Effect of Cerebral Arterial Occlusion on Cerebral Perivascular Innervation: A Histochemical and Immunohistochemical Study in the Rat

Takayuki KUROYANAGI, Hideaki HARA, and Shigeaki KOBAYASHI

Department of Neurosurgery, Shinshu University School of Medicine, Matsumoto, Nagano

Abstract

The effect of acute cerebral occlusion on the distribution of cerebral perivascular nerves containing catecholamine, neuropeptide Y, and vasoactive intestinal peptide was studied in the three commonly used rat models of cerebral ischemia: Unilateral permanent middle cerebral artery (MCA) occlusion induced with an intraluminal thread technique; unilateral MCA occlusion produced by extraluminal electrocoagulation of the MCA; and transient arterial occlusion of the whole brain induced extracranially by the four-vessel clasp occlusion method for 30 minutes. Animals were sacrificed 3 days after occlusion and the distribution of the perivascular nerves of the MCA studied. Intraluminally occluded MCAs showed a similar distribution of perivascular nerves to those of contralateral and sham-operated MCAs. Extraluminally occluded MCAs demonstrated a marked decrease in perivascular nerves containing catecholamine and peptides while the contralateral MCAs showed normal distribution of the nerves. Extracranial occlusion caused no discernible change in the distribution of perivascular nerves in occluded and sham-operated animals. This study indicates that the different methods of cerebral arterial occlusion have variable effects on the perivascular innervation. Arterial occlusion induced by intraluminal or transient extracranial procedures does not impair cerebral perivascular innervation at least up to 3 days post-occlusion. In contrast, cerebral arterial occlusion by extraluminal electrocoagulation diminishes the perivascular nerves around the occluded cerebral artery.

Key words: catecholamine, cerebral arterial occlusion, cerebral perivascular innervation, neuropeptide Y, vasoactive intestinal peptide

Introduction

Innervation of cerebral blood vessels by nerves containing catecholamine and neuropeptides occurs in several species. The function of cerebral perivascular innervation is not fully understood, but physiological studies show that the sympathetic nerves may modulate the range of autoregulation to maintain the cerebral circulation despite variations in systemic blood pressure. Recently, the trigemino-cerebrovascular and sphenopalatine-cerebrovascular nervous systems have also been implicated in neurogenic control of cerebral circulation. Cerebral ischemia results in parenchymal neural damage which leads to changes in the metabolism of neurotransmitters. Abnormal excitatory neurotransmitter release is probably related to ischemic neural damage. However, the function of cerebral perivascular nerves in the pathophysiology of cerebral ischemia and occluded vessels has received little attention. Here, we report our study of the effect of vessel occlusion on the distribution of cerebral perivascular nerves containing catecholamine, neuropeptide Y (NPY), and vasoactive intestinal peptide (VIP) in the three most common models of rat cerebral occlusion.

Materials and Methods

Forty-six adult male Sprague-Dawley rats weighing 300-350 g were used in the experiments. All animals were allowed access to food and water ad libitum under a 12-hour light and 12-hour dark cycle. Four rats provided the normal control group. Three groups of animals underwent induced cerebral ischemia according to different surgical procedures.
General anesthesia for surgery was maintained with 1.5–4% halothane.

Group 1: Thirteen animals underwent acute cerebral occlusion induced with a nylon thread technique. Rats were placed in the supine position and a median neck incision made under the operating microscope. The common carotid and external carotid arteries on the right were carefully exposed and coagulated with bipolar forceps. A 3 cm 3-0 monofilament nylon thread was introduced into the common carotid artery via a small hole made in the arterial wall and advanced intracranially to the anterior cerebral artery to block blood flow into the middle cerebral artery (MCA). Care was taken not to damage the superior cervical ganglion or internal carotid nerves. Neither the pterygopalatine nor vertebral artery was occluded. The intraluminal suture was left in place for 3 days in all animals. Five sham-operated animals received the same procedure but the nylon thread was not introduced. This method produced consistently unilateral cerebral ischemia as shown previously.

Group 2: Nine rats underwent MCA occlusion induced by a subtemporal approach. The right MCA was electrocoagulated between the cortical branches to the rhinal cortex and the lateral striate artery. Five sham-operated rats received the same procedure but the MCA was not coagulated.

Group 3: Five rats underwent transient arterial occlusion of the whole brain. The vertebral arteries were electrocauterized through the alar foramen of the first cervical vertebra and reversible clasps placed loosely around the common carotid arteries. Twenty-four hours later, the awake rats were restrained and the carotid clasps tightened for 30 minutes. Five animals in which both vertebral but not carotid arteries were occluded served as sham-operated group.

Three days after surgery, the animals in each group were anesthetized with pentobarbital (40 mg/kg, i.p.) and decapitated. The brain was immediately removed together with the major cerebral arteries at the base of the brain. The bilateral MCAs were carefully dissected in ice-cold phosphate buffered saline under the operating microscope. The MCAs were cut into 3–4 mm segments and processed for catecholamine histofluorescence and peptide immunohistochemical examination. The MCA segments from Groups 1 and 2 for immunohistochemical analysis were distal to the occluded site. Infarction size was measured using a coronal brain section including the frontoparietal cortex, caudate putamen, and medial preoptic area and placed in 2% 2,3,5-triphenyltetrazolium chloride (TTC) monohydrate at 37°C for 30 minutes.

