac function was studied in 12-month-SHR compared with the age-matched normotensive Wistar-Kyoto rats (WKY) and younger WKY (4-month-WKY) as well. Left ventricular end-diastolic pressure (LVEDP) and maximum rate of rise of left ventricular pressure (LV dp/dtmax) were significantly higher in SHR than in WKY and 4-month-WKY. Volume loading (saline, 2 ml/kg/min, i.v., for 10 min) caused a marked increase in LVEDP both in SHR and WKY, but a higher elevation of LVEDP was observed in SHR. LV dp/dtmax, however, was increased in WKY and more markedly in 4-month-WKY, while it was not increased in SHR. Administration of denopamine to SHR at a rate of 1 μg/kg/min, i.v., for 10 min before and during 10 min of volume loading produced a marked increase in LV dp/dtmax and a significant decrease in LVEDP. Under denopamine infusion, volume loading caused an increase in LV dp/dtmax and produced a LVEDP elevation to a similar level to that of WKY. Denopamine at this dose caused no significant effects on heart rate and mean arterial blood pressure, indicating a selective inotropic action. The present study suggests that functional cardiac reserve is reduced in the aged SHR and that denopamine increases the functional reserve of the hypertrophied heart.

In spontaneously hypertensive rats (SHR), cardiac function is maintained by the induction of compensatory hypertrophy (1–4) secondary to the increased afterload following spontaneous development of hypertension. It has been reported that myocardial hypertrophy in SHR starts at 11 to 13 weeks of age (5–8). However, in the aged SHR, such as those over 12 months of age, pumping ability of the heart tends gradually to decline and finally results in a decompensated state (9, 10). The abnormalities of hypertrophied heart muscle have been reported also in the isolated papillary muscle preparation, in which declines in the velocity of contraction and in the activity of actomyosin ATPase were found (11).

In this connection, we were interested in examining the effects of a cardiotonic agent on the cardiac function of the aged SHR.

Denopamine is a selective inotropic agent which is orally active and has a little effect on systemic blood pressure (12, 13). Although denopamine has a selective beta1-adrenoceptor agonistic property (12), it has been shown that the increases in heart rate and myocardial oxygen consumption by denopamine are weak (12, 14). The positive inotropic effect was also observed in the failing heart (15).

The aim of the present study is firstly to assess the left ventricular function by volume loading of 12-month-SHR in comparison with that of age-matched normotensive Wistar-Kyoto rats (WKY) and young matured Wistar-Kyoto rats (4-month-WKY) and secondly to examine the effect of denopamine on the cardiac function of the aged SHR. For this purpose, we used a simple technique for the recording of intraventricular pressure.
and rate of rise of left ventricular pressure (LV dp/dt) without thoractomy and major operation.

Materials and Methods

Animals and anesthesia: Male SHR of 12 months of age (380–430 g) and WKY of 4 months (300–380 g) and 12 months (390–480 g) of age were used. All rats were obtained from Charles River Japan and housed in our laboratory and provided with standard rat food and tap water ad libitum for several weeks or months.

After being anesthetized with pentobarbital sodium at an intraperitoneal dose of 50 mg/kg, animals were fixed in the supine position under spontaneous respiration. To maintain the anesthesia throughout the experiment, appropriate dosages of pentobarbital sodium were added to the animal intraperitoneally.

For the drug study, rats were allotted to have the same level of blood pressure between the control and the drug treated groups.

Measurements of cardiovascular variables: Blood pressure was measured by a pressure transducer (MPU-0.5, Nihon Kohden), connected to a polyethylene tubing inserted into the right femoral artery. Heart rate was measured with a cardiotachometer, triggered by arterial pressure pulse. Left ventricular (LV) pressure was measured by a high-fidelity pressure transducer (CP-01, Century Technology) via a short polyethylene tubing (PE-60) connected to a 22G needle, which was directly inserted into the left ventricle through the 6th or 7th intercostal space. LV dp/dt was obtained by a differential amplifier (EQ-600G, Nihon Kohden). All the measurements were recorded simultaneously on a recticorder (6000 series, Nihon Kohden).

Volume loading: Warmed saline (37°C) at a rate of 2 ml/kg/min was infused into the right femoral vein for 10 min.

