Peptide-Containing Nerves Around Blood Vessels of Stroke-Prone Spontaneously Hypertensive Rats

ROBERT M.K.W. LEE, MASATO NAGAHAMA, RICHARD MCKENZIE, AND EDWIN E. DANIEL

SUMMARY The distribution and density of nerves containing vasoactive intestinal polypeptide, substance P, and neuropeptide Y around the cerebral and peripheral blood vessels of stroke-prone spontaneously hypertensive rats (SHRSP) and normotensive Wistar-Kyoto rats (WKY) were studied using an indirect immunofluorescence technique. Neonatal sympathectomy of SHRSP with anti-nerve growth factor and guanethidine was also carried out to study the effect of sympathectomy on the distribution of these nerves. Vasoactive intestinal polypeptide nerve density was higher in the veins and superior mesenteric artery of SHRSP than of WKY and lower in the cerebral arteries of SHRSP than of WKY, but no difference was found in the muscular mesenteric arteries. Sympathectomy reduced the density of these nerves in all the peripheral vessels but had little effect on the cerebral arteries. Density of substance P nerves was similar between SHRSP and WKY in the peripheral vessels but higher in the cerebral arteries of WKY than of SHRSP. Sympathectomy reduced the density of these nerves in the peripheral vessels but increased the density in some cerebral arteries of SHRSP. Neuropeptide Y nerve density was higher in the peripheral blood vessels of SHRSP than of WKY, and no difference was found in the cerebral arteries. Sympathectomy almost completely removed these nerves in the peripheral vessels but had no effect on the cerebral arteries. We suggest that some of the differences in nerve density between SHRSP and WKY, especially those in the peripheral blood vessels, may be related to the development of hypertension in the SHRSP.

KEY WORDS • hypertension • mesenteric arteries • cerebral arteries • substance P • vasoactive intestinal polypeptide • neuropeptide Y

THE function of peptide-containing nerves around the blood vessels is generally unclear. Therefore, whether these nerves do play a role in the etiology of hypertension remains to be elucidated. For example, in Wistar rats, nerves containing dense substance P (SP) were found around superior mesenteric arteries. A sensory function has been suggested for SP in the vascular nerves, yet, neonatal treatment of spontaneously hypertensive rats (SHR) with capsaicin, which presumably depletes neuronal SP, also prevented hypertension development in these rats, suggesting SP may have other functions besides a sensory function. In our recent study on the effect of sympathectomy on hypertension development and vessel wall morphology, we found that despite the destruction of sympathetic nerves with our procedure, other nerve types were still present.

The primary aim in our present study was to locate and identify various peptide-containing nerves from the cerebral and peripheral blood vessels of stroke-prone SHR (SHRSP) and Wistar-Kyoto rats (WKY). Our intent was to relate the distribution of these nerves to the known action of the peptides so that we could gain some understanding concerning the possible involvement of these nerves in the development of hypertension.

Materials and Methods

SHRSP and WKY were obtained from rat colonies currently maintained at the McMaster University Animal Quarters. Male rats, 6 months of age or older, were used. Blood vessels were fixed by perfusion under pressure with 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer, pH 7.3, at room temperature for 20 minutes, followed by immersion fixation in the same fixative for another 2 hours at 4°C.

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These blood vessels were processed for the immunohistochemical demonstration of nerves containing SP, vasoactive intestinal polypeptide (VIP), and neuropeptide Y (NPY) using the method of Coons et al. The SP, VIP, NPY, and antisera to SP and VIP were purchased from Sigma Chemical (St. Louis, MO, USA). Antiserum to NPY was obtained from Professor N. Yanaihara of Japan. Antisera were used at a dilution of 1:1000. The site of the antigen-antibody reaction was revealed by antirabbit IgG labeled with fluorescein isothiocyanate (Sigma Chemical). Both nonspecific (buffer only) and specific controls for each specific neuropeptide were used. Whole mounts of blood vessels in glycerol were examined with a Zeiss (San Antonio, TX, USA) fluorescence microscope. A semi-quantitative scoring method, based on the density of nerves visible in each of the several viewing fields with 25 × objective, was noted for each vessel type. For each viewing field a score of 0 to 4 was assigned as follows: 0 = absent; 0.1 to 1.0 = one or two nerves found occasionally (e.g., 1 out of 10 viewing fields = 0.1); 2 = moderately dense in some and sparsely innervated in other viewing fields; 3 = moderately dense in all viewing fields; 4 = densely innervated in all viewing fields. This type of scoring is similar to that used by other investigators (e.g., References 1, 5) in the study of vascular peptide-containing nerves in mammals.

