Analysis of Cerebrovascular Sympathetic Nerve Density in Relation to Stroke Development in Spontaneously Hypertensive Rats

J.S. Smeda, PhD

Previous studies have shown that elevating the K+ levels from 0.75% to 2.11% in the diet of stroke-prone spontaneously hypertensive rats significantly retards the development of stroke and increases their lifespan. On the other hand, stroke-resistant spontaneously hypertensive rats fail to develop stroke even if they are fed the low-K+ version of this diet. Since sympathetic nerves surrounding the cerebral vasculature play an important role in protecting the brain from stroke during hypertension, I studied whether changes in sympathetic nerve density accounted for the differing incidences of stroke in stroke-prone spontaneously hypertensive rats fed high- and low-K+ diets and in stroke-resistant and stroke-prone spontaneously hypertensive rats fed a low-K+ diet. At 14 weeks of age, all 11 stroke-prone rats fed the low-K+ diet had evidence of cerebral hemorrhage while such lesions were virtually absent in the 11 littermates fed the high-K+ diet and totally absent in the eight stroke-resistant rats fed the low-K+ diet. Stroke-prone (regardless of diet) but not stroke-resistant rats exhibited greater sympathetic nerve densities in the left hemisphere than in the right. When stroke-prone rats were compared, in some areas of the cerebrovasculature, rats fed the high-K+ diet had greater mean sympathetic nerve densities than those fed the low-K+ diet. On the other hand, stroke-resistant and stroke-prone rats fed the low-K+ diet exhibited comparable sympathetic nerve densities in most cerebral arteries studied. I conclude that the small increases in cerebrovascular sympathetic nerve density observed in stroke-prone spontaneously hypertensive rats fed the high-K+ diet would be of minor benefit in retarding stroke development. Moreover, differences in sympathetic nerve density could not account for the different incidences of stroke observed between stroke-prone and stroke-resistant spontaneously hypertensive rats. (Stroke 1990;21:785-789)
SPSHR subjected to dietary manipulations and between SPSHR and SRSHR.

Materials and Methods

The 24 male SPSHR and the eight male SRSHR were bred at McMaster University’s Health Science Center. The SPSHR colony was started in 1985 by breeding rats obtained from Dr. H. Ito, Kinki University, Osaka, Japan, whereas the SRSHR colony has been bred for the last 10 years at McMaster University. Both SPSHR and SRSHR were weaned at 5–6 weeks of age. At weaning, male SPSHR in each litter were assigned to one of two groups. One group of 11 SPSHR as well as all eight SRSHR were fed a Japanese-style stroke-inducing diet (Zeigler Brothers, Inc., Gardners, Pennsylvania) containing 4% NaCl and 0.75% K⁺ (low-K⁺ diet), whereas the other group of 11 SPSHR were fed the same diet containing 2.11% K⁺ (high-K⁺ diet).

All rats were killed when they were 14–15 weeks of age. Their blood pressures were measured using a tail cuff compression method, and they were anesthetized with 65 mg/kg sodium pentobarbital and then exsanguinated by cutting the aortic arch. Their brains were removed from the skulls and placed in oxygenated Krebs’ solution. The cerebral blood vessels indicated in Figure 1 were dissected from the brains and placed in separate vials containing Krebs’ solution. The sympathetic nerves were prepared for fluorescent microscopy using the glyoxylic acid technique outlined by Furness and Costa. The cerebral arteries were viewed with a Zeiss fluorescent microscope (Thornwood, New York) at ×250 magnification and photographed using color slide film. Sympathetic nerves appeared as green-yellow fibers forming a plexus on the adventitial border of the artery.

To test whether the innervation was sympathetic, the right and left superior cervical sympathetic ganglia were surgically removed from the two remaining SPSHR. One week after surgery glyoxylic acid treatment of the cerebrovasculature of these rats indicated absence of a fluorescent periartrial nerve plexus.

Adventitial sympathetic nerve density was morphometrically analyzed using a projection unit produced by Wild Leitz USA, Inc. (Rockleigh, New Jersey). Color photographs of the arteries were projected onto a screen containing a rectangular grid (31.6×22.5 cm) containing 21 vertical and 15 horizontal lines forming a 315-point matrix. The number of times nerves crossed a horizontal or vertical line (In) and the number of times arteries touched a point (Pa) were recorded. Density (length of nerve plexus per unit of adventitial surface area) was calculated using a modification of the formula originally outlined by Weibel as (1.5708×In/LL)(Pa/Pa), where Pa is the total number of points in the matrix (315) and LL is the total length of the lines in the matrix (17,037 μm at projection magnification).

