Density of Sympathetic Nerve Terminals in Human Superficial Temporal Arteries: Potassium Permanganate Fixation and Monoamine Oxidase Histochemistry

Nobuyuki Oka, Ichiro Akiyamch, Kozo Matsubayashi, Masakuni Kameyama, Toshihiro Maeda, and Junichiro Kawamura

The density of sympathetic nerve terminals in human superficial temporal arteries from 5 cases at intra- and extracranial bypass surgery was examined with two histochemical methods, one with potassium permanganate fixation and the other with the new monoamine oxidase staining technique. By potassium permanganate fixation, small cored vesicles containing fibers of noradrenergic nerve terminals made up 29.2% of all nerve fibers in the adventitia. The monoamine oxidase-containing nerves in the adventitia made up 31.4%. According to this study, sympathetic nerve terminal density in human superficial temporal arteries was assumed to consist of approximately 30% of all adventitial nerve terminals. In periadventitial nerve bundles, some unmyelinated axons contained monoamine oxidase activity. Thus, staining is considered to be useful in demonstrating the periadventitial and intervaricose fibers as well as the nerve terminals of sympathetic nerves in human cerebral arteries. (Stroke 1987; 18:229-233)

ALTHOUGH sympathetic nerves play an important role in vasomotor activities and neurotrophic effects, there have been only a few histological studies of sympathetic innervation in human cerebral arteries. 1–5 Since intracranial arteries are not easily accessible in humans, the examination of the superficial temporal artery (STA) could be of help in understanding further histological evidence of vascular innervation of intracranial arteries. The advantage of examination of STA is twofold. One is that specimens are easily obtainable at intra- and extracranial (IC-EC) bypass surgery, and the other is that this artery is innervated by the same sympathetic chain originating from the superior cervical ganglion as the internal carotid artery. The functional similarities between intra- and extracranial arteries have been demonstrated by an in vitro study, 6 which showed the contractile activities of STA to various vasoactive agents such as vasopressin, serotonin, epinephrine, norepinephrine, and phenylephrine to be the same as those of the middle cerebral arteries.

In this study the density of sympathetic nerve fibers presumably innervating the STA was examined with potassium permanganate (KMnO4) fixation to detect sympathetic nerve terminals and with monoamine oxidase (MAO) staining 7,8 to demonstrate postganglionic sympathetic nerve fibers and their terminals.

Subjects and Methods

Pieces of STA were obtained from 5 patients who were subjected to IC-EC bypass surgery for transient ischemic attack or minor stroke. Their clinical backgrounds, diagnoses, and angiographic findings are briefly summarized in Table 1. The dissected segments of STA were immediately divided into smaller pieces approximately 1 mm in length for histological studies. For light microscopy they were fixed with 3% glyoxylic acid in 0.1 M phosphate buffer and observed with fluorescence microscopy as reported before 3 and then counterstained with hematoxylin and eosin. Segments for electron microscopy were processed for either permanganate fixation or MAO staining with the coupled peroxidation method. For the former 9,10 specimens were fixed in ice cold 3% KMnO4 for 1 hour and then rinsed in Ca2+-free Krebs-Ringer solution. For the latter 7,8 specimens were fixed in 1% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer for 1 hour and were postfixed in 0.2% paraformaldehyde and 15% sucrose in 0.1 M phosphate buffer at 4°C overnight. They were then incubated in MAO reaction medium consisting of 0.1% horseradish peroxidase, 0.005% 3,3′-diaminobenzidine tetrahydrochloride, 0.075% tyramine hydrochloride, 0.6% nickel ammonium sulfate, and 0.065% sodium azide in 0.05 M Tris-HCl buffer, at 4°C for 48–96 hours, and were postfixed in 1% osmium tetroxide for 1 hour. Both permanganate-fixed and MAO-stained specimens were dehydrated in graded alcohol solutions and embedded in Spurr’s epoxy resin. Thin sections were stained with uranyl acetate and examined in an Hitachi 600 electron microscope. All nerve terminals and axons in adventitia that were observed in each grid were photographed; later, the number of nerve terminals contain-
Table 1. Clinical Data in 5 Cases and Light Microscopic Findings of STA’s

<table>
<thead>
<tr>
<th>Case/age/sex</th>
<th>Type of CVA*</th>
<th>Angiograms†</th>
<th>Vascular Lesions‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/54/M</td>
<td>TIA</td>
<td>R-MCA branch occlusions</td>
<td>+ 1</td>
</tr>
<tr>
<td>2/57/M</td>
<td>Completed stroke (MCA)</td>
<td>L-MCA branch occlusions</td>
<td>+ 1</td>
</tr>
<tr>
<td>3/61/M</td>
<td>Completed stroke (MCA)</td>
<td>L-ICA stenosis</td>
<td>+ 2</td>
</tr>
<tr>
<td>4/63/M</td>
<td>Completed stroke (MCA)</td>
<td>L-MCA branch occlusions</td>
<td>+ 2</td>
</tr>
<tr>
<td>5/69/M</td>
<td>Completed stroke (MCA)</td>
<td>L-ICA stenosis</td>
<td>+ 2–+ 3</td>
</tr>
</tbody>
</table>

*CVA, cardiovascular accident; TIA, transient ischemic attack; MCA, middle cerebral artery.
†R, right; L, left; ICA, internal carotid artery.
‡Severity of intimal thickening and medial fibrosis: + 1, slight; + 2, moderate; + 3, severe.

