Unmyelinated Nerve Fibers of the Human Mandibular Nerve

By

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Summary: The aim of this research is to find and to evaluate morphometrically the unmyelinated nerve fibers in the human mandibular nerve using a light microscope. Our report demonstrates for the first time the presence of the unmyelinated nerve fibers of the human mandibular nerve stained by a special method. Our results also indicate that there is a morphometric change with aging in the unmyelinated axons of the nerve.

For a long time, the mandibular nerve, which is a branch of the trigeminal nerve, was believed to be composed essentially of myelinated nerve fibers. Although there have been several reports dealing with unmyelinated nerve fibers^{1,3–8)}, those concerning the trigeminal nerve are limited in numbers^{1,8)}. In general, there is a lack of sufficient data concerning the presence, numbers, distribution, sizes and functions of human unmyelinated nerve fibers. In the present study, we used a special staining method which enabled us to uncover the presence of unmyelinated nerve fibers in the human mandibular nerve and to conduct an evaluation that revealed morphometric changes during the aging process.

Material and Methods

Small sections of the mandibular nerve were removed from an area adjacent to the trigeminal ganglion from 6 human cadavers (4 males and 2 females) aged from 26 to 85 years (average age: 66.2 years). The causes of death indicated no direct or indirect connection with the nervous system. These were selected among 50 subjects under the condition that all teeth were present and normal. We consider all the branches of the trigeminal nerve to be normal. The methods employed in this

study are same as in our previous researches^{3–5,7)} concerning the fixation, washing, dehydration, embedding, sectioning, morphometry and statistical analysis; the only difference concerned the staining method. The sections were stained by Goto's modification of Masson-Goldner's method. The shrinkage ratio of sections was the same as in our previous reports ($10 \pm 0\%$ in length). For more details, see the references^{2–5,7)}.

Results

Microscopic findings of unmyelinated axons in the mandibular nerve will be explained regarding numbers, transverse areas and perimeters.

Numbers of Unmyelinated Axons

We counted the total number of unmyelinated axons within a unit area of $2192.5 \mu\text{m}^2$ in the human mandibular nerve as being between 10 and 29 (average: 18.8 ± 4.8). The myelinated nerve fibers have black axons with surrounding red myelin sheaths (Fig. 1), while the unmyelinated nerve fiber is shown with a black or dark purple axon without a surrounding red myelin sheath (Fig. 1). The number of unmyelinated axons did not change with age ($r = -0.0940$).

Transverse Area of Unmyelinated Axons

The transverse area of unmyelinated axons in the human mandibular nerve ranged from 0.77 to 1.71 (average: 1.37) μm^2 . A scatter diagram with regression analysis between the average transverse area and age reveals that the average area of unmyelinated axons in the transverse section increased with age ($r = 0.8564$), and so did the total transverse area per unit area ($r = 0.4847$).

Perimeter of Unmyelinated Axons

The average perimeter of unmyelinated axons in the human mandibular nerve was 4.13 μm . It, too, increased with age ($r = 0.9120$). Furthermore, we also noticed a slight correlation between age and the total perimeter of unmyelinated axons ($r = 0.2987$).

Discussion

The authors could not find any other reports in the literature regarding morphometric data on unmyelinated nerve fibers of the human mandibular nerve. As far as the methodology is concerned, fixation, embedding, staining and morphometry are the most important steps in this research. We tried several staining methods, such as LPH stain²⁾, Klüver-Barrera method, Mallory's azan stain, silver

impregnations, and others. We concluded that Goto's modification of Masson-Goldner's method is the best one at present for the morphometric evaluation of unmyelinated nerve fibers. Thanks to this staining method, we can easily distinguish unmyelinated from myelinated axons. Moreover, it is also possible to count the numbers and to measure the transverse areas and perimeters of unmyelinated axons with the use of an image-analyzer. Unmyelinated axons appeared dark blue, surrounded by a thin Schwann sheath stained in a light-blue color, whereas myelinated axons, stained in a color similar to that of unmyelinated axons, were surrounded by a thick myelin sheath stained in red. Regarding the size, unmyelinated axons were always smaller than myelinated axons. A regression analysis of unmyelinated axons disclosed a positive correlation between the transverse area and age ($r = 0.8564$), which meant the axonal area increased especially after 60 years of age (Fig. 2). The regression analysis also revealed that there was a significant correlation between the perimeter of the axon and age ($r = 0.9120$): it showed a definite increase of perimeters after 60 years of age (Fig. 3). There was, however, no correlation between the number of unmyelinated axons per unit area and age ($r = -0.0940$). As one grows older, an unmyelinated axon might become larger, although the number of unmyelinated axon practically does not

Fig. 1. Color microphoto of the mandibular nerve in 59-year-old man, Masson-Goldner's stain (Goto's modification).

There are many unmyelinated nerve fibers (black arrow) among myelinated nerve fibers (white arrow). *: degenerated nerve fibers, bar = 10 μm .

Fig. 2. Scatter diagram between the total transverse area of unmyelinated axons of the human mandibular nerve and age ($r = 0.8564$).

Fig. 3. Scatter diagram between the total perimeter of unmyelinated axons of the human mandibular nerve and age ($r = 0.9120$).

change ($r = -0.0940$). Before this research, the presence of unmyelinated nerve fibers in human beings had not been established, nor had the changes occurring during the aging process. Researchers used to try to count only the number of myelinated nerve fibers in general. This research, however, has for the first time brought to light the increase of axonal sizes of unmyelinated nerve fibers during the aging process. The function of the unmyelinated nerve fibers in the human mandibular nerve still remains unknown.

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