Combined Effect of Neonatal Sympathectomy and Adrenal Demedullation on Blood Pressure and Vascular Changes in Spontaneously Hypertensive Rats


Neonatal sympathectomy using a combined treatment with antiserum to nerve growth factor and guanethidine during the first 4 weeks after birth was carried out in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. Bilateral adrenal demedullation was performed in 4-week-old sympathectomized SHR and WKY rats. The development of hypertension in SHR was prevented by sympathectomy, but the blood pressure (BP) was still higher than in age-matched WKY rats. Demedullation reduced the BP of sympathectomized SHR to the same level as that of WKY rats. Heart rates of SHR and WKY rats were not affected by the treatments. Morphometric measurements of the mesenteric arteries showed that sympathectomy significantly reduced the medial mass in the mesenteric arteries of SHR, mainly through a reduction in the number of smooth muscle cell layers. In sympathectomized SHR, demedullation increased the lumen size of muscular arteries under maximally relaxed conditions; which might explain the further reduction in BP in these animals. Demedullation in sympathectomized SHR and WKY rats caused a decrease in smooth muscle cell layers in the superior mesenteric artery, but the same treatment resulted in a slight increase in the number of smooth muscle cell layers in the large and small mesenteric arteries of SHR and WKY rats. Adventitial area was increased in some mesenteric arteries of SHR and WKY rats by sympathectomy, and demedullation caused a further increase in the size of adventitia in WKY rats. Heart weight in SHR was normalized to the level found in WKY rats by sympathectomy and demedullation. We conclude that in sympathectomized SHR, the elevated BP was maintained by the adrenal medulla. (Circulation Research 1991;69:714–721)

In the spontaneously hypertensive rat (SHR), the importance of the sympathetic nervous system in the initiation and maintenance of hypertension has been emphasized by many authors, mainly because in these animals increased sympathetic activity was present in very young animals and destruction of the sympathetic nervous system either prevented or attenuated the development of hypertension. In our recent studies in which SHR were sympathectomized neonatally using a combined treatment of anti-nerve growth factor and guanethidine, the blood pressure (BP) of treated SHR (mean systolic BP, 139±2 mm Hg) was still higher than that of untreated and treated normotensive Wistar-Kyoto (WKY) rats (115±4 and 112±5 mm Hg, respectively), despite a total absence of sympathetic nerves in the peripheral arteries, such as the mesenteric and tail arteries, and the prevention of structural changes of the mesenteric arteries. Sympathectomy of SHR with anti-nerve growth factor alone was also not effective in preventing hypertension, because the BP of treated SHR was ~160 mm Hg or higher and because cardiac hypertrophy was still present. It is evident that other systems may be involved in maintaining a higher BP in these sympathectomized SHR. In view of the findings that adrenal medullary secretion was enhanced when the sympathetic nerve activity is suppressed (e.g., by fasting) and the suggestion that the adrenal glands are important for the development and maintenance of hypertension in SHR, it

From the Departments of Anaesthesia (R.M.K.W.L., J.T.) and Biomedical Sciences (M.C.), McMaster University, Hamilton, Ontario; the Robarts Research Institute (K.R.B.), London, Ontario; and the University of Ottawa Heart Institute (F.H.H.L., J.T.), Ottawa Civic Hospital, Ottawa, Ontario, Canada.

Supported by the Heart and Stroke Foundation of Ontario. R.M.K.W.L. is a Career Scientist supported by the Ontario Ministry of Health. F.H.H.L. is a Career Investigator supported by the Heart and Stroke Foundation of Ontario.

Address for correspondence: Dr. Robert M.K.W. Lee, Department of Anaesthesia, McMaster University, Health Sciences Centre, Hamilton, Ontario L8N 3Z5, Canada.

Received March 28, 1990; accepted May 2, 1991.
is possible that a complete removal of the adrenal medulla is probably needed to lower the BP of SHR to the level found in WKY rats. The role of the adrenal glands in SHR hypertension development is unclear, mainly because of the conflicting results from different studies. Bilateral adrenal demedullation of juvenile SHR attenuated, but did not prevent, the development of hypertension, whereas demedullation of adult SHR either had no effect on the BP or lowered the BP of SHR. In contrast, complete bilateral adrenalectomy in prehypertensive and adult SHR actually reduced the BP of SHR to normotensive levels, even though some reduction in BP was also observed in WKY rats. Furthermore, adrenalectomy also lowered the BP of SHR treated with anti-α1-receptor antagonists and/or guanethidine. Nevertheless, the role of the adrenal medulla in the maintenance of hypertension in SHR is unclear.