Measurements of ventricular weights: At the end of each experiment, the heart was excised and the atria were removed. The ventricles were blotted free of blood, the right ventricular free wall was dissected from the left ventricle, and both ventricles were weighed.

Drug administration: Denopamine was dissolved in the corresponding amount of HCl and diluted by saline. Denopamine (1 μg/kg/min) or saline was infused into the left femoral vein at a rate of 0.04 ml/min by an infusion pump (Truth A-II, Nakagawa Seikohdoh) for 10 min before and during 10 min of volume loading.

Statistical analysis: All data in the tables and figures are expressed as the mean±S.E.M. The paired t-test was used for evaluation of data. The differences were considered statistically significant if P values were less than 0.05.

Results

1. The ventricular weights and cardiovascular variables in aged SHR (SHR) and age-matched WKY (12-month-WKY) and 4-month-WKY

Body and ventricular weights of SHR and 12-month-WKY are shown in Table 1. Both LV weight and LV weight per body weight in SHR were higher than those in 12-month-WKY of the same age. On the other hand, the right ventricular weight per body weight was only slightly larger in SHR than 12-month-WKY.

Table 2 summarizes the cardiovascular variables.

<table>
<thead>
<tr>
<th>Table 1. Body and ventricular weights and ratios of SHR and WKY</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
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<tr>
<td>SHR (12 months, n=11)</td>
</tr>
<tr>
<td>WKY (12 months, n=6)</td>
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Values are means±S.E.M. LV: left ventricular, RV: right ventricular. **P<0.01 versus WKY.
parameters of SHR and 12- and 4-month-WKY in the control state. In SHR, mean arterial pressure, heart rate, LV dp/dt\(_{\text{max}}\) and LV end-diastolic pressure (LVEDP) were higher than those in 12- and 4-month-WKY.

2. Effects of volume loading on cardiac function and blood pressure in SHR, 12- and 4-month-WKY

**LVEDP:** Volume loading caused an increase in LVEDP gradually over 10 min in all groups (Fig. 1). The level of the LVEDP at 10 min of volume loading was higher in SHR (11.5 mmHg) than those in 12- and 4-month-WKY. Volume loading produced a greater increase in LVEDP in 12-month-WKY than 4-month-WKY, in spite of the same control level of LVEDP in both groups.

**LV dp/dt\(_{\text{max}}\):** Following volume loading, maximum increases in LV dp/dt\(_{\text{max}}\) were 25% and 12% above the control in 4- and 12-month-WKY, respectively, whereas LV dp/dt\(_{\text{max}}\) was not increased in SHR (Fig. 2).

**Mean arterial pressure:** Volume loading caused a slight but significant increase in mean arterial pressure in 4-month-WKY (Fig. 3). However, volume loading produced no effect on arterial pressure in 12-month-WKY. On the other hand, mean arterial pressure decreased significantly at 10 min of volume loading in SHR.

### Table 2. Cardiovascular variables in SHR and WKYs

<table>
<thead>
<tr>
<th></th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>LV dp/dt(_{\text{max}}) (mmHg/sec)</th>
<th>LVEDP (mmHg)</th>
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<tr>
<td>SHR</td>
<td>175±5.9**</td>
<td>355± 7.3**</td>
<td>10550±406**</td>
<td>5.5±0.43**</td>
</tr>
<tr>
<td>(12 months, n=11)</td>
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<tr>
<td>WKY</td>
<td>104±5.7</td>
<td>276±16.8</td>
<td>7090±286</td>
<td>3.0±0.31</td>
</tr>
<tr>
<td>(12 months, n=6)</td>
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</tr>
<tr>
<td>WKY</td>
<td>99±5.1</td>
<td>287± 5.0</td>
<td>7490±339</td>
<td>2.7±0.45</td>
</tr>
<tr>
<td>(4 months, n=7)</td>
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Values are means±S.E.M. MAP: mean arterial pressure, HR: heart rate, LV dp/dt\(_{\text{max}}\): maximum rate of rise of left ventricular pressure, LVEDP: left ventricular end-diastolic pressure. **P<0.01 versus 4 and 12 months WKY.