The glyoxylic acid-induced fluorescence method was used for catecholamine fluorescence histochemistry. The MCA specimens were immersed in 2% glyoxylic acid solution (pH 7.0) and then heated for 5 minutes at 95°C. For NPY and VIP immunohistochemistry, the arteries were immersed in ice-cold fixative containing 2% formaldehyde and 15% saturated picric acid solution in pH 7.2 0.1 M phosphate buffer. All specimens were left in the fixative for 24 hours, followed by rinsing in Tyrode's solution containing 10% sucrose at 4°C for 48 hours. The arteries were then mounted on glass slides and processed for immunohistochemical evaluation of NPY and VIP using an indirect immunofluorescence technique. The specimens were incubated for 16 hours at 4°C in rabbit anti-NPY (Incstar, Stillwater, Minn., U.S.A.; diluted 1:300) or VIP antisera (Incstar; diluted 1:500). After washing, specimens were then incubated at room temperature for 60 minutes in fluorescein-conjugated goat anti-rabbit immunoglobulin (Cooper Biomedical, Malvern, Pa., U.S.A.; diluted 1:100). The specificity of the anti-NPY and anti-VIP antibodies were tested by preincubation with 10⁻³ M concentration of NPY or VIP, respectively, for 24 hours at 4°C prior to use, or omission of the primary antibody, both of which resulted in prevention of nerve immunostaining. The anatomical nomenclature for the rat brain was adapted from the atlas of Paxinos and Watson.

Nerves containing catecholamine and peptides on the MCA wall were viewed and photographed at magnification ×125 using a fluorescence microscope (Nikon, Tokyo). The nerve densities were estimated from photographs taken at uniform exposure and development times. Five standardized areas in each specimen were sampled. The number of nerve fibers crossing the diagonal of a 0.2 mm square in each of the five standardized areas was counted. The mean number of nerves in each 0.04 sq mm area was expressed as an overall mean ± SEM. Data were compared using the unpaired two-tailed Student's t-test. Significance was accepted at p < 0.05.

Results

The normal animals demonstrated numerous catecholamine histofluorescence and NPY- and VIP-immunoreactive perivascular nerve fibers in the MCA walls. The sham-operated rats in all groups showed similar catecholamine histofluorescence and peptide immunoreactivity in the perivascular nerve fibers (Table 1).

Neurum Med Chir (Tokyo) 34, February, 1994
Table 1  Number of perivascular nerves containing catecholamine and peptides on the MCA wall

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Non-operated side</th>
<th>Operated side</th>
<th>Non-operated side</th>
<th>Operated side</th>
<th>Non-operated side</th>
<th>Operated side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 4)</td>
<td>5.8 ± 0.44</td>
<td></td>
<td>5.0 ± 0.44</td>
<td></td>
<td>4.9 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with occlusion (n = 13)</td>
<td>5.8 ± 0.23</td>
<td>5.4 ± 0.20</td>
<td>5.2 ± 0.14</td>
<td>5.1 ± 0.13</td>
<td>5.3 ± 0.05</td>
<td>4.7 ± 0.34</td>
</tr>
<tr>
<td>with sham operation</td>
<td>5.5 ± 0.39</td>
<td>5.4 ± 0.39</td>
<td>5.2 ± 0.35</td>
<td>5.3 ± 0.33</td>
<td>5.2 ± 0.30</td>
<td>5.0 ± 0.25</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with occlusion (n = 9)</td>
<td>5.4 ± 0.23</td>
<td>0.9 ± 0.23*</td>
<td>5.2 ± 0.18</td>
<td>0.6 ± 0.17*</td>
<td>5.3 ± 0.22</td>
<td>0.4 ± 0.11*</td>
</tr>
<tr>
<td>with sham operation</td>
<td>5.3 ± 0.07</td>
<td>5.6 ± 0.41</td>
<td>5.3 ± 0.40</td>
<td>5.4 ± 0.19</td>
<td>5.1 ± 0.34</td>
<td>5.0 ± 0.38</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with occlusion (n = 5)</td>
<td>5.6 ± 0.37</td>
<td></td>
<td>5.2 ± 0.32</td>
<td></td>
<td>5.2 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>with sham operation</td>
<td>5.6 ± 0.41</td>
<td></td>
<td>5.4 ± 0.42</td>
<td></td>
<td>5.2 ± 0.29</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. Values for Group 3 are the mean values of measurements from both hemispheres.
*Significance of data compared with normal rats (p < 0.01).

