Comparative Study of Sinoaortic Denervated Rats and Spontaneously Hypertensive Rats

Chao-Yu Miao, Wen-Jun Yuan, and Ding-Feng Su

Background: Both hypertension and high blood pressure variability (BPV) are involved in cardiovascular damage. This comparative study was designed to explore the possible effects of both of these phenomena on the cardiovascular system.

Methods: The high BPV model of 16-week sinoaortic denervated (SAD) rats and the hypertension model of spontaneously hypertensive rats (SHR) were used for comparison at the same age of 26 weeks. The comparison was focus on hemodynamics, cardiovascular hypertrophy, and hemodynamic responses to ketanserin. Linear regression analysis was performed to study the role of hemodynamics in cardiovascular hypertrophy.

Results: In SHR, hypertension was accompanied by a moderately high BPV, whereas in SAD rats, substantially high BPV existed alone, without hypertension. Left ventricular hypertrophy was severe in SHR but was mild in SAD rats. Aortic hypertrophy was present in SAD rats but was absent in SHR. In SAD rats, the hypertrophy was correlated with BPV but not with blood pressure (BP) level. However, in SHR, hypertrophy was correlated with both BP and BPV level. The BP-lowering effect of ketanserin was comparable in both models, whereas its BPV-lowering effect was greater in SAD rats than in SHR. This hypersensitivity was associated with basal BPV level in SAD rats.

Conclusions: These results indicate that hypertension may be more important than high BPV in causing left ventricular hypertrophy, and that the aorta may be more sensitive to substantially high BPV. Am J Hypertens 2003;16:585–591 © 2003 American Journal of Hypertension, Ltd.

Key Words: Blood pressure, blood pressure variability, hypertension, hypertrophy, sinoaortic denervation.
BPV model of SAD rats, and the genetically hypertensive model of spontaneously hypertensive rats (SHR). Our comparison focused on hemodynamics, cardiovascular hypertrophy, and their relationship. The potential difference of hemodynamic responses to the drug was also explored by intravenous administration of ketanserin, a 5-HT2A receptor antagonist with weak blockade of α1 receptors.26

Methods

During the experiments, animals were housed with controlled temperature (23°C to 25°C) and lighting (8 AM to 8 PM light, 8 PM to 8 AM dark) and with free access to tap water and rat chow. All procedures were in accordance with institutional animal care guidelines and were approved by the local institutional committee.

Animal Models

SAD Rats Male Sprague-Dawley rats were purchased from Sino-British SIPPR/BK Lab Animal Ltd. At the age of 10 weeks, SAD was performed according to that described by Krieger9 with minor modification. Briefly, rats were anesthetized with a mixture of ketamine (50 mg/kg intraperitoneally) and diazepam (5 mg/kg intraperitoneally) and were then medicated with atropine sulfate (0.5 mg/kg, intraperitoneally) and procaine benzylpenicillin (60,000 U intramuscularly). After a midline neck incision and bilateral isolation of the neck muscles, aortic baroreceptor denervation was carried out bilaterally by cutting the superior laryngeal nerves near the vagi, removing the superior cervical ganglia including a small section of the sympathetic trunk, and sectioning aortic depressor nerves. The carotid sinus baroreceptors were denervated bilaterally by stripping the carotid bifurcation and its branches, followed by application of 10% phenol (in 95% ethanol) to the external, internal, and common carotid arteries and the occipital artery. In control rats (designated here as sham rats), sham operation was performed with the midline neck incision and bilateral isolation of the neck muscles, aortic baroreceptor denervation was carried out bilaterally by cutting the superior laryngeal nerves near the vagi, removing the superior cervical ganglia including a small section of the sympathetic trunk, and sectioning aortic depressor nerves. The carotid sinus baroreceptors were denervated bilaterally by stripping the carotid bifurcation and its branches, followed by application of 10% phenol (in 95% ethanol) to the external, internal, and common carotid arteries and the occipital artery. In control rats (designated here as sham rats), sham operation was performed with the midline neck incision and bilateral isolation of the neck muscles. Phenol was also applied in sham rats to each common carotid artery to control for any potential effects of phenol in the SAD group.6,19 After operation, these rats were allowed to survive for 16 weeks. SAD and sham control rats were used at the age of 26 weeks for measurements and comparisons.

