Ultrastructure of the Perineurial Cell of the Sciatic Nerve in Rats

—A Transmission and Scanning Electron Microscopic Study—

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Summary: The perineurial cells of the sciatic nerve of mature albino rats were investigated by using light microscope (LM), transmission electron microscope (TEM) and scanning electron microscope (SEM). The perineurium of the sciatic nerve was composed of several lamellae of thin perineurial cells which were held together by the zonula occludens and also by the zonula adherens. The TEM view of the perineurium revealed that the plasmalemma of the perineurial cells presented numerous caveolae similar to those described in the endothelial cells of blood capillaries. By SEM, the perineurial cells were very thin, flattened out with various shapes from rectangular to hexagonal, and arranged closely to each other in the fashion of a stone pavement around the nerve fascicles. It was possible to isolate the perineurium from the nerve and to demonstrate the cellular border of the perineurial cell by silver impregnation method. The SEM observation of the perineurial cells ascertained the figure of the cells which were seen in the silver-impregnated specimen under LM. The present authors have interest in the fact that shape and arrangement of the perineurial cells of spinal nerves resemble closely those of the myoid cells in the limiting lamina of the seminiferous tubules of the mammalian testes. The meaning and significance of the similarity between these two types of cells was discussed.

Key words: perineurium — peripheral nerve — transmission electron microscope (TEM) — scanning electron microscope (SEM) — silver impregnation

Introduction

The fascicles of peripheral nerve trunks are surrounded by a multilayered sheath, the perineurium, composed of several alternating cellular and connective tissue layers. The perineurium was discovered by histologists late in the 19th-century (Ranvier, 1871, 1872; Key and Retzius, 1873, 1876), and Key and Retzius (1873, 1876) first described the presence of endo-

thel-like cells in the perineurium covering the nerve trunk. The presence of the flat endothel-like cells in the perineurium was confirmed by many workers using the light microscope (LM) (Lehmann, 1953, 1957; Clara and Özer, 1959; Shanthaveerappa and Bourne, 1962a, b, 1963; Martin, 1964), and the transmission electron microscope (TEM) (Pease and Pollie, 1959; Röhlich and Knoop, 1961; Shanthaveerappa et al. 1963; Thomas,

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1963; Gamble, 1964; Cravioto, 1966; Waggener and Beggs, 1967; Burkel, 1967; Liebermann, 1968; Saito and Zacks, 1970; Kerjaschki and Stockinger, 1970; Haller and Low, 1971; Akert et al. 1976; Klemm, 1970; Reese and Olsson, 1970; Olsson and Reese, 1971; Kristensson and Olsson, 1971; Krishnan et al. 1979). The TEM studies clearly show that the perineurial cells are interconnected by tight junctions and form a cellular tube around the nerve fascicles. In spite of the numerous previous LM and TEM studies, there is no report on the three-dimensional structure of the perineurial cell layers using the scanning electron microscope (SEM). Therefore, in order to obtain information on this account, several experiments were undertaken in which the extracellular substances of the perineurium were chemically digested away prior to the SEM observation.

In addition, the outline of the cellular borders of the perineurial cells were visualized under the LM by the silver impregnation method.

Materials and Methods

Adult albino rats (200~300g) anesthetized by intramuscular injection of ketamine hydrochloride were used in this study.

1. TEM studies:

The tissues were fixed by perfusion via the abdominal aorta with physiological saline followed by a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) at room temperature. The sciatic nerve trunks were dissected out and immersed in the same fixative for 4hr at 4°C. After this the specimens were washed for several hours in the cacodylate buffer containing 7.5% sucrose and were post-

fixed in 2% OsO₄ with 0.1M phosphate buffer (pH 7.4) for 1.5 hr, at 4°C. They were then dehydrated in graded acetone, transferred into dibutyl-glycidyl ether and embedded in Epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with an Hitachi H-500 TEM.