In this study, we investigated the combined effect of neonatal sympathectomy and adrenal demedullation on the BP and the structure of the mesenteric arteries of SHR and WKY rats. The results are compared with those in unoperated and neonatally sympathectomized SHR and WKY rats.

Materials and Methods

SHR and WKY rats were obtained from the rat colonies currently maintained in the animal quarters of McMaster University, Hamilton, Ontario, Canada. These colonies were initiated with the rats obtained from Charles River Laboratories Inc., Wilmington, Mass. Neonatal sympathectomy of SHR and WKY rats using anti-α1-receptor antagonists and guanethidine was carried out as outlined previously. Bilateral adrenal medullectomy was performed in 4-week-old sympathectomized SHR and WKY rats under ether anesthesia via flank incisions. BP and heart rate were measured every 2 weeks using the indirect method through the tail until 30 weeks of age, when the rats were killed. Rats were anesthetized with sodium pentobarbital (45 mg/kg i.p.). The mesenteric vascular bed was cannulated for perfusion fixation of the arteries, using our established methods for tissue sampling and processing, where the vessels were fixed under maximally relaxed conditions. From each rat, one adrenal gland, the brain, left kidney, and vasa deferentia were obtained before perfusion fixation of the arteries and immediately frozen for the measurement of catecholamines. The remaining adrenal gland was prepared for routine light microscopy to assess the extent of demedullation and tissue regeneration as follows. The adrenal gland was fixed in 2.5% glutaraldehyde and embedded in paraffin. Sections were treated with 3% aqueous potassium dichromate (chromatization) and subsequently stained with hematoxylin and eosin. In such a preparation, catecholamines become brownish, thereby making the identification of chromaffin cells in the adrenal medulla easier. In a subgroup of treated and untreated SHR and WKY rats, an indwelling catheter inserted into a carotid artery was put in place. Forty-eight hours after surgery, BP and heart rate measurements were carried out through this arterial line in conscious, unrestrained rats. Mesenteric arteries were subsequently removed from these rats. Tissue concentrations of norepinephrine and epinephrine were measured using an electrochemical detector in a high-performance liquid chromatography system.

Values are expressed as mean±SEM. The number of rats used (n) is indicated in all tables. Student’s unpaired t test was used for comparison between various experimental groups. Linear regression analysis was also used to test whether there was a correlation between BP and vessel wall parameters. Differences were considered significant at p<0.05.

Results

Blood Pressure and Body and Organ Weights

Sympathectomy lowered BP in SHR, but the BP of treated SHR was still higher than that of sympathectomized WKY rats (Table 1). A combined sympathectomy and demedullation lowered the BP of SHR to the level found in untreated WKY rats, whereas similar treatments lowered the BP of WKY rats only transiently (Figures 1 and 2). Sympathectomy alone reduced the body weight of SHR. Additional demedullation did not appear to have a further effect on the body weight of SHR (Table 1). Neither sympathectomy nor the combined treatment had any effect on the heart rate of SHR, but sympathectomy lowered the heart rate of WKY rats. In all instances, the heart rate of SHR was always higher than that of the corresponding WKY rats in the same treatment group.

In the control groups, the weight of the kidneys from SHR was higher than that in WKY rats (Table 1). In SHR, sympathectomy alone or the combined treatment reduced the kidney weights to the same levels found in WKY rats. Neither sympathectomy alone nor combined sympathectomy and demedullation affected the weight of the kidneys in WKY rats. The weight of the heart and adrenal glands in control SHR was higher than that in control WKY rats, and sympathectomy and demedullation eliminated the difference between the two groups.