Neonatal sympathectomy with guanethidine and anti-nerve growth factor was carried out as outlined in Lee et al.3

Results

Systolic blood pressure of SHRSP and WKY was 244 ± 11 mm Hg (mean age ± SEM, 37 ± 5 weeks) and 125 ± 7 mm Hg (age, 30 ± 5 weeks), respectively. Sympathectomy reduced the blood pressure of SHRSP to 160 ± 2 mm Hg (age, 33 ± 2 weeks). Mean body weight of control and treated SHRSP was similar (304 g). The mean body weight of WKY was slightly lower (285 g). The distribution of various peptide-containing nerves is summarized in Table 1. Peptide-containing nerves were absent in the aorta and common carotid arteries.

### Vasoactive Intestinal Polypeptide

Among the peripheral blood vessels, the density of VIP nerves was generally higher in the SHRSP than in the WKY except in the large and small mesenteric arteries, where VIP nerve density was similar in SHRSP and WKY. In the cerebral arteries, the VIP nerve density was higher in the circle of Willis and superior cerebellar arteries of WKY than of SHRSP. With the exception of some cerebral arteries, neonatal sympathectomy reduced the density of VIP nerves in the mesenteric arteries and the veins from SHRSP. In both SHRSP and WKY, VIP nerve density was higher in the small mesenteric arteries than in the superior mesenteric and large mesenteric arteries. The veins in WKY were either not innervated (large mesenteric) or poorly innervated. Among the cerebral arteries of SHRSP and WKY, some were highly innervated (e.g., circle of Willis; Figure 1A), whereas others were poorly innervated (e.g., middle cerebral; Figure 1B).

### Substance P

The distribution of SP nerves in the three types of mesenteric arteries was similar in SHRSP and WKY. Sympathectomy reduced the density of SP nerves in the large and small mesenteric arteries (see Figure 1, C and D). The veins were poorly innervated. Sympathectomy also reduced the density of SP nerves in the superior and large mesenteric veins. Among the cerebral arteries, the density of SP nerves was usually lower in the SHRSP than in the WKY. Sympathectomy either increased the SP nerves in the SHRSP to a density