2. SEM studies:

The sciatic nerve, after fixation by perfusion through the cannulated aorta as described above, was immersed in the fixative over two days at 4°C. The sciatic nerve was prepared under a dissecting microscope by removing most of the epineurial connective tissue. After several rinses in 0.1M cacodylate buffer (pH 7.4) containing 7.5% sucrose, the specimens were placed in 5N KOH solution for 3 to 20 min at 60°C. They were, then, thoroughly washed in the cacodylate buffer and immersed in collagenase (Sigma, type II) solution at a concentration of 200 unit/ml in 0.1M phosphate buffer (pH 6.8) for 3 hrs at 37°C. After a rinse in distilled water, the samples were post-fixed in 2% OsO₄ solution for 2-3 hrs (4°C), dehydrated through a graded series of acetone, and transferred to iso-amyl acetate. They were critical point-dried using liquid CO₂, coated with gold in an ion-sputter coater and observed with an Hitachi S-800 SEM.

In some animals, fresh sciatic nerves were quickly dissected out just after flushing out the blood with the aortic perfusion. Prior to the fixation, the sciatic nerves were cut into small pieces and placed in the collagenase (Sigma, type II) solution at a concentration of 200-600 unit/ml in 0.1M phosphate buffer (pH 6.8) for 20 to 90 min at 37°C. The specimens were thereafter fixed with the aldehyde fixative, post-fixed with OsO₄ and were treated as above for SEM observation.

3. Silver impregnation:

The fresh nerve fascicles which were obtained from the animals were cut into small short pieces, about 2 mm in length, and were kept in 0.5% silver nitrate in the dark for 48 to 72 hrs. Then, the epineurial connective tissue covering was removed under a binocular dissecting microscope, with the help of fine watch-maker's forceps, scissors and fine needles. The denuded perineurium was slit, leaving it flat on the glass slide, and the nerve fascicles with endoneurium was removed according to the method of Shanthaveerappa and Bourne (1962b, 1964). These isolated perineurial sheets were mounted on glycerin and, after sealing the edges of the coverglass with nail polish, were exposed to sunlight to reduce the silver nitrate till the mounted tissue

became brown. The specimens were observed by LM and were photographed.

Results

1. Observations with light microscopy:

Semithin sections, obtained from Epoxy-resin-embedded material, were stained with 0.5% toluidine blue and observed under the LM.

The perineurial sheet of the sciatic nerve trunk measured $3\mu\text{m}$ in thickness (Figs. 1, 2), and formed a continuous tube around the nerve fascicles. The perineurium appeared to be composed of two to three layers of flattened cells, perineurial cells, interspersed with extracellular connective tissue components (Fig. 2). The nerve fiber fascicles, thor-

Figs. 1, 2. Semithin sections of nerve fascicles from a specimen of sciatic nerve embedded in epoxy-resin and stained with 0.5% toluidine blue.

Ep: Epineurium, Pe: Perineurium, En: Endoneurium

Fig. 1: $\times 240$ Fig. 2: $\times 490$

oughly surrounded by the perineurium, were further invested from the outside with the relatively loose connective tissue sheath, the epineurium (Figs. 1, 2).

2. TEM studies:

The perineurium of the sciatic nerve trunk was composed of alternating 4-6 layers of cells and connective tissue (Fig. 3-6). The individual perineurial cells were identified by their elongated nuclei, thin and flat cytoplasmic processes, numerous caveolae in the plasmalemma and the presence of the basal lamina. Mitochondria, rough endoplasmic reticulum (rER), and Golgi apparatus were usually encountered in the perikaryon, although the mitochondria and rER were infrequently

seen in the attenuated cytoplasmic processes. Extensive arrays of fine filaments and dense patches were frequently observed in the cytoplasm along the plasmalemma (Figs. 3-6). The perineurial cells were invested by the basal lamina not only on the epineurium-side but also on the endoneurium-side surface. This basal lamina was not, however, present over the entire cell surface (Figs. 5, 6). In some perineurial cells, a continuous basal lamina was present on one side but was absent or discontinuous on the other side. The plasmalemmal caveolae were relatively prominent, seen on both sides of the plasmalemma of the perineurial cells and occurred throughout all layers of the perineurial sheath. The elongated pro-

Figs. 3, 4. TEM view of the perineurium. The perineurium is composed of alternating 4-6 layers of cells and connective tissue.