Light Microscopy and Tissue Level of Catecholamines

In light-microscope sections, the central part of the adrenal glands in almost all the demedullated rats was totally devoid of medullary tissue. Instead, a central cavity surrounded by scar tissue was found. A few chromaffin cells were found in some demedullated rats (two of 10 rats). In control groups, tissue levels of catecholamines were similar between SHR and WKY rats in most of the tissues (Table 2). The exception was in the heart, where the level of norepinephrine was higher in WKY rats than in SHR. Sympathectomy and demedullation significantly re-
TABLE 1. Physical Characteristics of Spontaneously Hypertensive Rats and Normotensive Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>C</th>
<th>SX</th>
<th>SX+D</th>
<th>C vs. SX</th>
<th>C vs. SX+D</th>
<th>SX vs. SX+D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>216±4</td>
<td>5</td>
<td>139±2</td>
<td>8</td>
<td>120±6 6</td>
<td>&lt;0.0005  &lt;0.0005  &lt;0.0005</td>
</tr>
<tr>
<td>WKY</td>
<td>115±4</td>
<td>11</td>
<td>112±5</td>
<td>8</td>
<td>108±1 5</td>
<td>NS</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>379±6</td>
<td>6</td>
<td>358±12</td>
<td>8</td>
<td>363±10 6</td>
<td>NS</td>
</tr>
<tr>
<td>WKY</td>
<td>359±9</td>
<td>11</td>
<td>323±9</td>
<td>7</td>
<td>328±15 5</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body wt (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>347±6</td>
<td>11</td>
<td>312±7</td>
<td>8</td>
<td>327±4 6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>WKY</td>
<td>343±14</td>
<td>11</td>
<td>296±17</td>
<td>8</td>
<td>304±14 5</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney wt (g wet wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>1.31±0.03</td>
<td>6</td>
<td>1.17±0.05</td>
<td>8</td>
<td>1.24±0.04 6</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>WKY</td>
<td>1.05±0.09</td>
<td>5</td>
<td>1.03±0.07</td>
<td>8</td>
<td>1.09±0.05 5</td>
<td>NS</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart wt (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>1.14±0.03</td>
<td>6</td>
<td>NA</td>
<td>1.00±0.01</td>
<td>6</td>
<td>...</td>
</tr>
<tr>
<td>WKY</td>
<td>0.89±0.02</td>
<td>5</td>
<td>NA</td>
<td>0.92±0.06</td>
<td>5</td>
<td>...</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.005</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal gland wt (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>25.2±1.25</td>
<td>5</td>
<td>NA</td>
<td>10.3±0.72</td>
<td>5</td>
<td>...</td>
</tr>
<tr>
<td>WKY</td>
<td>20.3±0.72</td>
<td>5</td>
<td>NA</td>
<td>12.4±0.73</td>
<td>5</td>
<td>...</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.025</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. C, control rats; SX, sympathectomized rats; SX+D, rats given a combined treatment of sympathectomy and demedullation; n, number of rats in each group; SHR, spontaneously hypertensive rats; WKY, normotensive Wistar-Kyoto rats; NS, not significant; NA, results not available.

duced the catecholamine levels in the adrenal glands, mesenteric arteries, heart, kidneys, and vasa deferentia of SHR and WKY rats, and the reduction was generally more pronounced in SHR than in WKY rats (Table 2). However, there were exceptions. In the vasa deferentia, sympathectomy and demedullation reduced the tissue levels of catecholamines more in WKY rats than in SHR. Sympathectomy and demedullation did not affect the catecholamine levels in the brain of SHR and WKY rats.

FIGURE 1. Plot showing blood pressure profile of control spontaneously hypertensive rats (SHR), sympathectomized (SX) SHR, and SHR given a combined treatment with sympathectomy and demedullation (SX+D).

