Sympathetic Nerve Terminals in the Tunica Media of Human Superficial Temporal and Middle Cerebral Arteries: Wet Histofluorescence

ICHIRO AKIGUCHI, M.D.,* HIDENAO FUKUYAMA, M.D.,* MASAKUNI KAMEYAMA, M.D.,*
TSUNEMARO KOYAMA, M.D.;† HIROSHI KIMURA, M.D.;‡
AND TOSHIHIRO MAEDA, M.D.‡

SUMMARY In specimens from the superficial temporal artery (STA) and middle cerebral artery (MCA), obtained during STA-MCA anastomosis, green fluorescent varicose fibers of sympathetic nerves were clearly visible with both formaldehyde-glutaraldehyde and sucrose-potassium phosphate-glyoxylic acid wet-histofluorescent techniques. These fibers were fairly thick, were densely packed and had a meshwork-like arrangement. Fluorescent terminals were seen both in the adventitia and in the outer muscular layer of the media in both STA and MCA specimens. They were more often observed in patients with prominent atherosclerosis in these vessels. The present study suggests the possible role of sympathetic nerve terminals in the development of vasospasm and occlusive lesions in cerebral vessels. It may also help to explain the marked constriction and transient occlusion following a STA-MCA bypass procedure.

Materials and Methods

The formaldehyde-glutaraldehyde (FaGlu) and sucrose-potassium phosphate-glyoxylic acid (SPG) wet-histofluorescent methods are useful because of their simplicity and constant visualization of catecholamine terminal fibers as compared to conventional Falck-Hillarp histofluorescent methods.5 7

These methods were used on arterial specimens obtained at surgery. Five MCA and 8 STA specimens were obtained from 8 patients with transient ischemic attacks (TIAs), reversible ischemic neurological deficits, or completed stroke between the ages of 37 and 67 during 9 STA-MCA anastomosis (table 1). Each specimen was immediately immersed into either FaGlu (4% formaldehyde, 0.5% glutaraldehyde, and 30% sucrose in phosphate buffer) or SPG (2% glyoxylic acid and 30% sucrose in phosphate buffer at pH 7.4) mixtures at 0–4°C for 4–6 hours. They were then processed according to FaGlu and SPG techniques. The specimens were examined and photographed under ultraviolet light (Olympus BH-RFL).

Results

In the sections from the STA and the cortical branch of MCA, green fluorescent varicose fibers were clearly visible with both FaGlu and SPG techniques. Cathecholamine fluorescence was more clearly visible with the SPG technique than with the FaGlu technique (fig. 1). Fluorescent varicose fibers in these specimens were seen around the border zone of the media and the adventitia of the vascular wall in both STA and MCA (fig. 2). Most of them were observed in the collagen and elastic tissue of the innermost layer of the adventitia. They were fairly thick in diameter, had a high density of fluorescence and appeared in a meshwork-like or ring-like arrangement.

Fluorescent terminals were seen not only in the adventitia but also in the outer muscular layer of the media in 7 out of 13 specimens in both STA and MCA (table 1). In case 5 with minor completed stroke and left carotid siphon stenosis, strongly fluorescent catecholamine fibers were seen within the outer one fifth of the media. They appeared alongside elastic fibers (fig. 3, 4). Furthermore, sympathetic nerve terminals were seen in the atherosclerotic lesions of the specimens which were present both in the adventitia and the media. In case 6 with reversible ischemic neurological deficit and internal carotid stenosis, intimal thickening and proliferation of collagenous and elastic tissue were prominent, and some fluorescent catecholamine fibers were visible in this proliferated connective tissue (fig. 5), which appeared to be invading into the muscular layer of the media. There was no distinct correlation between the severity of vascular lesions and the density of sympathetic nerves in either STA or MCA specimens. These terminals, within the media,
SYMPATHETIC TERMINALS IN HUMAN CEREBRAL ARTERIES/Akiguchi et al.