### Table 1. Density of Immunoreactive Fibers in Various Blood Vessels

<table>
<thead>
<tr>
<th>Vessel</th>
<th>SHRSP</th>
<th>SHRSP (SX)</th>
<th>WKY</th>
<th>SHRSP</th>
<th>SHRSP (SX)</th>
<th>WKY</th>
<th>SHRSP</th>
<th>SHRSP (SX)</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Common carotid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral arteries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior mesenteric</td>
<td>1.67</td>
<td>0.75</td>
<td>0.88</td>
<td>1.75</td>
<td>1.46</td>
<td>1.30</td>
<td>0.83</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Large mesenteric</td>
<td>1.12</td>
<td>0.50</td>
<td>1.40</td>
<td>2.25</td>
<td>1.63</td>
<td>2.75</td>
<td>3.38</td>
<td>0.17</td>
<td>2.75</td>
</tr>
<tr>
<td>Small mesenteric</td>
<td>2.50</td>
<td>0</td>
<td>2.0</td>
<td>2.5</td>
<td>2.10</td>
<td>2.25</td>
<td>3.0</td>
<td>0</td>
<td>1.88</td>
</tr>
<tr>
<td>Veins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal</td>
<td>1.67</td>
<td>0.17</td>
<td>0.10</td>
<td>0.78</td>
<td>0.67</td>
<td>0.27</td>
<td>2.0</td>
<td>0.25</td>
<td>2.0</td>
</tr>
<tr>
<td>Superior mesenteric</td>
<td>1.50</td>
<td>1.0</td>
<td>1.0</td>
<td>1.25</td>
<td>0</td>
<td>1.17</td>
<td>2.67</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>Large mesenteric</td>
<td>1.0</td>
<td>0.33</td>
<td>0</td>
<td>1.25</td>
<td>0.25</td>
<td>0.67</td>
<td>3.0</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>Cerebral arteries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Basilar</td>
<td>1.06</td>
<td>0.57</td>
<td>0.76</td>
<td>0.73</td>
<td>1.5</td>
<td>2.5</td>
<td>1.0</td>
<td>0.33</td>
<td>0.25</td>
</tr>
<tr>
<td>Circle of Willis</td>
<td>1.57</td>
<td>1.67</td>
<td>2.83</td>
<td>1.33</td>
<td>2.83</td>
<td>2.83</td>
<td>1.67</td>
<td>1.83</td>
<td>2.17</td>
</tr>
<tr>
<td>Superior cerebellar</td>
<td>1.17</td>
<td>0.33</td>
<td>3.0</td>
<td>0.33</td>
<td>0.17</td>
<td>0.17</td>
<td>1.0</td>
<td>0.33</td>
<td>0.75</td>
</tr>
<tr>
<td>Middle</td>
<td>1.50</td>
<td>1.0</td>
<td>0.5</td>
<td>0.3</td>
<td>2.5</td>
<td>2.33</td>
<td>1.5</td>
<td>0.93</td>
<td>2.0</td>
</tr>
<tr>
<td>Anterior</td>
<td>2.0</td>
<td>2.0</td>
<td>2.33</td>
<td>1.25</td>
<td>4.0</td>
<td>2.67</td>
<td>1.0</td>
<td>1.0</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Scoring system is described in Materials and Methods. Numbers in parentheses refer to the number of animals involved. (SX) = sympathectomized.
similar to that of WKY (e.g., circle of Willis and middle cerebral arteries; Figure 1, E and F) or higher than that of WKY (anterior cerebral; Figure 2A). In the cerebral arteries from SHRSP and WKY, the density of SP nerves decreased dramatically in the side branches (Figure 2B). In the basilar artery, SP nerve density was higher at the proximal end than at the distal end.

Neuropeptide Y

NPY nerve density in the superior mesenteric artery was similar in SHRSP and WKY. In the large and small mesenteric arteries, however, it was higher in the SHRSP than in the WKY. Nerve density was also higher in the large mesenteric artery than in the small mesenteric artery in both SHRSP and WKY (Figure 2, C and D). Sympathectomy almost completely eliminated the presence of the NPY nerves in the peripheral vessels of SHRSP. NPY nerves in the portal veins of SHRSP (Figure 2E) and WKY were similar in density, but they were absent in the superior mesenteric and large mesenteric veins of WKY. Their density was generally low in the cerebral arteries (Figure 2F), and there was no difference between SHRSP and WKY. Sympathectomy reduced the density of NPY nerves slightly in some cerebral arteries, but generally did not appear to have much affect on NPY nerve density.

Discussion

The major findings in this study were that the density of peptide-containing nerves around the peripheral and cerebral arteries of SHRSP is different from that in WKY, and that neonatal sympathectomy alters the density of these nerves in the SHRSP. These observations may be relevant to our understanding of the etiology of hypertension in SHRSP, as discussed below.

