Morphological Changes of Unmyelinated Nerves in the Cerebral Arteries after Removal of the Pterygopalatine Ganglion
—An Electron Microscopic Study—

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Abstract
To investigate the participation of the pterygopalatine ganglion in the parasympathetic innervation of the cerebral arteries, Wallerian degeneration was evaluated in the unmyelinated nerves of the major cerebral arteries following the removal of the bilateral pterygopalatine ganglia in dogs. Several days after removal, transmission electron microscopy demonstrated typical features of degeneration in about one-third of the unmyelinated nerves, which are generally considered to be mostly autonomic, i.e., sympathetic and/or parasympathetic. Accordingly, removal of the pterygopalatine ganglion causes Wallerian degeneration of the sympathetic and/or parasympathetic nerves innervating the cerebral arteries. A supplementary study using monoamine fluorescence histochemistry demonstrated that there was no degeneration of the sympathetic nerves innervating the cerebral arteries. Therefore, the degenerated unmyelinated nerves are almost all parasympathetic. These experimental results support the concept that most parasympathetic nerves innervating the cerebral arteries originate in the pterygopalatine ganglion.

Key words: parasympathetic nerve, unmyelinated nerve, facial nerve, cerebral artery, pterygopalatine ganglion, Wallerian degeneration

Introduction
The cerebral arteries are innervated by both sympathetic and parasympathetic nerves.6,7,15 The sympathetic nerves originate mainly in the ipsilateral superior cervical ganglion.7,13 The origin of the parasympathetic nerves that innervate the cerebral arteries remained unclear until 1985, when stereotactic destruction of the pterygopalatine ganglion was shown to cause reduced acetylcholinesterase (AChE)-positive innervation of the major cerebral arteries in the rat.9 AChE-positive nerves are believed to correspond almost specifically to parasympathetic nerves.4,7 The same procedure also causes reduced vasoactive intestinal polypeptide (VIP)-containing innervation of the major cerebral arteries.5,18,22 VIP-containing nerves are thought to co-exist with parasympathetic nerves.1,4,5,10,11 Removal of the pterygopalatine ganglion causes the density of choline acetyltransferase (ChAT)-positive nerves in the major cerebral arteries to decrease.19 The ChAT immunohistochemistry technique visualizes the parasympathetic nerves more specifically than AChE histochemistry.17,19 All these results show that Wallerian degeneration occurs in the parasympathetic nerves of the major cerebral arteries after removal of the pterygopalatine ganglion. However, these three staining methods are not completely specific for parasympathetic nerves.4,5,7,10,11,17-19,22

We report an ultrastructural study to evaluate Wallerian degeneration in the unmyelinated nerves distributed in the adventitia of the major cerebral

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arteries after removal of the pterygopalatine ganglion, and a supplementary study using monoamine fluorescence histochemistry to determine whether the sympathetic nerves degenerate following the removal of the pterygopalatine ganglion.

Materials and Methods

The uni- or bilateral pterygopalatine ganglia was removed under the operating microscope in eight adult mongrel dogs of both sexes weighing 8–12 kg. The animals were anesthetized by intravenous sodium pentobarbital (25 mg/kg), intubated, and artificially ventilated using pancuronium bromide (0.1 mg/kg, i.v.). Sodium pentobarbital (10 mg/kg, i.v.) was given periodically. The anterior unilateral zygomatic arch was removed. After extirpation of the zygomatic gland, found only in the dog and cat and located just ventral to the zygomatic arch, the lateral aspect of the periorbita and the pterygoid muscle at the orbital base were exposed. By retracting the periorbita medially, the pterygopalatine ganglion situated medial to the maxillary nerve at the orbital base was exposed and removed. The maxillary nerve was kept intact. A sham operation, in which the bilateral pterygopalatine ganglia were exposed but preserved with intact afferent and efferent nerves, was performed in one animal.

Four to 6 days after removal of the bilateral pterygopalatine ganglia, four animals were anesthetized and perfused through the bilateral common carotid arteries with heparinized saline (4°C), then a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde (pH 7.4, 4°C, 0.1 M phosphate buffer). The internal carotid, anterior cerebral, middle cerebral, posterior communicating, posterior cerebral, superior cerebellar, basilar, and vertebral arteries were dissected from the brain, then heated at 90°C for 6 minutes. The cerebral arteries from two unoperated animals were similarly processed as controls. The treated arteries were observed under the fluorescence microscope (BH-RFL; Olympus, Tokyo).

Results

Electron microscopy showed bundles of unmyelinated nerve axons at intervals in the adventitia of the cerebral arteries. Each bundle was enveloped by a Schwann cell. Some axons in the bundles had no neurotubules or mitochondria, only neurofilaments in the axoplasm. Most of these axons were enclosed by laminated, flattened processes of Schwann cells (Fig. 1). Other axons contained vacuoles, amorphous electron-dense materials, or laminated structures in the axoplasm. Darkening, swelling, or vacuolization appeared in the mitochondria of some axons. Increased numbers of mitochondria occurred in a few other axons. Occasionally, the axons were completely replaced by empty spaces, debris, or membranous structures (Fig. 1). These histological findings were demonstrated in about one-third of the

Fig. 1 Bundles of unmyelinated nerve axons in the adventitia of the right middle cerebral artery 4 days after removal of the bilateral pterygopalatine ganglia. Some axons (a), with only neurofilaments in the axoplasm, are enclosed by laminated, flattened processes of Schwann cells. Some other axons (b) are completely replaced by empty spaces or debris. Bar = 1 μm.
unmyelinated nerve axons examined, while the other two-thirds were structurally normal. There were no abnormal histological findings in the unmyelinated nerve axons of the control or sham-operated animals.

