Myelinated Nerve Fiber Analysis of the Human Greater Splanchnic Nerve

By

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Summary: The purpose of the present study is to analyze the human greater splanchnic nerve in relation to aging. We adopted a new staining method which makes it possible to discriminate various structures of the nervous tissue. We examined 25 human greater splanchnic nerves from cadavers for anatomy dissection. We measured the number, area and perimeter of axons. The results reveal that: (1) there is no correlation between age and the number of axons; (2) the mean area and perimeter of axons increase with age, but not the total area and perimeter. We compared these results with those for the lesser splanchnic nerve. These morphological changes in the greater splanchnic nerve may indicate a kind of compensation through axon hypertrophy for hypofunctions in abdominal organ control.

In general, physical functions in the human being deteriorate with aging. These changes have already been studied in relation to motor or sensory functions, but little research has been conducted from a morphological point of view. In the present study, we dealt with this problem through analysis of the number, area and perimeter of myelinated axons in the human greater splanchnic nerve.

Material and Methods

Greater splanchnic nerves were removed from 25 human cadavers (16 males and 9 females) for anatomical dissection. The average age was 73 years (44–96 years). As no medical problems were found in the central or peripheral nervous system, these specimens were considered to be normal.

Fixation

The fixation procedure consisted of two steps: as primary fixation, the material was kept in 3.7% formaldehyde solution for about one week. This was followed by secondary fixation with a mixture of 5% potassium dichromate and 5% potassium chromate (1:4 in volume) for three weeks: two weeks at room temperature, and another week at 37°C. The volume of the fixative was at least ten times the volume of the specimen.

Washing

After the fixation, the tissue blocks fitted with a nylon-mesh bag or a plastic tissue-basket were washed in running water with a siphon-operated automatic pipette washer for several hours.

Dehydration and embedding

The tissue blocks were dehydrated with ethanol at 50%, 70%, 90%, 95% and pure, and then were embedded in celloidin (Shiojirin). The shrinkage of the nerve tissue sections during fixation and embedding amounted to approximately 10% in length.

Sectioning

The blocks were sectioned transversely into 20μm thick slices.

Staining

The sections were processed with LPH (Luxol fast blue-periodic acid-Schiff-hematoxylin) triple stain.

Morphometry

The systemic sampling method was employed...
for the measurement of axons. The center of the greater splanchnic nerve was selected as a sampling site. Microscope images at 1,600 times magnification were used to count the transverse area and perimeter of axon through a eyepiece grid. The instruments employed in the present study are as follows: a microscope (BH2, Olympus, Tokyo, Japan), a drawing tube (BH2-DA, Olympus, Tokyo, Japan), a digitizer (Crystizer α KC 3300, Graphtec, Yokohama, Japan), a computer (PC-9801VX2, NEC, Tokyo, Japan), and running original image analyzing software. The data were analyzed statistically in order to determine the relationship between age and number, average area, total area, average perimeter and total perimeter of axons respectively (Scheffe's multiple comparison test).

Results

Microscopic findings

The myelinated nerve fibers of the greater splanchnic nerve were rather well preserved even in old persons (Fig. 1). They were scattered among the many non-myelinated nerve fibers.

Number of myelinated axons

We estimated the total number of myelinated axons in the human greater splanchnic nerve at between 5 and 13 (mean 8.43) within a unit area of 0.0016 x 0.0016 mm. The data for all the nerves are listed in Table 1. The regression analysis showed no correlation between the number of axons in the greater splanchnic nerve and age (r = 0.180).

Transverse areas of myelinated axons

The transverse area of myelinated axons ranged from 3.68 to 9.16 (mean 5.37) μm² (Table 1). The regression analysis showed no significant correlation between the mean transverse area and age (r = 0.0326), or between the total transverse area and age (r = 0.042).

Perimeter of myelinated axons

The mean perimeter of myelinated axons ranged from 6.59 to 11.69 (mean 8.54) μm (Table 1). The

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Mean 73.1 8.43 5.37 44.33 8.54 71.39
SD 13.6 2.16 1.30 11.79 1.18 17.63
regression analysis showed no significant correlation between the mean perimeter of myelinated axons and age \((r = 0.1366)\), or between the total perimeter and age \((r = 0.0016)\).