Stroke was assessed by two methods (see Reference 8). Focal areas of hemorrhage on the brain surface were counted, and the amount of brain surface involved in hemorrhage was graded subjectively on a 0–4 scale (0, no hemorrhage; 4, severe involvement) by the same observer. For each method, mean±SEM was calculated for each group.

The unpaired two-tailed t test was used to compare SPSHR fed the low- and high-K⁺ diets and to compare SPSHR and SRSHR fed the low-K⁺ diet. The paired t test was used to compare differences between sympathetic nerve densities of the right and left hemispheres with the null hypothesis of no difference in both SPSHR and SRSHR. The level of significance was chosen to be p<0.05.

Table 1. Physical Characteristics of SPSHR and SRSHR Fed Low- and High-K⁺ Diets

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Dietary K⁺ (wk)</th>
<th>Age (wk)</th>
<th>Weight (g)</th>
<th>Blood pressure (mm Hg)</th>
<th>Rats exhibiting cerebral hemorrhage</th>
<th>Brain areas involved Mean±SEM</th>
<th>Stroke severity index (0–4 scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPSHR</td>
<td>11</td>
<td>0.75%</td>
<td>14.3±0.4</td>
<td>224±11</td>
<td>227±11</td>
<td>11</td>
<td>3.2±0.6</td>
<td>1–7</td>
</tr>
<tr>
<td>SPSHR</td>
<td>11</td>
<td>2.11%</td>
<td>14.3±0.2</td>
<td>244±3</td>
<td>227±6</td>
<td>2†</td>
<td>0.3±0.2*</td>
<td>0–2</td>
</tr>
<tr>
<td>SRSHR</td>
<td>8</td>
<td>0.75%</td>
<td>14.3±0.1</td>
<td>278±5*</td>
<td>214±5</td>
<td>0</td>
<td>0*</td>
<td>0*</td>
</tr>
</tbody>
</table>

SPSHR, stroke-prone spontaneously hypertensive rats; SRSHR, stroke-resistant spontaneously hypertensive rats. Data are mean±SEM. *p<0.05 different from SPSHR fed low-K⁺ diet by unpaired two-tailed t test.
†Pinpoint hemorrhages.
Smeda  Cerebral Sympathetic Innervation in SHR  787

Table 2. Density of Adventitial Sympathetic Nerves in Cerebrovasculature of SPSHR and SRSHR Fed Low- or High-K+ Stroke-Inducing Diets