N: Nucleus of perineurial cell, BL: Basal lamina, MN: Myelinated nerve, EN: Endoneurium, Mf: Microfibrils

Fig. 3: $\times 8,800$ Fig. 4: $\times 12,000$

cesses of the perineurial cells were linked mostly by zonulae occludens and occasionally by zonulae adherens (Figs. 5,6).

The extracellular spaces between the cellular layers contained small amounts of collagenous fibrils running longitudi-

nally and circumferentially (Fig. 6). There were also bundles of microfibrils (Fig. 4) of the type associated with elastic fibers, but no dense elastin material was observed.

Figs. 5, 6. TEM view of the perineurium. The thin, attenuated cytoplasmic processes of perineurial cells are interconnected with tight junctions (arrows in Fig. 5), and occasionally with zonula adherens (arrows in Fig. 6). Note the discontinuous basal lamina (arrowhead).

Pe: Perineurial cell, BL: Basal lamina, Col: Collagenous fibers

Fig. 5: $\times 12,000$ Fig. 6: $\times 20,000$

Figs. 7, 8. LM view of the silver-impregnated perineurium.
 Fig. 7: View of the specimen at low magnification. Fig. 8: Closer
 view of a part of the silver-impregnated perineurium.
 Fig. 7: $\times 26$ Fig. 8: $\times 270$

3. Silver impregnation of the perineurial cell border:

The cellular border of perineurial cells was clearly demonstrated by silver impregnation method under LM. The individual perineurial cells were squamous shaped and extremely flat. The cells were rectangular to hexagonal in shape and arranged close to each other in the fashion of a stone pavement (Fig. 8). There is no stoma or gaps between the cells, even at the corners where three perineurial cells join together. In the present specimens, up to three layers of the perineurial cell sheets were successfully impregnated with silver nitrate, but, in practice only one or two of these layers were adequately photographed with alterations of the fine adjustment on LM.

4. SEM studies:

1) KOH treatments;

The aldehyde-fixed materials were chemically treated with KOH for the purpose of removing the epineurial con-

nective tissue. Specimens which were placed in 5N KOH for 10 min at 60°C revealed the successfully exposed outer-surface of the outermost perineurial cell lamella (Figs. 9-11). When the treatment with the strongly alkaline solution was longer than 20 min, most of the materials were excessively digested and broken into pieces. On the contrary, too short treatments with the alkaline solution resulted in incomplete digestion of the epineurial collagenous fibers. The adequately exposed perineurial cells formed a thin, continuous cellular sheath around the nerve fascicles. The perineurial cell sheath was composed of rectangular cells which were tightly joined to each other, with the appearance of a stone pavement (Figs. 10, 11). There was no stoma or gap between the neighboring perineurial cells. The region where the cell nucleus was located occasionally showed a mild bulging (Fig. 11) but was, usually, flat (Fig. 10). In some cases, one or two of the perineurial cell lamellae were partially

Fig. 9. SEM photomicrograph of the nerve fascicle after treatment with 5N KOH (10 min, 60°C). $\times 700$

Fig. 10. The perineurial cells are squamous, rectangular and are tightly joined to each other. $\times 1,200$

Fig. 11. The region where the cell nucleus is located shows occasionally a mild bulging, although it is usually almost flat as depicted in Fig. 10. $\times 1,200$

Fig. 12. One or two of the perineurial cell lamellae are occasionally observed simultaneously under SEM because some parts of them may peel off after KOH digestion. $\times 1,000$

Fig. 13. The SEM photomicrograph shows three portions of the nerve fascicles; 1) outermost perineurial cell sheet, 2) inner perineurial cell sheet, and 3) endoneurium and nerve fibers. $\times 550$