TABLE 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Type of CVA</th>
<th>Arteriograms</th>
<th>Methods</th>
<th>Vascular lesions</th>
<th>MCA</th>
<th>STA§</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 RT</td>
<td>57M</td>
<td>TIAs</td>
<td>L-MCA branch occlusions</td>
<td>FaGlu</td>
<td>mild</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2 HK</td>
<td>60M</td>
<td>TIAs</td>
<td>R-MCA branch occlusions</td>
<td>FaGlu</td>
<td>moderate</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3 YY</td>
<td>67M</td>
<td>CS*, mild</td>
<td>R-ICA stenosis</td>
<td>SPG</td>
<td>mild</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4 KM</td>
<td>61M</td>
<td>RIND†</td>
<td>L-ICA stenosis</td>
<td>SPG</td>
<td>mild</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5 TK</td>
<td>58M</td>
<td>CS, mild</td>
<td>L-carotid siphon stenosis</td>
<td>SPG</td>
<td>mild</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6 UT</td>
<td>65F</td>
<td>CS, mild</td>
<td>L-ICA stenosis</td>
<td>SPG</td>
<td>moderate</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>7 TK</td>
<td>37M</td>
<td>CS, mild</td>
<td>R-MCA branch occlusions</td>
<td>SPG</td>
<td>mild</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8 SO</td>
<td>62M</td>
<td>CS, mild</td>
<td>R-MCA branch occlusions</td>
<td>SPG</td>
<td>moderate</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

CS* = completed stroke; RIND† = reversible ischemic neurological deficit; vascular lesions‡ = severity of intimal thickening and medial fibrosis; MCA, STA§ = visualization of nerve terminals within the media of STA or MCA.

more often visualized in patients in whom vascular lesions were prominent. In the group of patients with moderate vascular lesion, these terminals within the media could be seen in 3 out of 4 specimens, while in the group of patients with mild lesion in 4 out of 9 specimens (table 1).

Discussion

A well-developed sympathetic nerve supply to the cerebral vascular bed was first presented in 1967 by Nielsen and Owman, using the Falck-Hillarp histofluorescent technique. These observations have been confirmed in both humans and in a number of laboratory animals. Electron microscopic studies have also shown that adrenergic nerve terminals come in close contact with the outer smooth muscle layer of the media, lose their Schwann cell sheath, and approach to within 80 to 100 nm of the smooth muscle cells. Therefore, there is no doubt that the arteries and arterioles of the human brain are enclosed by a plexus of adrenergic nerves which are superimposed on the media, and covered by the adventitia.

Marked spasm is often seen angiographically during the first few days following an STA-MCA bypass procedure. Transient postoperative occlusion of STA-MCA branch anastomosis in three patients was shown by angiography within 12 days of operation. Allen et al. studied, in vitro, the contractile activity of vasoactive agents on human STA and the cortical branch of MCA. They showed that the sympathetic nerves supplying STA as well as MCA may play a physiological role in determining both small and large changes in the

FIGURE 1. Green fluorescence of sympathetic adrenergic fibers is visible in the innermost part of the adventitia by both formaldehyde-glutaraldehyde (FaGlu) and sucrose-potassium phosphate-glyoxylic acid (SPG) wet-histofluorescent techniques. Note the clearer visualization of catecholamine fluorescence with SPG technique than with FaGlu technique. Right: STA, Case TK 58y-o M, Completed Stroke (mild), L-Internal carotid artery (ICA) Stenosis, SPG technique, × 200 in original magnification. Left: STA, Case HK 60y-o M, TIAs, R-MCA Branch Occlusions, FaGlu technique, × 200 in original magnification.

FIGURE 2. Sympathetic adrenergic nerve terminals in STA and the cortical branch of MCA. Fluorescence varicose fibers are seen around the border zone of the media and the adventitia. a) STA, Case KM 61y-o M, reversible ischemic neurological deficit (RIND), L-Internal carotid artery (ICA) Stenosis, SPG technique, × 200 in original magnification. b) MCA, Case KM 61y-o M, RIND, L-ICA Stenosis, SPG technique, × 200 in original magnification.
diameter of these vessels, and postulated that the sympathetic nerves could be the cause of spasm in these arterial segments.

Catecholamine fluorescent intensity in the sympathetic nerve terminals of vessel walls was reduced after experimental subarachnoid hemorrhage.\textsuperscript{12-15} The amount of visible fluorescent nerve terminals in the adventitia was markedly reduced in experimental sub-
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FIGURE 5 a), b) Sympathetic adrenergic nerve terminals are seen within the vascular lesions in STA. Note the intimal thickening and proliferated collagenous and elastic tissue with supporting ground substance both in the adventitia and the media. Some of the fluorescence catecholamine fibers are observed along with this proliferation (arrows).

Intracranial hemorrhage in the cat, while unusual accumulations of catecholamine within the media were observed during the reversible stage of acute vasospasm. Moreover, marked depletion of noradrenaline fluorescence was observed in the vessel wall with irreversible spasm, where myonecrosis was observed by electron microscopy. These results suggest that noradrenaline liberation to the vascular media plays an important role in the development of cerebral vasospasm and myonecrosis.