The adventitia of the cerebral arteries demonstrated synapses of unmyelinated nerves in close proximity to the medial muscles. Some synapses possessed vacuoles, electron-dense materials, laminated structures, or debris in the cytoplasm. Histological findings in some mitochondria of the synapses were similar to those in the axons. Deformities or swelling were seen in the synaptic vesicles of some synapses. Other synapses contained fewer or no synaptic vesicles. In places, the synapses were replaced completely by multivesicular vacuoles. The cytoplasm of some synapses was homogeneously or mottled dark. Some darkened cytoplasm showed fragmentation (Fig. 2). These findings occurred in about one-third of the synapses of unmyelinated nerves examined. The other two-thirds of the synapses had normal structures. No abnormal histological findings were detected in the synapses of the unmyelinated nerves in control or sham-operated animals.

Macrophages were seen regularly in the adventitia of the cerebral arteries, located near or adjacent to the axon bundles or synapses of unmyelinated nerves. Some had axon-like or synapse-like struc-

Fig. 2 Synapses of the unmyelinated nerves in the adventitia of the right anterior cerebral artery 4 days after removal of the bilateral pterygopalatine ganglia. The cytoplasm of one synapse (a) is homogeneously dark and fragmented. Furthermore, this synapse had vacuoles. Another two synapses (b) appear normal. F: fibroblast, M: medial muscles. Bar = 1 μm.

Fig. 3 Macrophages in the adventitia of the left anterior cerebral artery 6 days after removal of the bilateral pterygopalatine ganglia. A synapse-like structure is seen in the cytoplasm (arrow). Bar = 1 μm.

Fig. 4 Monoamine fluorescent fibers of the right (upper) and left (lower) anterior cerebral arteries 10 days after removal of the right pterygopalatine ganglion. No difference was observed in the density of monoamine fluorescent fibers. Bar = 50 μm.
tures in their cytoplasm (Fig. 3). Macrophages were rarely seen in the control and sham-operated animals.

No abnormal histological findings were demonstrated in the myelinated nerves of the adventitia of the cerebral arteries in the control, sham-operated, and operated animals.

Monoamine fluorescence histochemistry demonstrated green fluorescent fibers forming a network around the cerebral arteries in the control animals. A few thick fiber bundles were also observed. Monoamine fluorescent fibers were densely distributed in the internal carotid, anterior cerebral, and middle cerebral arteries, and less densely in the posterior communicating, posterior cerebral, and superior cerebellar arteries. The density was lowest in the basilar and vertebral arteries.

The density of the monoamine fluorescent fibers in the cerebral arteries of the operated animals was compared with that in the non-operated control animals. Each artery was examined in several visual fields under the fluorescence microscope at a magnification of 100. The four animals from which the pterygopalatine ganglia had been removed uni- or bilaterally demonstrated a density of monoamine fluorescent fibers in all arteries almost the same as that in the control animals (Fig. 4).

**Discussion**

Our electron microscopic study demonstrated several abnormal histological findings in the axons and synapses of the unmyelinated nerves. The axons showed loss of neurotubules and mitochondria, possibly due to Wallerian degeneration of the unmyelinated nerves. Most degenerated axons were enclosed by laminated, flattened processes of Schwann cells. Several types of abnormal structures appeared in the axons and synapses of the unmyelinated nerves, suggesting degeneration. The decrease or complete absence of the synaptic vesicles observed is certainly due to degeneration, as is the appearance of macrophages near the degenerating unmyelinated nerves. Several of the abnormal features observed in the mitochondria, disappearance of axons, and deformities of the axons and synapses are probably due to degeneration. Increased numbers of mitochondria in a few axons may imply the localized regeneration of the unmyelinated nerve axons.

These ultrastructural findings are typical features of Wallerian degeneration of unmyelinated nerves. Unmyelinated nerves of the cerebral arteries are generally thought to be mostly autonomic ones. The results of our electron microscopic study therefore indicate that removal of the pterygopalatine ganglion caused Wallerian degeneration of the autonomic, i.e., sympathetic and/or parasympathetic nerves innervating the cerebral arteries. The ultrastructural findings cannot be interpreted in greater detail, since parasympathetic nerves cannot be identified specifically by transmission electron microscopy. To evaluate the ultrastructural findings more fully, the experiments using monoamine fluorescence histochemistry were performed.

The monoamine fluorescent fibers in the cerebral arteries correspond specifically to the sympathetic nerves. Therefore, our results using monoamine fluorescence histochemistry show that, after removing the pterygopalatine ganglion, no degeneration occurred in the sympathetic nerves innervating the cerebral arteries. Monoamine fluorescence histochemistry has shown that there are no adrenergic neurons or terminals in the pterygopalatine ganglion, so our experimental results are compatible. Accordingly, most of the degenerated unmyelinated nerves demonstrated by our electron microscopic study were probably parasympathetic, not sympathetic nerves.

The results of our experiment have provided additional evidence that most parasympathetic nerves innervating the major cerebral arteries originate in the pterygopalatine ganglion. Strictly speaking, the pterygopalatine ganglion is not purely parasympathetic but contains some sensory nerves of trigeminal origin. Substance P- and calcitonin gene-related peptide (CGRP)-immunoreactive nerve fibers, which originate in the trigeminal ganglion, exist around the cerebral arteries. The pterygopalatine ganglion contains a few CGRP-immunoreactive cells and scattered fibers immunoreactive to both CGRP and substance P distributed in a similar pattern. Therefore, it is possible that some sensory nerves, after leaving the trigeminal ganglion, pass through the pterygopalatine ganglion and reach the cerebral arteries. The substance P-immunoreactive fibers are unmyelinated C-fibers. Further experiments must establish whether the degenerated unmyelinated nerves in our experiments correspond to the sensory nerves, although Suzuki et al. have excluded this experimentally.

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References


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