Discussion

The present study demonstrated that the distribution of the cerebral perivascular nerves containing catecholamine and peptides was unaltered by intraluminal (Group 1) or extracranial maneuvers (Group 3) causing MCA occlusion. In contrast, cerebral arterial occlusion by extraluminal electrocoagulation reduced the perivascular innervation of the occluded MCA (Group 2). These findings suggest that the methods of cerebral arterial occlusion have variable effects on the cerebral perivascular innervation which implies that interrupted blood flow in the cerebral artery does not influence the distribution of the cerebral perivascular nerves at least up to 3 days post-occlusion. This study examined relatively short post-occlusion periods since the general condition of Group 2 animals deteriorated over 7-10 days.

Since perivascular nerves containing catecholamine, VIP, and NPY are located in the adventitia and adventitia-media border of the cerebral arteries, methods of arterial occlusion such as electrocoagulation, ligation, and clipping of the cerebral arteries will easily damage the perivascular nerves together with the cerebral adventitia. This mechanical effect on the perivascular innervation was studied previously in the rat common carotid artery. The surgical procedure including clamping of vessel wall and removing superficial connective tissue from the vessel caused extensive damage to the sympathetic nerves innervating the artery.

VIP-immunoreactive nerves in the adventitia of the cerebral arteries arise from the sphenopalatine and otic ganglia, and the microganglia at the base of the brain. Catecholamine- and NPY-contain-
ing nerves around cerebral arteries originate peripherally, mostly from the superior cervical ganglion. Catecholamine and NPY coexist in the superior cervical ganglion cell and in the adrenergic cerebral perivascular nerve. The origins and sources of these nerves predict that the perivascular nerves will run along the cerebral arteries centrifugally and will be damaged by electrocoagulation distally. In contrast, Capra et al. produced cerebral infarction by clip occlusion of the MCA in the cat, finding that the unilateral occlusion induced loss of fluorescence in the bilateral cerebral perivascular nerves. The difference between this study and ours may be explained by the differences in surgical preparations and in species. Since subarachnoid hemorrhage may reduce cerebral perivascular innervation, we strictly excluded animals which demonstrated subarachnoid hemorrhage during electrocoagulation of the MCA.

Our study showed that the perivascular nerve fibers were not impaired by the intraluminal MCA occlusion, although ischemic damage was consistently induced in the brain parenchyma. We did not evaluate the collateral blood flow to the intraluminal-ly occluded MCA which might nourish the perivascular nerves. However, the area of paren-

Neurol Med Chir (Tokyo) 34, February, 1994
chymal ischemic damage suggests that the collateral circulation was as small as that in brain with extraluminally occluded MCA. This suggests that the perivascular nerves are more resistant to ischemic insult than the brain parenchyma, as suggested previously with special reference to the vasa vasorum and cerebrospinal fluid circulation.\textsuperscript{12,23}

The functional significance of the cerebral perivascular nerves in the pathophysiology of the ischemic brain still remains unclear. Recently, Kano \textit{et al.}\textsuperscript{10} suggested a protective role for the parasympathetic nerves in focal cerebral ischemia. Rat cerebral ischemia caused by MCA ligation followed by temporary common carotid artery occlusion demonstrated that chronic lesions in parasympathetic efferent nerves to the circle of Willis increased the infarction volume. Taken together with our results, the protective function of the parasympathetic innervation in their study is not a direct effect to the occluded vessels, but is apparently related to the activation of the non-occluded vessels which maintain the collateral blood flow, since extraluminally occluded vessels have no perivascular innervation. Further studies of an animal model using intraluminally occluded MCA. This suggests that the cerebral perivascular nerves in the pathophysiology of thromboembolic stroke are awaited. The surviving perivascular nerves in the wall of the intraluminally occluded cerebral artery may be therapeutically significant. If so, the cerebral perivascular nerves may act directly on the occluded cerebral artery. A recent physiological study demonstrated that electrical stimulation of the sphenopalatine ganglion increased cerebral blood flow in the ipsilateral parietal cortex associated with a rise in tissue \textit{PO}$_2$ and a decrease in tissue \textit{PCO}$_2$, suggesting the neurogenic vasodilatory potential of the sphenopalatine-cerebrovascular system.\textsuperscript{26} Therefore, whether stimulation of the parasympathetic nerve has a protective function for the ischemic brain and occluded vessels is of great interest.

Clearly, the thread technique for unilateral occlusion does not cause degeneration of perivascular innervation at least up to 3 days post-occlusion, and is therefore the optimum method to study the roles of the perivascular nerves in the pathophysiology of thromboembolic ischemia.

\textbf{Acknowledgments}

This work was supported in party by a Grant-in-Aid for General Scientific Research from the Ministry of Education, Science and Culture of Japan (No. C 03670680).

\textbf{References}

14) Jackowski A, Crockard A, Burnstock G, Lincoln J: Alterations in serotonin and neuropeptide Y content

\textit{Neurol Med Chir (Tokyo)} 34, February, 1994


31) Zervas NT, Liszczak TM, Mayberg MR, Black PM: Cerebrospinal fluid may nourish cerebral vessels through pathways in the adventitia that may be analogous to systemic vasa vasorum. *J Neurosurg* 56: 475-481, 1982


*Address reprint requests to: T. Kuroyanagi, M.D., Department of Neurosurgery, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390, Japan.*

*Neurol Med Chir (Tokyo)* 34, February, 1994