<table>
<thead>
<tr>
<th>Artery</th>
<th>SPSHR Low-K+ diet</th>
<th>SPSHR High-K+ diet</th>
<th>SRSHR Low-K+ diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertebrobasilar system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L vertebral</td>
<td>3.11±0.40</td>
<td>3.42±0.17</td>
<td>3.07±0.49</td>
</tr>
<tr>
<td>Basilar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior section</td>
<td>3.13±0.13</td>
<td>3.75±0.20*</td>
<td>3.27±0.30</td>
</tr>
<tr>
<td>Anterior section</td>
<td>2.67±0.41</td>
<td>2.95±0.24</td>
<td>2.41±0.36</td>
</tr>
<tr>
<td>L superior cerebellar</td>
<td>3.58±0.36</td>
<td>3.17±0.34</td>
<td>2.37±0.24*</td>
</tr>
<tr>
<td>Circle of Willis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior-middle cerebral junction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>4.68±0.48</td>
<td>5.37±0.43</td>
<td>5.88±0.38</td>
</tr>
<tr>
<td>R</td>
<td>3.52±0.41</td>
<td>4.84±0.33*</td>
<td>5.16±1.08</td>
</tr>
<tr>
<td>Internal carotid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>3.93±0.53</td>
<td>4.63±0.35</td>
<td>5.45±0.20*</td>
</tr>
<tr>
<td>R</td>
<td>4.02±0.39</td>
<td>3.92±0.69</td>
<td>4.78±1.33</td>
</tr>
<tr>
<td>Posterior communicating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>4.46±0.39</td>
<td>4.62±0.23</td>
<td>3.35±0.19*</td>
</tr>
<tr>
<td>R</td>
<td>2.38±0.72</td>
<td>3.14±0.54</td>
<td>1.32±0.35</td>
</tr>
<tr>
<td>Posterior cerebral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>3.53±0.46</td>
<td>3.86±0.37</td>
<td>2.48±0.25</td>
</tr>
<tr>
<td>R</td>
<td>3.59±0.29</td>
<td>2.97±0.24</td>
<td>1.80±0.33*</td>
</tr>
<tr>
<td>Middle cerebral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal segment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>3.18±0.43</td>
<td>4.30±0.23*</td>
<td>2.83±0.23</td>
</tr>
<tr>
<td>R</td>
<td>2.54±0.50</td>
<td>3.32±0.32</td>
<td>2.59±0.24</td>
</tr>
<tr>
<td>Middle segment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>4.19±0.53</td>
<td>4.21±0.18</td>
<td>3.32±0.24</td>
</tr>
<tr>
<td>R</td>
<td>2.27±0.38</td>
<td>2.99±0.42</td>
<td>2.78±0.50</td>
</tr>
<tr>
<td>Distal segment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>4.05±0.29</td>
<td>4.65±0.29</td>
<td>3.87±0.60</td>
</tr>
<tr>
<td>R</td>
<td>2.89±0.02</td>
<td>3.97±0.28*</td>
<td>3.53±0.48</td>
</tr>
<tr>
<td>Anterior cerebral artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>5.79±0.39</td>
<td>6.21±0.36</td>
<td>5.59±0.61</td>
</tr>
<tr>
<td>R</td>
<td>2.97±0.54</td>
<td>2.64±0.32</td>
<td>2.57±0.37</td>
</tr>
</tbody>
</table>

SPSHR, stroke-prone spontaneously hypertensive rats; SRSHR, stroke-resistant spontaneously hypertensive rats; L, left; R, right. Data are mean±SEM length of nerve plexus per unit adventitial surface area x 10^-4 (μm/μm^2).

*p<0.05 different from SPSHR low-K+ diet by unpaired two-tailed t test.

n values for each determination: 5–8 rats, SPSHR low-K+ diet; 5–9 rats, SPSHR high-K+ diet; and 5 or 6 rats, SRSHR low-K+ diet.

Results

Table 1 summarizes the physical characteristics of the rats. All hemorrhages in the SPSHR fed the low-K+ diet occurred in the cerebral hemispheres; none occurred in the cerebellum or brainstem (pons, medulla, or spinal cord). The pinpoint hemorrhages in the SPSHR fed the high-K+ diet all occurred in the parietal region. No SRSHR fed the low-K+ diet exhibited evidence of cerebral hemorrhage.

Table 2 lists mean±SEM sympathetic nerve densities of various arteries for the three groups. Marked regional differences were observed. Highest densities were found in the anterior cerebral arteries; density decreased markedly in the anterior cerebral artery trunk, and its distal regions were virtually void of sympathetic innervation (data not shown). This feature was common to both SPSHR and SRSHR. Density remained quite high throughout the internal carotid artery segment of the circle of Willis and decreased in the more distal regions of the posterior cerebral arteries. This decrease was more pronounced in SRSHR than in SPSHR. Sympathetic nerve density also decreased in the middle cerebral artery from its junction with the anterior cerebral artery at the circle of Willis to its proximal segment; from there density remained constant over the areas measured. This decrease was more pronounced in SRSHR than in SPSHR. Very distal segments of the middle cerebral arteries (not shown) were virtually devoid of sympathetic innervation. The vertebobasilar system was less densely innervated than the circle of Willis. The posterior section of the basilar artery...
The cerebral and noncerebral vascular beds of SRSHR have greater densities of sympathetic nerves than those of Wistar-Kyoto normotensive rats. A quantitative increase in sympathetic innervation could protect the cerebrovasculature of SRSHR from hemorrhage during hypertension by either trophically influencing the vasculature to develop a thickened vascular wall. This could make the blood vessels more resistant to rupture, and promote a greater degree of cerebrovascular vasoconstriction, which in turn could prevent the hyperperfusion of smaller blood vessels of the brain.