SHR Male SHR and Wistar-Kyoto (WKY) control rats were purchased from the Shanghai Institute of Hypertension. At the age of 26 weeks, they were used for measurements and comparisons.

Hemodynamic Monitoring in Conscious Unrestrained Rats

Rats were anesthetized as described above. A polyethylene catheter (PE-10 connected to PE-50) was inserted into the left femoral vein for drug administration. Both catheters were tunneled subcutaneously, exteriorized between the scapulae, and fixed on the saddle. After 2 days of recovery, BP was continuously recorded for 3 h in conscious unrestrained rats with a computerized technique.27 Briefly, the BP signals, transmitted to the electric signals by a transducer, were digitized and processed on a personal computer, which calculated on-line the BP and HP beat by beat. Only the data over the last 1-h period were used for off-line analysis. The mean of beat-to-beat BP values was used as an index of BP, and the SD of beat-to-beat BP values as an index of BPV.17,27 The same method was used for the calculation of HP and HP variability (HPV). In SAD rats, arterial baroreflex function for heart rate control was also assessed by intravenous injection of phenylephrine 2 to 5 µg/kg. If SAD rats exhibit a bradycardia of less than 20 beats/min, when phenylephrine-induced increase in BP is greater than 50 mm Hg, they are considered as successful baroreceptor denervation and included in the study.15

Measurement of Ventricular and Aortic Weights

After hemodynamic monitoring, the animal was weighed, anesthetized, and killed by exsanguination. The heart and thoracic aorta were excised and rinsed in cold physiologic saline. The atria and vessels were then removed from the ventricles. The right ventricular free wall was separated from the left ventricle and septum, and they were blotted and weighed separately. At the same time, the aorta was cleaned of adhering fat and connective tissue. Just below the branch of the left subclavicular artery, a 22-mm-long segment of thoracic aorta was harvested, blotted, and weighed. As an index of ventricular hypertrophy, the ratio of ventricular weight to body weight was determined, and as an index of aortic hypertrophy, the ratio of aortic weight to length was calculated. These parameters are commonly used for evaluating cardiovascular hypertrophy.18,28

Determination of Drug Effects on Hemodynamics

In additional groups of SAD rats and SHR, ketanserin (kindly provided by Janssen Research Foundation, Beerse, Belgium) was used for comparing drug effects on hemodynamics between two models. Catheterization and recovery periods were the same as stated above. The protocol for hemodynamic monitoring is described as below. After 1 h of stabilization, three 30-min sampling periods, each with 5-min intervals, were programmed. Data from the first period were used as baseline. Before the second period, 1 mL/kg of saline was injected intravenously, and data from the second period were used as vehicle control. Before the third period, ketanserin dissolved in saline was injected intravenously and flushed with saline. The total volume of injected solution was <1 mL/kg, and the dose of injected ketanserin was 3 mg/kg. Data from the third
period were the values obtained after ketanserin. During every 30-min period, BP, BPV, HP, and HPV were calculated.

Statistical Analysis

Statistical analysis was performed using the SAS statistical program (SAS, Cary, NC). Data are reported as mean ± SD. Differences between two groups were evaluated by the Student unpaired t test. Hemodynamic data before and after drug administration were compared by the Student paired t test. The relationship between two variables was assessed by linear regression analysis. Statistical significance was judged at $P < .05$.