![Fig. 1. Effects of volume loading on LVEDP in SHR (● solid line, 12 months) and WKYs (Δ, 4 months; ○, 12 months). Volume loading (2 ml/kg/min, i.v., infusion) was started at 10 min and continued to 20 min; it caused a significant (P<0.05 versus values at 10 min) increase in LVEDP in SHR and WKYs. Denopamine (broken line) was administered intravenously to SHR at a rate of 1 μg/kg/min for 20 min; it lowered LVEDP before volume loading and diminished the elevation of LVEDP following volume loading in SHR (P<0.05). *P<0.05. **P<0.01 versus values before medication.](image-url)
Heart rate: Volume loading to SHR caused a small but significant decrease in heart rate, while it produced no effect on heart rate in 12- and 4-month-WKY (Fig. 4).

3. Cardiovascular effects of denopamine in SHR

Administration of denopamine to SHR at a rate of 1 μg/kg/min, i.v., caused a decrease in LVEDP and a progressive increase in LV dp/dt_{max} without affecting the mean arterial pressure and heart rate (Figs. 1–4). LVEDP was decreased to a level similar to that of 12- and 4-month WKY, and LV dp/dt_{max} was increased by 24% at 10 min of infusion.
These responses to denopamine reached almost steady state by 10 min of the drug infusion, when volume loading was started. Volume loading produced an increase in LVEDP under the drug infusion, but the level of LVEDP stayed lower than that of untreated SHR and was similar to or even lower than those of 12- and 4-month-WKY. LV dp/dt\(_{max}\) was further increased by volume loading, and the increase reached to 32% at 10 min of loading. Mean arterial pressure was not affected, but heart rate tended to be increased by volume loading under the drug infusion.

Discussion

In the present study, we used a simple technique for the measurement of intraventricular pressure. This method provides continuous monitoring of LVEDP and LV dp/dt without major surgery and thoracotomy, with a minimum trauma to animals. Several cardiovascular variables in 12-month-SHR such as basal levels of LV dp/dt\(_{max}\), LVEDP and heart rate were significantly higher than those in WKY. Pfeffer et al. showed that the level of LV dp/dt\(_{max}\) in 18-month-SHR was lowered and LVEDP of the male SHR was elevated compared with those of normotensive rats of a corresponding age under ether anesthesia (10). With respect to heart rate, they observed no difference between SHR and the corresponding WKY (9, 10). The differences in cardiovascular variables in SHR between our study and previous reports may be due to the differences in experimental conditions such as the anesthesia used and thoracotomy.

In 4-month-WKY, volume loading caused a more marked increase in LV dp/dt\(_{max}\) and a lower elevation of LVEDP than those in 12-month-WKY, and it produced a significant increase in blood pressure. These results may partly reflect an age-associated difference of the myocardial function.

In aged SHR, the elevation of LVEDP was higher than that in WKY, and volume loading did not cause an increase in LV dp/dt\(_{max}\). Moreover, basal LVEDP in SHR was significantly higher than that in WKY, in spite of basal levels in LV dp/dt\(_{max}\) and in spite of having a heart rate much higher than those of WKY. These facts suggest that the functional reserve of the heart in 12-month-SHR is limited by both aging and continued hypertension, so that the heart showed a kind of decompensatory response to volume loading.

Administration of denopamine to SHR increased LV dp/dt\(_{max}\) and decreased LVEDP, showing typical positive inotropic actions. On the other hand, denopamine produced no significant effects on mean arterial pressure and heart rate. Therefore, denopamine showed a selectivity to a positive inotropic action when compared with a chronotropic action and with an effect on blood pressure in aged
SHR. The inotropic selectivity of denopamine has been reported in several experimental animals (12) and in humans as well (16). In aged SHR, volume loading in the control study produced no increase in LV dp/dt max, while denopamine could cause a volume-induced increase in LV dp/dt max as observed in WKYs, showing the restoration of cardiac functional reserve. An elevation of LVEDP caused by volume loading was kept to a level similar to those of WKYs under denopamine infusion. These effects of denopamine may result from its positive inotropic actions.

The results suggest that the left ventricle in 12-month-SHR cannot afford to respond thoroughly to the functional requirement under volume loading conditions and that denopamine increases the functional reserve of the left ventricle in the aged SHR.

References
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