FIGURE 2. Plot showing blood pressure profile of control Wistar-Kyoto (WKY) rats, sympathectomized (SX) WKY rats, and WKY rats given a combined treatment with sympathectomy and demedullation (SX+D).
<table>
<thead>
<tr>
<th>Tissues</th>
<th>Control</th>
<th>SX+D</th>
<th>p</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal glands</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE (10^2 ng/g tissue wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>58.83±10.65</td>
<td>3.36±0.94</td>
<td>&lt;0.01</td>
<td>94</td>
</tr>
<tr>
<td>WKY</td>
<td>55.53±1.11</td>
<td>15.86±6.83</td>
<td>&lt;0.005</td>
<td>71</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (10^2 ng/g tissue wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>472.06±72.80</td>
<td>78.39±8.52</td>
<td>&lt;0.005</td>
<td>83</td>
</tr>
<tr>
<td>WKY</td>
<td>551.71±8.68</td>
<td>293.11±60.48</td>
<td>&lt;0.025</td>
<td>47</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mesenteric arteries</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE (10^2 ng/g tissue wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>9.48±0.70</td>
<td>2.86±0.60</td>
<td>&lt;0.005</td>
<td>70</td>
</tr>
<tr>
<td>WKY</td>
<td>11.03±0.67</td>
<td>NM</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (10^2 ng/g tissue wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>24.22±13.31</td>
<td>NM</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>WKY</td>
<td>21.77±7.80</td>
<td>9.98±1.01</td>
<td>NS</td>
<td>54</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE (10^2 ng/g tissue wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>4.81±0.25</td>
<td>2.63±0.28</td>
<td>&lt;0.0005</td>
<td>45</td>
</tr>
<tr>
<td>WKY</td>
<td>6.26±0.44</td>
<td>4.04±0.23</td>
<td>&lt;0.0005</td>
<td>35</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.025</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE (10^2 ng/g tissue wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>3.12±0.34</td>
<td>3.15±0.53</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>WKY</td>
<td>2.37±0.44</td>
<td>2.19±0.37</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (10^2 ng/g tissue wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>0.13±0.13</td>
<td>0.92±0.92</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>WKY</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE (10^2 ng/g tissue wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>0.64±0.06</td>
<td>0.08±0.01</td>
<td>&lt;0.0001</td>
<td>88</td>
</tr>
<tr>
<td>WKY</td>
<td>0.85±0.03</td>
<td>0.05±0.01</td>
<td>&lt;0.0001</td>
<td>94</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (10^2 ng/g tissue wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>0.02±0.01</td>
<td>0.02±0.01</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>WKY</td>
<td>0.01±0.01</td>
<td>0.00±0.00</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vas deferens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE (10^2 ng/g tissue wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>109.98±4.08</td>
<td>76.56±7.46</td>
<td>&lt;0.0001</td>
<td>30</td>
</tr>
<tr>
<td>WKY</td>
<td>110.29±6.52</td>
<td>3.72±0.69</td>
<td>&lt;0.0001</td>
<td>97</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E (10^2 ng/g tissue wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>0.14±0.02</td>
<td>0.02±0.01</td>
<td>&lt;0.001</td>
<td>86</td>
</tr>
<tr>
<td>WKY</td>
<td>0.09±0.01</td>
<td>0.00±0.00</td>
<td>&lt;0.001</td>
<td>100</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. SX+D, rats given a combined treatment of sympathectomy and demedullation; NE, norepinephrine; SHR, spontaneously hypertensive rats; WKY, normotensive Wistar-Kyoto rats; E, epinephrine; NS, not significant; NM, no measurable amount was found. For control rats, n=6 for SHR and n=5 for WKY; for SX+D, n=3 for SHR and n=5 for WKY.

*No measurable amount of epinephrine was present.
Morphometry

Morphometric results on the vessel wall dimensions in the three groups of mesenteric arteries are given in Table 3. In the superior mesenteric artery, which is an elastic, conduit vessel, in the control groups, total vessel wall cross-sectional area as well as cross-sectional area of each of the components of the vessel wall (e.g., media, adventitia) were significantly larger for SHR than for WKY rats. The number of smooth muscle cell layers was similar between SHR and WKY rats. Sympathectomy only reduced the medial area of SHR but increased the adventitial area in WKY rats. A combined treatment, however, reduced the total vessel wall in SHR, mainly through the reduction in the cross-sectional area of the medial and adventitial layers. Decrease in the medial area in SHR was partly related to the decrease in the number of smooth muscle cell layers. In contrast, in WKY rats, an increase in the vessel wall area was present; this increase was mainly due to a larger adventitial cross-sectional area. The number of smooth muscle cell layers was also reduced in WKY rats, but the decrease did not have a significant effect on the overall size of the medial layer.

Comparison of the sympathectomy group with the group given the combined treatment showed that demedullation increased the medial area in WKY rats and decreased the adventitial area in SHR. In both SHR and WKY rats, demedullation decreased the number of smooth muscle cell layers in the media. There was a positive correlation between BP and medial area ($r=0.64, p<0.001, df=36$) as well as between BP and the number of smooth muscle cell layers ($r=0.33, p<0.05, df=36$).