Results

Hemodynamic Changes in SAD Rats and SHR

Table 1 summarizes the hemodynamic data obtained from conscious rats. Compared with sham controls, the average level of systolic BP, diastolic BP, and mean BP remained unchanged, whereas systolic BPV, diastolic BPV, and mean BPV were substantially increased in SAD rats. In contrast to SAD rats, both BP and BPV were increased in SHR as compared with WKY controls. However, the increase in BPV was significantly higher in SAD rats (117% to 122%) than in SHR (28% to 60%). No significant changes in HP and HPV were found in these two models.

Ventricular and Aortic Hypertrophy in SAD Rats and SHR

Body weights were significantly lower in both SAD rats and SHR compared with the respective controls. From hypertrophy indexes, there existed left and right ventricular hypertrophy and aortic hypertrophy in SAD rats, whereas only left ventricular hypertrophy was found in SHR (Table 2). The left ventricular hypertrophy index was increased by 19% in SAD rats and by 37% in SHR, indicating more advanced left ventricular hypertrophy in SHR than in SAD rats. The ratio of left to right ventricular weight was unchanged in SAD rats, but markedly elevated in SHR, indicating mass imbalance of two ventricles in SHR. This parameter, together with ventricular hypertrophy index, further suggested that ventricular hypertrophy occurred in both the left and right ventricles of SAD rats and only in the left ventricles of SHR.

Table 1. Hemodynamic changes in conscious SAD rats and SHR

<table>
<thead>
<tr>
<th></th>
<th>Sham $(n = 10)$</th>
<th>SAD $(n = 10)$</th>
<th>Difference (%)</th>
<th>WKY $(n = 8)$</th>
<th>SHR $(n = 8)$</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>139 ± 6</td>
<td>140 ± 6</td>
<td>1</td>
<td>141 ± 8</td>
<td>180 ± 15*</td>
<td>28</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>93 ± 7</td>
<td>98 ± 9</td>
<td>5</td>
<td>98 ± 5</td>
<td>137 ± 13*</td>
<td>40</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>108 ± 6</td>
<td>112 ± 7</td>
<td>4</td>
<td>112 ± 6</td>
<td>151 ± 14*</td>
<td>35</td>
</tr>
<tr>
<td>SBPV (mm Hg)</td>
<td>6.3 ± 1.0</td>
<td>13.7 ± 4.0*</td>
<td>117</td>
<td>8.2 ± 1.6</td>
<td>10.5 ± 1.5†</td>
<td>28</td>
</tr>
<tr>
<td>DBPV (mm Hg)</td>
<td>5.2 ± 0.8</td>
<td>11.5 ± 3.0*</td>
<td>121</td>
<td>6.3 ± 0.9</td>
<td>10.1 ± 3.5†</td>
<td>60</td>
</tr>
<tr>
<td>MBPV (mm Hg)</td>
<td>5.5 ± 0.8</td>
<td>12.2 ± 3.4*</td>
<td>122</td>
<td>6.3 ± 1.0</td>
<td>10.1 ± 2.9†</td>
<td>60</td>
</tr>
<tr>
<td>HP (msec)</td>
<td>164 ± 15</td>
<td>158 ± 14</td>
<td>-4</td>
<td>170 ± 26</td>
<td>179 ± 13</td>
<td>5</td>
</tr>
<tr>
<td>HPV (msec)</td>
<td>23.7 ± 4.3</td>
<td>23.0 ± 3.9</td>
<td>-3</td>
<td>24.1 ± 3.8</td>
<td>25.5 ± 4.1</td>
<td>6</td>
</tr>
</tbody>
</table>

SAD = sinoaortic denervated rats; SHR = spontaneously hypertensive rats; Sham = sham-operated control rats; WKY = Wistar-Kyoto rats; SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure; SBPV = SBP variability; DBPV = DBP variability; MBPV = MBP variability; HP = heart period; HPV = HP variability.

Values are mean ± SD.

* $P < .01$; † $P < .05$ vs respective control.