Vasoactive Intestinal Polypeptide

Under in vitro conditions, VIP has been shown to cause relaxation of blood vessels. VIP is noted to be a potent hypotensive agent under in vivo conditions through its effects on resistance and capacitance vessels. It is conceivable that a deficiency of these nerves in the blood vessels may be one of the causal factors of hypertension. However, our results showed that VIP nerve density was similar between SHRSP and WKY in the muscular, reactive arteries (large and small mesenteric), thus suggesting that VIP nerves are probably not involved in the primary cause of hypertension. VIP nerve density in the veins and superior mesenteric arteries of SHRSP was actually higher than in WKY. It is possible that increased VIP nerve density in these vessels may be a secondary response to
hypertension. Further studies of VIP nerve density in young SHRSP before the establishment of stable hypertension will be useful in determining whether changes in VIP nerve density are secondary to hypertension development. A reduction in VIP nerve density by neonatal sympathectomy was unexpected. Previous studies of various mammals showed that surgical or chemical sympathectomy had no effect on the VIP nerves, and that these nerves are closely associated with the cholinergic nerves. It is likely that reduction of VIP nerve density in sympathectomized SHRSP was related to the reduction of blood pressure as discussed above.

Substance P

Among the peripheral blood vessels, the density of SP fibers was similar in SHRSP and WKY, suggesting that these fibers may not be involved in hypertension development in SHRSP. Whether SP nerves have a vasomotor function is unclear. Barja et al. found that SP produced constriction of the veins but was basically without effect on arteries, except the carotid artery in which a dose-dependent relaxation was observed. This is despite the fact that SP nerves were absent around the carotid artery. A sensory function is generally assigned to SP nerves. The mechanism through which neonatal capsaicin treatment prevented the development of hypertension in SHR is unclear, but it is unlikely that SP nerves were involved, at least not in the peripheral blood vessels. It is possible that other neurotransmitters or neuromodulators are involved. Recent studies have shown that capsaicin is capable of depleting additional neuropeptides from primary afferents, which include corticotropin releasing factor, calcitonin gene-related peptide, galanin, and substance K (neurokinin A; see Reference 7).

Reduction of SP nerves in the peripheral blood vessels of SHRSP by neonatal sympathectomy is contrary to the results of Barja et al., who reported that treatment of adult Wistar rats with 6-hydroxydopamine had no effect on SP fibers. Differences in the treatment method may be the cause of such variation. In our study, newborn SHRSP were treated with antibody to nerve growth factor for 6 days, followed by daily treatment with guanethidine for another 3 weeks. Since SP nerves are highly dependent on nerve growth factor for nerve development and maintenance of neural protein synthesis, it is not surprising that their density was affected by sympathectomy.

The significance of a higher density of SP nerves in the cerebral arteries of WKY than of SHRSP is not known. It may be related to lower blood pressure in the WKY, because lowering blood pressure by sympathectomy increased the density of SP nerves in SHRSP to the level of WKY. However, the effect of sympathectomy cannot be discounted. Further studies involving other means of lowering the blood pressure (e.g., vasodilators) should be carried out in order to find out whether SP nerve density in the cerebral arteries is related to pressure.

Neuropeptide Y

NPY has been shown to coexist with norepinephrine in sympathetic nerves. In SHR, the parental stock of SHRSP, adrenergic innervation of large and small mesenteric arteries was denser than in WKY. Therefore, it is not surprising that NPY nerves in SHRSP were also found to be denser than in WKY. The NPY nerve density in the veins was also higher in SHRSP than in WKY. No information is available on the density of adrenergic nerves in the veins of SHRSP.

The sympathectomy procedure that we used had little effect on the NPY nerves in the cerebral arteries of SHRSP. This is consistent with our previous observation that sympathetic nerves in the cerebral arteries of SHR were not affected by our procedure. It is possible that our procedure is not effective against nerves that are already established, because at birth, the cerebral arteries of SHR are already innervated, whereas innervation in the mesenteric arteries occurs after birth (E.I. Mangiarua and R.M.K.W. Lee, unpublished observations, 1987).

In conclusion, our study showed that VIP, SP, and NPY nerves are present in the peripheral and cerebral blood vessels of SHRSP and WKY, and there are some differences in the density of these nerves between SHRSP and WKY. Lowering of the blood pressure in sympathectomized SHRSP was probably achieved through the removal of the influence of hyperinnervated sympathetic and NPY nerves in the peripheral blood vessels.

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References

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