In the large mesenteric artery, an example of a large muscular artery, all of the vessel wall dimensions except lumen area were larger in control SHR than in control WKY rats. In the two treated groups, such differences were still maintained in some parameters, such as the cross-sectional area of the intima, media, and adventitia and the number of smooth muscle cell layers, suggesting parallel shifts in structural changes in these rats. The main effects of sympathectomy were a decrease in the medial area of SHR and a decrease in the number of smooth muscle cell layers in both SHR and WKY rats. The adventitial area was increased in both SHR and WKY rats. Additional demedullation actually resulted in larger arteries, in that the luminal area and medial area in both SHR and WKY rats became larger and there was a slight increase in the number of smooth muscle cell layers in WKY rats. There was a further increase in the adventitial area in the demedullated WKY rats, but not in SHR. Because of such differential changes in the various components of the vessel wall, the net result was that in SHR there was no overall change in the total vessel wall area that was due to treatment(s), whereas in WKY rats there was a stepwise increase in the total vessel area that was due to the treatments. There was a positive correlation between BP and medial area ($r=0.57, p<0.001, df=37$) and between BP and the number of medial smooth muscle cell layers ($r=0.72, p<0.001, df=37$).

In the small mesenteric arteries, which are examples of small muscular arteries or arterioles, most of the changes due to treatments were found in SHR. In the control groups, most of the vessel wall parameters except lumen were larger in SHR than in WKY rats. Such differences were present in most of the treated groups. Lumen area was smaller in SHR than in WKY rats in the control group, and treatments of SHR resulted in arteries with a larger lumen, thereby eliminating the difference between SHR and WKY rats. Sympathectomy actually reduced the size of the media in SHR, as indicated by the decrease in the media to lumen ratio. The number of smooth muscle cell layers was reduced by sympathectomy in both SHR and WKY rats. Demedullation somehow increased the medial area in SHR and increased the number of smooth muscle cell layers in SHR and WKY rats as compared with the rats that received only sympathectomy. There was a positive correlation between BP and the medial area ($r=0.46, p<0.01, df=41$) and between BP and the number of smooth muscle cell layers ($r=0.45, p<0.01, df=41$).