Table 2. Ventricular and aortic weights in SAD rats and SHR

<table>
<thead>
<tr>
<th></th>
<th>Sham $(n = 11)$</th>
<th>SAD $(n = 15)$</th>
<th>Difference (%)</th>
<th>WKY $(n = 9)$</th>
<th>SHR $(n = 12)$</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>508 ± 36</td>
<td>433 ± 35*</td>
<td>-15</td>
<td>430 ± 50</td>
<td>322 ± 32*</td>
<td>-25</td>
</tr>
<tr>
<td>LVW/BW (mg/g)</td>
<td>2.01 ± 0.17</td>
<td>2.39 ± 0.10*</td>
<td>19</td>
<td>2.13 ± 0.16</td>
<td>2.92 ± 0.13*</td>
<td>37</td>
</tr>
<tr>
<td>RVW/BW (mg/g)</td>
<td>0.59 ± 0.08</td>
<td>0.67 ± 0.06*</td>
<td>14</td>
<td>0.63 ± 0.09</td>
<td>0.68 ± 0.06</td>
<td>8</td>
</tr>
<tr>
<td>VW/BW (mg/g)</td>
<td>2.60 ± 0.23</td>
<td>3.05 ± 0.14*</td>
<td>17</td>
<td>2.76 ± 0.20</td>
<td>3.60 ± 0.15*</td>
<td>30</td>
</tr>
<tr>
<td>LVW/RVW</td>
<td>3.46 ± 0.32</td>
<td>3.61 ± 0.29</td>
<td>4</td>
<td>3.42 ± 0.41</td>
<td>4.28 ± 0.39*</td>
<td>25</td>
</tr>
<tr>
<td>AW/length (mg/mm)</td>
<td>1.41 ± 0.16</td>
<td>1.59 ± 0.15*</td>
<td>13</td>
<td>1.38 ± 0.12</td>
<td>1.32 ± 0.11</td>
<td>-4</td>
</tr>
</tbody>
</table>

AW = aortic weight; BW = body weight; LVW = left ventricular weight; RVW = right ventricular weight; VW = LVW + RVW; other abbreviations as in Table 1.

Values are mean ± SD.

* $P < .01$ vs respective control.
Relationship Between Hypertrophy and Hemodynamics in SAD Rats and SHR

Linear regression analysis showed that in SAD and sham rats, hypertrophy indexes of the left and right ventricle and aorta were all positively correlated with BPV but not with BP level. In SHR and WKY rats, both BP and BPV were positively correlated with hypertrophy index of the left ventricle, but not hypertrophy indexes of the right ventricle and aorta (Table 3).

Effects of Ketanserin on Hemodynamics in SAD Rats and SHR

There were no significant differences in BP, BPV, HP, and HPV between baseline and vehicle control. Hemodynamic data before (second period) and after (third period) drug administration were compared. Both HP and HPV remained unchanged after intravenous injection of ketanserin. Ketanserin reduced BP and BPV in both SAD rats and SHR. The BP lowering effect was comparable in SAD rats and SHR, whereas it was significantly greater in SAD rats than in SHR (Fig. 1). Further analysis showed that in SAD rats, BPV reduction by ketanserin was correlated with the BPV level before drug administration (Fig. 2). There were no similar findings for BPV reduction in SHR or for BP reduction in SAD rats and SHR.

Discussion

This is the first report to demonstrate qualitative or quantitative differences in hemodynamics, cardiovascular hypertrophy, and drug responsiveness between SAD rats and SHR. The differences can be summarized as follows. First, in SHR, hypertension was accompanied by moderately high BPV; however, in SAD rats, substantially high BPV existed alone, without sustained hypertension. Second, left ventricular hypertrophy was more advanced in SHR than SAD rats. Right ventricular hypertrophy and aortic hypertrophy was present in SAD rats but was absent in SHR. Third, the BPV reduction by ketanserin was greater in SAD rats than in SHR, although the BP reduction was comparable in both models.