Compared with SPSHR fed the low-K⁺ diet, many cerebral arteries of SPSHR fed the high-K⁺ diet had higher mean sympathetic nerve densities; in some arteries the differences were significant. Such changes in nerve density may be of benefit in protecting the cerebral vasculature from stroke and could have been potentially modified further if an even higher K⁺ concentration (i.e., 4%) had been included in the diet. However, in view of the modest magnitude of such changes in some cerebral arteries and the variability observed among rats within a group, the potential benefit of such changes in nerve density with respect to stroke must be questioned. Furthermore, if such changes were important in retarding stroke development in SPSHR fed the high-K⁺ diet, it is difficult to explain why SRSHR are virtually protected from stroke despite the fact that their sympathetic nerve densities are comparable to that in SPSHR fed the low-K⁺ diet. It is possible that the capacity of the sympathetic nervous system to change quantitatively is limited in both SRSHR and SPSHR. The sympathetic nervous system of spontaneously hypertensive rats has proven to be quite resistant to immunologic and chemical agents that readily produce sympathectomy in normal rats. In view of this, changes in sympathetic nerve density possible in response to dietary manipulations might also be quite limited.

An interesting observation was that the middle cerebral artery and certain points along the circle of Willis (at the anterior–middle cerebral artery junction and the posterior communicating artery) contained a greater sympathetic nerve density in the left than in the right hemisphere. This bilateral difference existed only in SPSHR (regardless of diet), and its significance is unknown. It is tempting to relate this difference in cerebrovascular sympathetic innervation to that in stroke incidence for SPSHR and SRSHR. However, such a relation is difficult to establish. If sympathetic nerves were actively involved in contracting the cerebrovasculature, the presence of more nerves would favor a situation in which cerebral blood flow might be shunted to the right at the expense of the left hemisphere. Hemorrhagic stroke might therefore be favored in the right over the left hemisphere. However, I found the incidence of hemorrhage to be equal in the right and left hemispheres of SPSHR fed the low-K⁺ diet. Furthermore, my previous study and a study by Yamori et al have indicated that when a bilateral difference in the incidence of hemorrhagic stroke exists, stroke occurs more often in the left than in the right side of the cerebrum.

The sympathetic nervous system might still play a role in protecting the cerebrovasculature of SPSHR fed a high-K⁺ diet or SRSHR from stroke; however, such protection may be due to functional as opposed to quantitative differences. In this regard, Mueller et

<table>
<thead>
<tr>
<th>Artery</th>
<th>SPSHR</th>
<th>SRSHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior–middle cerebral junction</td>
<td>1.06±0.33*</td>
<td>−0.12±1.46</td>
</tr>
<tr>
<td>Middle cerebral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal segment</td>
<td>0.63±0.27*</td>
<td>0.41±0.70</td>
</tr>
<tr>
<td>Middle segment</td>
<td>1.44±0.31*</td>
<td>−0.24±0.16</td>
</tr>
<tr>
<td>Distal segment</td>
<td>0.82±0.27*</td>
<td>0.02±1.09</td>
</tr>
<tr>
<td>Internal carotid</td>
<td>−0.15±0.47</td>
<td>0.88±1.34</td>
</tr>
<tr>
<td>Posterior communicating</td>
<td>1.59±0.57*</td>
<td>2.00±0.39*</td>
</tr>
<tr>
<td>Posterior cerebral</td>
<td>0.61±0.29</td>
<td>0.90±0.53</td>
</tr>
</tbody>
</table>

SPSHR, stroke-prone spontaneously hypertensive rats; SRSHR, stroke-resistant spontaneously hypertensive rats. Data are mean±SEM (left–right) length of nerve plexus per unit adventitial surface area x 10⁻² (µm²/µm²).

* p<0.05 different from 0 by paired t test.

n values for each determination: 6–15 SPSHR; 4 SRSHR.
all observed that the cervical sympathetic nerve activity of SPSHR was quite similar to that of Wistar-Kyoto normotensive rats and lower than that of SRSHR. It was suggested that the lack of elevated cervical sympathetic activity in SPSHR as opposed to SRSHR makes the former more susceptible to stroke.

References

KEY WORDS • cerebral hemorrhage • sympathetic nervous system • rats
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J S Smeda

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