Discussion

In most animal models of hypertension, destruction of the sympathetic nervous system also resulted in the prevention of hypertension development (see Reference 3 for review). However, in SHR, the studies to date do not show a correlation between the degree of sympathectomy and the degree of hypertension prevention.3 Failure of some sympathectomy studies to prevent hypertension was mainly because the sympathectomy procedures that were used only resulted in partial elimination of the sympathetic nerves.5,16 It is possible that, in instances when sympathectomy was not complete, the remaining nerves may act as a trigger in initiating hypertension, such that partial sympathectomy, instead of proportionately decreasing BP, could still trigger and initiate the development of hypertension in SHR.16 This idea is compatible with the suggestion of Yamori et al17 that in SHR a small portion of norepinephrine tissue content is sufficient for the development and maintenance of hypertension. In view of the fact that the adrenal medulla contributes a significant amount of circulating catecholamines and that stimulation of the sympathetic nerves to the adrenal medulla causes large quantities of norepinephrine and epinephrine to be released into the circulating blood,18 it is conceivable that maintenance of hypertension in partially sympathectomized SHR might be accomplished through the compensatory role of the adrenal glands. Our results indeed provide evidence that a higher BP was maintained by the adrenal medulla in sympathectomized SHR than in age-matched WKY rats, because demedullation lowered the BP of SHR to the same level found in WKY rats.
Table 3. Vessel Wall Dimensions of Mesenteric Arteries from Control, Sympathectomized, and Sympathectomized and Demedullated Spontaneously Hypertensive Rats and Normotensive Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>Superior mesenteric artery (10^4 μm²)</th>
<th>Large mesenteric artery (10^4 μm²)</th>
<th>Small mesenteric artery (10^4 μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>SX</td>
<td>SX+D</td>
</tr>
<tr>
<td></td>
<td>Mean n</td>
<td>Mean n</td>
<td>Mean n</td>
</tr>
<tr>
<td>Total vessel wall area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>3.23±0.19 5</td>
<td>2.85±0.14 8</td>
<td>2.51±0.19* 6</td>
</tr>
<tr>
<td>WKY</td>
<td>1.94±0.11 5</td>
<td>2.20±0.10 8</td>
<td>2.26±0.06* 5</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.0005</td>
<td>&lt;0.005 NS</td>
<td>NS</td>
</tr>
<tr>
<td>Luminal area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>4.41±0.22 5</td>
<td>3.82±0.25 8</td>
<td>3.41±0.25* 6</td>
</tr>
<tr>
<td>WKY</td>
<td>3.71±0.26 5</td>
<td>3.64±0.23 8</td>
<td>3.53±0.10 5</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05 NS</td>
<td>NS</td>
<td>&lt;0.0005 NS</td>
</tr>
<tr>
<td>Wall/lumen ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>0.74±0.05 5</td>
<td>0.77±0.07 8</td>
<td>0.75±0.06 6</td>
</tr>
<tr>
<td>WKY</td>
<td>0.53±0.03 5</td>
<td>0.62±0.05 8</td>
<td>0.64±0.01* 5</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.005 NS</td>
<td>&lt;0.05 NS</td>
<td>&lt;0.0005 NS</td>
</tr>
<tr>
<td>Intimal area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>...</td>
<td>...</td>
<td>0.19±0.01 5</td>
</tr>
<tr>
<td>WKY</td>
<td>...</td>
<td>...</td>
<td>0.12±0.06 5</td>
</tr>
<tr>
<td>p</td>
<td>...</td>
<td>...</td>
<td>&lt;0.0005&lt;0.05 NS</td>
</tr>
<tr>
<td>Medial area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>1.85±0.10 5</td>
<td>1.60±0.08* 8</td>
<td>1.55±0.11* 6</td>
</tr>
<tr>
<td>WKY</td>
<td>1.15±0.06 5</td>
<td>1.03±0.06 8</td>
<td>1.19±0.04* 5</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.0005&lt;0.005 NS</td>
<td>&lt;0.025&lt;0.005</td>
<td>&lt;0.0005&lt;0.005 NS</td>
</tr>
<tr>
<td>Media/lumen ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>0.42±0.02 5</td>
<td>0.43±0.03 8</td>
<td>0.46±0.03 6</td>
</tr>
<tr>
<td>WKY</td>
<td>0.31±0.01 5</td>
<td>0.29±0.02 8</td>
<td>0.34±0.01* 5</td>
</tr>
<tr>
<td>p</td>
<td>0.005&lt;0.005 NS</td>
<td>&lt;0.005 NS</td>
<td>&lt;0.005 NS</td>
</tr>
<tr>
<td>Adventitial area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>1.38±0.10 5</td>
<td>1.25±0.11 8</td>
<td>0.96±0.09* 6</td>
</tr>
<tr>
<td>WKY</td>
<td>0.78±0.59 5</td>
<td>1.17±0.08* 8</td>
<td>1.06±0.04* 5</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.005 NS</td>
<td>NS</td>
<td>&lt;0.005 NS</td>
</tr>
<tr>
<td>No. smooth muscle cell layers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>6.08±0.06 5</td>
<td>5.97±0.06 8</td>
<td>5.60±0.18* 6</td>
</tr>
<tr>
<td>WKY</td>
<td>5.98±0.08 5</td>
<td>5.91±0.17 8</td>
<td>5.48±0.07* 5</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.0005 NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SX, sympathectomized rats; SX+D, rats given a combined treatment of sympathectomy and demedullation; n, number of rats used in each group; SHR, spontaneously hypertensive rats; WKY, normotensive Wistar-Kyoto rats; NS, not significant.
*p<0.05 compared with Control; †p<0.05 compared with SX.
The mechanism through which BP reduction was accomplished was probably different between sympathectomy and demedullation, because different types of remodeling of the vessel wall structure were involved. In sympathectomized SHR, BP reduction was mostly related to a decrease in the contractile mass of the arteries, whereas a further reduction in BP by demedullation was due to a decrease in flow resistance because of a larger lumen size in the large and small mesenteric arteries. These arteries are within the range of resistance vessels involved in the regulation of BP.19 In normotensive sympathectomized rats, resistance to blood flow in the hindquarters was slightly higher than in control rats.20 It was suggested that, in the absence of functional sympathetic innervation to the vasculature, a normal or heightened vasoconstrictor tone is maintained through the effect of blood-borne catecholamines from the adrenal medulla.20 Demedullation significantly reduced the resistance to blood flow in the hindquarters of these sympathectomized rats, but the mechanism was unknown.20 It is possible that a similar mechanism (i.e., larger lumen size) might be involved in these animals as well.

Neonatal sympathectomy was without effect on the heart weight of SHR,3,6 but sympathectomy and demedullation reduced the heart weight to the level found in WKY rats. It is unlikely that prevention of cardiac hypertrophy was due to normalization of BP, because the most drastic drop in BP was achieved with sympathectomy. It is possible that in sympathectomized animals the persistence of cardiac hypertrophy may be due to an enhanced catecholamine secretion by the adrenal medulla. In the mesenteric arteries, the differential effects of sympathectomy alone and of combined sympathectomy and demedullation on the structure of different types of vessels suggest that different mechanisms might be involved in causing vascular changes in hypertension, as explained below.