Accumulating evidence from human and animal studies suggests that both hypertension and high BPV (ie, pressure lability) are risk factors for cardiovascular damage, including ventricular and aortic hypertrophy. Our present study further supports the role of hypertension and high BPV in cardiovascular hypertrophy. In addition, these comparative data imply that hypertension and high BPV

Table 3. Linear regression coefficients between hemodynamic parameters and organ weights in SAD rats and SHR

<table>
<thead>
<tr>
<th></th>
<th>SAD + Sham (n = 20)</th>
<th></th>
<th>SHR + WKY (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LVW/BW (g/kg)</td>
<td>RVW/BW (g/kg)</td>
<td>AW/Length (g)</td>
</tr>
<tr>
<td>SBP</td>
<td>0.2306</td>
<td>0.1401</td>
<td>0.0270</td>
</tr>
<tr>
<td>DBP</td>
<td>0.2450</td>
<td>−0.0300</td>
<td>0.3136</td>
</tr>
<tr>
<td>MBP</td>
<td>0.2730</td>
<td>0.0205</td>
<td>0.2630</td>
</tr>
<tr>
<td>SBPV</td>
<td>0.7540*</td>
<td>0.5999*</td>
<td>0.6498*</td>
</tr>
<tr>
<td>DBPV</td>
<td>0.7980*</td>
<td>0.5998*</td>
<td>0.7500*</td>
</tr>
<tr>
<td>MBPV</td>
<td>0.7830*</td>
<td>0.6042*</td>
<td>0.7105*</td>
</tr>
</tbody>
</table>

Abbreviations as in Tables 1 and 2.
* P < .01; † P < .05

FIG. 1. Systolic blood pressure (SBP) and its variability (SBPV) before (□) and after (■) intravenous injection of ketanserin 3 mg/kg in sinoaortc denervated (SAD) rats (n = 10) and spontaneously hypertensive rats (SHR) (n = 10).
may act on different targets, leading to different consequences.

In the present study, we found that left ventricular hypertrophy was severe in SHR but mild in SAD rats; left ventricular hypertrophy index increased by 37% in SHR and by 19% in SAD rats. In the previous independent studies, we and others also reported that left ventricular hypertrophy was modest in SAD rats (11% to 16% increase in left ventricular hypertrophy index at 6, 10, and 16 weeks after SAD) compared with that in commonly used hypertensive rats (30% in SHR and 32% in two-kidney, one-clip hypertensive rats). These indicate that hypertension is more of a critical factor than high BPV for causing left ventricular hypertrophy. Alternatively, the severe left ventricular hypertrophy may be caused by synergism of hypertension and high BPV, as both BP and BPV were positively correlated with left ventricular hypertrophy in SHR.

Our present findings that aortic hypertrophy was present in SAD rats but absent in SHR at the age of 26 weeks, further support our most recent conclusion that the aorta is a sensitive organ to substantially increased BPV. In that study, we found that aortic hypertrophy occurred earlier than left ventricular hypertension in SAD rats, and the hypertrophy was positively correlated with BPV, but not with BP level. The absence of aortic hypertrophy has been described to be associated with upregulation of aortic nitric oxide synthase activity in SHR at the age of 15 weeks. Another possible explanation is that vascular lesions in genetic hypertension of SHR at this age may mainly involve small arteries. It should be noted, however, that one cannot interpret these results to indicate that there was no aortic response to hypertension in SHR. In fact, most recently, we used the weight measurements of thoracic aorta and geometric morphometric measurements of abdominal aorta, and found that aortic hypertrophy was present in SHR at 50 weeks of age but not at 26 weeks of age. Also, severe left ventricular hypertrophy was found in SHR of both ages; the left ventricular hypertrophy index was increased by 36% at the age of 26 weeks and by 42% at the age of 50 weeks. These unpublished data demonstrate that left ventricular hypertrophy is an earlier pathologic change than aortic hypertrophy in SHR, indicating the aorta is less sensitive to hypertension than is the left ventricle. In this study, we applied the commonly used parameter, the ratio of aortic weight to length, to evaluate aortic hypertrophy. The value of this parameter is associated with the aortic position and length because of the different size of aorta at different position. To make the measurements relatively accurate, we took the same position and the same length of aorta to measure aortic weight, as described in Methods. The aortic weight was greater in SAD than in sham rats, despite the lesser body weight in SAD than in sham rats, indicating aortic hypertrophy exists in SAD rats.