**Discussion**

The peripheral nervous system can be divided into two groups: somatic and autonomic. The somatic nervous system controls motor and sensory functions, while the autonomic nervous system regulates the contraction of smooth and cardiac muscles and the secretion of various glands. There are few reports available on the morphology of the somatic nervous system\(^{1-4,7}\). Although research on the autonomic nervous system has been carried out mainly with experimental work in the field of physiology or pharmacology, there has been little research on its morphometry, especially in the human being\(^6\). The reason for that may be that although studies on ganglion cells can be done by morphometric methods, studies on nerve fibers are more limited due to a lack of suitable methods to evaluate them.

Various staining methods have been employed for examining nervous tissue up to now. However, no method had until recently been developed which could evaluate various structural elements of the nervous system including axons. Staining methods can be classified into two types: conventional methods and special methods used for the nervous system, such as myelin sheath stain, luxol fast blue-cresyl violet (LFB-CV or K-B method) or silver impregnation methods. But these methods present various problems in the case of morphometric analysis. We employed a new staining method: LPH triple stain. This method is preferable in many respects, but especially on two points: improved staining discrimination and minimized shrinkage ratio. Details of the LPH triple stain are mentioned in an article by Goto published in 1987\(^3\).

Many studies about age-related changes in various nerves have been reported since the LPH staining was introduced\(^{1,2,6-11}\). Fujii et al.\(^1\) reported that the transverse areas and circularity ratios of the axons in the facial nerve did not change with age, but the numbers of axons became reduced with age. In another article\(^2\), they reported that the transverse axonal areas of the cochlear nerve were reduced with age, while the number and transverse areas of the amyloid bodies in the vestibulocochlear nerve increased with age; furthermore, the number of cochlear nerve fibers did not decrease with age. Yanagisawa et al.\(^7\) reported that the number, transverse area and perimeter (mean and total) of the human deep peroneal nerve axons decreased with age. In particular, disappearance of large axons was the main feature of the aging process. In the report by Zhang et al.\(^9\) myelinated axons of the posterior funiculus at C6 level were studied to measure their transverse area and to count their number. This research revealed that a decrease in size and number of axons was one of the changes occurring with the aging process.

We also examined 30 human lesser splanchnic nerves\(^6\). A comparison with the greater splanchnic nerve gives the following results: as far as the number of myelinated fibers is concerned, no significant difference was noted between the lesser splanchnic nerve and the greater splanchnic nerve. However, a morphometric analysis revealed a decrease with age of the axonal areas and perimeters in the lesser splanchnic nerve, but, on the contrary, an increase with age in the mean area and perimeter of axons in the greater splanchnic nerve, although in the latter case, the total areas and perimeters of axons remained more or less the same.

The lesser splanchnic nerve is thought to affect the function of the suprarenal body and the kidney through the ramus renalis, while the greater splanchnic nerve is thought to affect the functions of several viscerae such as the stomach, liver, gall bladder and pancreas. In the clinical field, Fujii et al.\(^2\) revealed that the presbyacusis could be due to a reduction of axonal areas in the cochlear nerve. In the aged, the causes of renal hypertension, diabetes mellitus, sexual hypofunction and protein hypo-metabolism etc. might be due to suprarenal hypofunction including steroid hyposecretion. Up to now, it was not known if any morphological changes due to aging affected the nerves connected with gastrointestinal digestion, metabolism and detoxication by the liver, and internal or external pancreatic secretion. From morphometric results, however, we can say that there is a definite correlation between aging and hypofunction of the organs controlled by the lesser splanchnic nerve. On the other hand, the partial increase we found in the size of myelinated axons in the greater splanchnic nerve, could be interpreted as a sort of hypertrophy meant to compensate for the hypofunctions of the organs controlled by this nerve.

**References**

3) Goto N. Discriminative staining methods for the nervous system.


Explanation of Figures

Plate I

Fig. 1. High-power view of the greater splanchnic nerve in 85-year-old woman, transverse section, LPH stain, scale bar = 10 μm. Myelinated nerve fibers are well preserved even in an old person, and are scattered among the many non-myelinated nerve fibers.