Results

In each specimen green fluorescent varicose fibers of sympathetic nerves were observed in the adventitia with the glyoxylic acid wet histofluorescent technique. Hematoxylin-eosin-stained sections showed mild or moderate structural changes such as intimal thickening and/or medial fibrosis in all 5 cases (Table 1). Electron microscopy studies of permanganate-fixed specimens revealed nerve terminals containing either small cored vesicles or agranular vesicles in the adventitial layer (Figure 1). Both types of nerve terminals were frequently enclosed in the same Schwann cell cytoplasm. The nerve terminals containing permanganate-positive vesicles, which measured approximately 50 nm in diameter, were observed in 76 (29.2%) nerve terminals of 260 that were examined on the photographs. Among the 255 axons examined on MAO-stained sections, 80 (31.4%) were MAO positive (Figure 2, Table 2). Nerve bundles consisting of both myelinated and unmyelinated fibers were occasionally observed in the adventitia.
Discussion

Innervation of cerebral arteries has been documented histochemically in various animals, but only a few human studies have been reported. Edvinsson et al examined human fetuses and reported that adrenergic plexuses were well-developed in the vessels of the rostral brain and decreased in density toward the brainstem. With a wet fluorescence method the segments of STA's and middle cerebral arteries obtained at IC-EC bypass surgery have shown fluorescent nerve terminals in both the adventitia and the medial layers of those vessels.

It is still unclear whether the perivascular nerves are functionally different in different innervated arteries. Morphometric studies of the vesicles included in nerve terminals might give us some clues to understand such a situation. In rabbit basilar arteries it was reported that 98% of the nerve terminals examined contained small granular vesicles that would serve for vasoconstriction. In cat pial arteries, however, granular vesicles were observed in only 36–41% of the nerve terminals examined. This figure was in accordance with findings in the present study, in which 29.2% of the nerve terminals of human STA's contained small granular vesicles with permanganate fixation. Furthermore, with the use of a recently elaborated histochemical method to demonstrate MAO, 31.4%, or 80 axons, of 255 observed in the adventitia of STA's from 5 patients contained MAO. These axons were considered to be postganglionic sympathetic nerve fibers. This figure corresponds well with the findings in the permanganate-fixed specimens in the present study.

Table 2. Nerve Terminals Containing Permanganate-Fixed Small Cored Vesicles and MAO-Containing Fibers

<table>
<thead>
<tr>
<th>Case</th>
<th>KMnO&lt;sub&gt;4&lt;/sub&gt;</th>
<th>MAO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19/69** (27.5%)</td>
<td>91/28†† (32.1%)</td>
</tr>
<tr>
<td>2</td>
<td>11/48 (22.9%)</td>
<td>12/39 (30.8%)</td>
</tr>
<tr>
<td>3</td>
<td>11/35 (31.4%)</td>
<td>20/61 (32.8%)</td>
</tr>
<tr>
<td>4</td>
<td>19/61 (31.1%)</td>
<td>23/82 (28.0%)</td>
</tr>
<tr>
<td>5</td>
<td>16/47 (34.0%)</td>
<td>16/45 (35.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>76/260 (29.2%)</td>
<td>80/255 (31.4%)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>29.4 ± 4.3%</td>
<td>31.9 ± 2.8%</td>
</tr>
</tbody>
</table>

*Number of nerve terminals containing small cored vesicles.
**Total number of nerve terminals observed.
†Number of MAO-containing fibers.
††Total number of axons observed.
phometric studies of synaptic vesicles or MAO-containing axons are useful in investigating the functional aspects of the vascular nerves. The population of granular vesicles in the nerve terminals appears to be influenced by hemodynamic and pathological changes of the vascular walls. This was shown in the renal arteries of hypertensive sheep when Burnstock et al. \(^\text{15}\) demonstrated a remarkable increase in both the number of small granular vesicles and the size and density of the granules in the sympathetic nerve terminals supplying the renal arteries. Clinically, after IC-EC bypass surgery transient vasospasm or vascular occlusion around the anastomosed site are occasionally observed. Surgical manipulation of the arteries might stimulate the sympathetic perivascular nerves causing vascular constriction or occlusion.

It is still unknown how the number of granular vesicles in nerve terminals would reflect the functional aspect of nerves innervating the vascular wall. Both the permanganate fixation and MAO staining with coupled peroxidation methods are useful to demonstrate adrenergic nerve terminals or their axons in the vessel wall. Further morphometric studies of perivascular nerves with these methods would give us more information about the functional role of perivascular nerves.

In periadventitial nerve bundles, MAO activity was present in Schwann cell cytoplasm. A previous study has demonstrated that in the rat sciatic nerve, MAO activity is present in Schwann cell cytoplasm and in the wall of endoneurial vessels.\(^\text{\textsuperscript{9}}\) This extraaxonal MAO may play a role in preventing an influx or escape of biogenic amines.

**References**

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**KEY WORDS** • sympathetic nerve terminals • superficial temporal artery • MAO staining
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