With regard to right ventricular hypertrophy in SAD rats, although the results showed a positive correlation between the right ventricular index and BPV, the biological significance of BPV in SAD–induced right ventricular hypertrophy remains to be further discussed. One study demonstrated that right ventricular hypertrophy after SAD was a consequence of carotid chemoreceptor denervation produced by SAD, suggesting that right ventricular hypertrophy after SAD may mainly result from possible changes in pulmonary circulation. The occurrence of SAD–induced right ventricular hypertrophy was first reported by Van Vliet et al. They unexpectedly found that right ventricular hypertrophy was much greater than left ventricular hypertrophy in SAD rats 6 weeks after operation (39% and 11% increases in the hypertrophy indexes of the right and left ventricles, respectively). This finding was confirmed in their later study (30% and 9% increases in hypertrophy indexes of the right and left ventricles, respectively). In these two studies, rat body weight was significantly decreased by 9% to 10% after SAD. Compared with their studies, right ventricular hypertrophy was so much less in our present study that it may be considered as the result of body weight loss after SAD, since the 14% increase in the ratio of right ventricular weight to body weight was comparable to the 14.7% decrease in the body weight in SAD rats. In another study of ours without haemodynamic measurements, an 11% increase in the ratio of right ventricular weight to body weight was found in Sprague-Dawley rats 16 weeks after SAD, with an 8.6% decrease in the body weight, also indicating less right ventricular hypertrophy. However, our recent study showed that in WKY rats 32 weeks after SAD, the absolute value of right ventricular weight was significantly increased by 14%, and the ratio of right ventricular weight to body weight was significantly increased by 22%, with a slight (5.8%) but nonsignificant decrease in body weight (unpublished data). Taken together, these findings lead us to believe that the increase in the ratio of right ventricular weight to body weight is not
the sole consequence of weight loss after SAD. The quantitative differences in right ventricular hypertrophy among different studies may be due to the difference in rats used in the studies: Long-Evans rats, Sprague-Dawley rats (present and unpublished studies), and WKY rats (unpublished study).

Ketanserin is an antihypertensive drug and has been used for treatment of hypertensive patients for more than 10 years. It is a 5-HT2A receptor antagonist with weak blockade of α1 receptor. Our previous study has shown that in SHR, the BP-lowering effect of ketanserin is mainly mediated by central 5-HT2A receptors. In the present study, ketanserin was selected as an example for comparison of drug responsiveness in these two models. It was found that the BP lowering effect of ketanserin was similar in both models, whereas its BP lowering effect was greater in SAD rats than in SHR. In addition, hypertensitivity was correlated with basal BPV level in SAD rats. These findings further suggest that, in vivo, cardiovascular function is different in SAD rats and SHR, and SAD rats may be a sensitive model for testing new compounds for the development of new drugs that specifically decrease BPV for cardiovascular protection.

In summary, our comparative study provides evidence of differences in cardiovascular function and morphology between SAD rats, an experimental model of high BPV alone, and SHR, a genetic model of hypertension accompanied by moderately high BPV. Our results indicate the following: 1) hypertension and high BPV may act on different targets, thereby leading to different consequences; 2) hypertension is more important than high BPV in causing left ventricular hypertrophy; and 3) high BPV is a more sensitive stimulus for the aorta. These data are meaningful for understanding the independent effects of hypertension and high BPV on the cardiovascular system. Further comparison using other hypertensive models (such as low renin hypertension of genetically Lyon hypertensive rats and experimentally deoxycorticosterone acetate-salt hypertensive rats) is needed for extending our conclusions.

References