In this study, abundant sympathetic nerve terminals were observed not only in the adventitia but also in the outer muscular layer of the media of both STA and MCA. There was no distinct correlation between the severity of arteriosclerosis and the density of sympathetic nerve terminals. However, sympathetic nerve terminals within the media could be more often visualized in patients in whom vascular lesions such as intimal thickening and fibrosis were prominent. Sympathetic adrenergic terminals were observed in the media of the sheep carotid artery, rabbit saphenous artery, and aorta and mesenteric artery of various animals, but such terminals have not been demonstrated within the media of human cerebral vessels except by silver impregnation methods.

It is necessary to study sympathetic nerve terminals within the medial layer of intracranial arteries of normal experimental animals and humans without vascular lesions. Recent electron microscopic studies showed no structural differences in the sympathetic arterial innervation of any of the organs studied; it also demonstrated that sympathetic nerve terminals come in close contact with the smooth muscle cell layer of the media.

In the present study, sympathetic nerve terminals were also seen in the fibrous lesions of the vascular wall, where collagen and elastic tissue proliferate. Some of these terminals could be traced to the site of this proliferation. Several reports referred to a possible relation between monoamine liberation to the vascular wall and increased collagen synthesis. For instance, monoamine oxidase would deaminate lysine to produce the bridge formation of collagen, and cyclic AMP levels might modulate the differentiation of normal fibroblasts which produce collagen. These findings suggest that the sympathetic nervous system in the arterial wall may play a significant role in the development of cerebral atherosclerosis and in the development of cerebral vasospasm. However, it is not clear in our study whether sympathetic nerves sprouted into the collagen and elastic tissue or unusual noradrenaline liberation induced the collagen production in the neighboring area. It is also unclear whether the sympathetic nerves visualized in the present study, within the media might be due to ontogenically developed sympathetic nerve fibers in the arterial wall.

Acknowledgments
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References
Comparison of Local Cerebral Blood Flow Determined by Thermal and Hydrogen Clearance

CASEY GAINES, M.D., L. PHILIP CARTER, M.D., AND ROBERT M. CROWELL, M.D.

SUMMARY Local cerebral blood flow (ICBF) was measured simultaneously in ten cats with (1) a large surface thermal diffusion probe resting on the cortex and (2) hydrogen clearance curves from implanted electrodes surrounding the thermal probe. A close correlation was found between ICBF values obtained by the two methods. Since hydrogen clearance is accepted as quantitative, the data suggest that the thermal diffusion technique is a reliably quantitative means of measuring local cerebral blood flow.

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THE HEAT CLEARANCE METHOD for determining local cerebral blood flow (ICBF) may be useful for monitoring clinical patients. In 1973, Carter and Atkinson described a surface thermal probe which incorporates a Peltier stack and may be safely and conveniently applied to the brain surface during craniotomy. The method allows continuous monitoring of ICBF for detection of rapid changes. This probe has been used in both laboratory and clinical settings and has been shown to be a reproducible and apparently quantitative means of measuring flow.

A major criticism of the heat clearance method has been its lack of reliable quantitation. Although data derived by heat clearance are related in a linear way with ICBF determined by Kr washout, previous investigations have indicated that the slope of the line is different in different preparations, and the intercept differs under different recording conditions.

To examine further the quantitative capability of the surface thermal probe, we have compared it to a hydrogen clearance method for simultaneous measurements of flow rates under a variety of conditions. The hydrogen clearance technique was selected because it has been widely accepted as quantitative, and it can be repeated frequently.

Methods

General Preparation

Ten mongrel cats (2.3–4.2 kg) were initially anesthetized with Ketamine hydrochloride (10 mg/kg IM, Bristol Laboratories) for placement of arterial and venous catheters. Arterial blood pressure and blood gases were monitored. Anesthesia was maintained with increments of intravenous pentobarbital (10–15 mg/kg) and pancuronium (0.2 mg/kg) as needed. The animals were placed on a ventilator (Harvard small animal respirator), and the PCO2 was maintained from 80 to 100 torr. PCO2 was manipulated from 25 to 50 torr to change ICBF. The rectal temperature was maintained from 36.5°C to 38.5°C with an electric heating blanket. A right parietal craniectomy was performed, and the thermal flow probe was placed on the cortex. Three
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