In the superior mesenteric artery where the density of innervation was less (0.4% of total vessel wall) as compared with that in the large mesenteric artery (1.35%) and small mesenteric arteries (2.5%),21 normal proliferation of the smooth muscle cells may be under the influence of catecholamines from the adrenal medulla, because sympathectomy alone was without effect, but additional demedullation reduced the number of smooth muscle cell layers in both SHR and WKY rats. In contrast, in the muscular arteries of SHR, an increased proliferation of the smooth muscle cells takes place immediately after birth,22 mainly under the influence of the sympathetic nervous system, because sympathectomy maintained the number of smooth muscle cell layers at the level found in untreated WKY rats in the large and small mesenteric arteries, whereas additional demedullation was without effect on this parameter.

Reduction in the adventitial area in the superior and small mesenteric arteries of SHR given the combined treatment might be related to the reduction in the BP of SHR, because in most hypertensive models, lowering of the BP also resulted in a decrease in the amount of connective tissue.16,23 However, in normal animals, sympathectomy actually resulted in an increase in vascular connective tissue content, as occurred in superior and large mesenteric arteries from WKY rats. Increased collagen content24 and increased collagen synthesis25 were observed in sympathectomized rabbits. The mechanism of collagen increase due to sympathectomy is unknown.

In the aortic coarctation hypertensive rat model, wall thickening in the arterioles of cremaster muscle was present independent of intra-arterial pressure, and sympathectomy with guanethidine and adrenal demedullation did not affect such vascular hypertrophy, suggesting that humoral growth factors may be involved.26 It is not known whether such factors are involved in vascular changes in SHR. Fetal transfer studies between SHR and WKY rats suggest that circulating hypertensinogenic factors may not be involved in SHR, at least in very young rats, because an increased wall/lumen ratio in the carotid artery and aorta was still present in SHR transferred to WKY dams, whereas no significant change was found in WKY transferred to SHR dams.27

Demedullation and sympathectomy caused a drastic reduction in the catecholamine level in the adrenal glands of SHR and WKY rats, with a greater reduction in SHR than WKY. Regeneration of the medulla or incomplete demedullation in some WKY rats might account for the presence of catecholamines in the tissues of some of these rats, such as mesenteric arteries and kidneys. The presence of some chromaffin cells in the adrenal glands from some demedullated rats supported this hypothesis. However, in some tissues, such as in the heart and vasa deferentia, the degree of reduction of catecholamines was ≤50%. This may be due to the resistance of some tissues to chemical sympathectomy, especially in tissues from SHR.12,23,26,29

The role of glucocorticoids in the development of hypertension in SHR is not clear. It was suggested that the glucocorticoids are essential for the maintenance of high BP.8 Injection of cortisol or deoxycorticosterone to adrenalectomized SHR apparently raised the BP of these rats from 120 to 200 mm Hg.10 However, our present study actually showed that without sympathetic nerves and adrenal medulla, the presence of adrenal cortex was not sufficient to cause hypertension in SHR. It is apparent that the role of glucocorticoids in hypertension development in SHR is at best a passive one, probably secondary to the influence of sympathetic nervous system. This is similar to the situation in the deoxycorticosterone/NaCl model of hypertension, where sympathectomy had been shown to prevent the development of hypertension as well.30

In conclusion, we have shown that in sympathectomized SHR the adrenal medulla contributes to the maintenance of a BP higher than in WKY rats. We have also demonstrated that in contrast with the
effect of sympathetic tone, where BP lowering was due to prevention of smooth muscle cell hyperplasia, further reduction in BP associated with demedullation was related to an increase in lumen size in the muscular arteries.

Acknowledgments

We thank Mr. R. McKenzie for his excellent technical assistance and Ms. S. Seaman for her secretarial help.

References


KEY WORDS • hypertension • sympathectomy • adrenal medulla • vascular structure • catecholamines
Combined effect of neonatal sympathectomy and adrenal demedullation on blood pressure and vascular changes in spontaneously hypertensive rats.
R M Lee, K R Borkowski, F H Leenen, J Tsoporis and M Coughlin

Circ Res. 1991;69:714-721
doi: 10.1161/01.RES.69.3.714

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/69/3/714