Fig. 14. Collagenase treatment of the fresh nerve fascicle. Some of the perineurial cells are desquamated in spots, showing the rectangular figures of single perineurial cells. $\times 240$

Fig. 15. Endoneurium just beneath the perineurial investment.
Upper half: overview of the endoneurium at low magnification
Lower half: high magnification view of the portion enclosed by the rectangle in the upper half of the figure. Upper photograph: $\times 230$ Lower photograph: $\times 2,300$

or totally peeled off because the inter-lamellar connective tissue or the epineurial connective tissue was digested away by the KOH treatment (Figs. 12, 13). The configurations, sizes and modes of arrangements of perineurial cells were very similar in different cell layers.

2) Collagenase treatment of the fresh nerve fascicles;

The fresh sciatic nerves which were quickly taken from the anesthetized animals and were enzymatically digested with collagenase solution showed good exposure of the outer surface of the perineurial cell lamella. In the present study, the optimal time for digesting the epineurial connective tissue components while preserving the perineurial cell sheets was 60 to 75 min (at 37°C). This method was expected to expose the outer surface of the perineurial cell layers and also partially disjoin neighboring perineurial cells. In fact, some of the perineurial cells were desquamated in spots from the exposed perineurial cell layer, showing the rectangular outline of single perineurial cells (Fig. 14).

When the whole perineurial cell layer was taken away by the collagenase treatment, the endoneurial connective tissue just beneath the perineurial sheath became widely exposed (Fig. 15).

Discussion

The LM and TEM studies of the thin sections of the nerve fibers have ascertained that the perineurium is a very thin, membranous tube surrounding bundles of nerve fibers and is composed of several alternating cellular and connective tissue layers. The silver impregnation of the fresh perineurial sheets has demonstrated the cellular border of the individual perineurial cells similar to the figures reported by Key and Retzius

(1873, 1876), Lehmann (1957) and Shanthaveerappa and Bourne (1962*a, b*, 1963, 1984). The three dimensional contour of the perineurial cells and the arrangement of the cells were shown by SEM in the present study. The SEM observation of the cellular sheets of the perineurium indicated that the rectangular to hexagonal flat perineurial cells were arranged close to each other, forming a thin cellular tube encompassing the nerve fascicles. The figures of the perineurial cell layer which were seen under SEM were surprisingly compatible to those of LM view of the specimens treated by silver impregnation method. Furthermore, it was also clearly shown by EM (Figs. 3-6, 9-13) that there are no gaps between perineurial cells and that the cells were joined together with the zonulae occludens and with the zonulae adherens. These findings are in good accordance with many reports concerning the ultrastructure and function of the sheets of perineurial cells. Namely, many authors who have previously observed the cellular junctions between the adjoining perineurial cells using the freeze replica method and also studied the permeability of various tracer substances through the perineurial cell layer have described that the perineurial cell layer acts as a diffusion-barrier to these substances (Shanthaveerappa and Bourne, 1962*a*, 1984; Martin, 1964; Olsson and Reese, 1971; Olsson et al. 1971; Thomas and Olsson, 1984; Olsson, 1990). Furthermore, the perineurial cellular sheets, which have a considerable stiffness owing to the well-developed intercellular junctional complexes and to the presence of alternating connective tissue sheets with numerous collagenous as well as elastic fibers, are supposed to have an important role in maintaining a positive endoneurial fluid pressure (Sunderland and Bradley, 1952; Low et al. 1977; Myers et al. 1978; Olsson, 1990). The TEM view of the per-

ineurial cells has disclosed that the cytoplasm contains bundles of filaments similar in appearance to the myofilaments of smooth muscle. Ross and Reith (1969) who studied the perineurial sheets of sciatic nerves of adult mice using TEM, were the first to note that the perineurial cells contain filaments and associated opaque regions similar to those observed in smooth muscle cells. They proposed that the perineurial cells have contractile properties, although this concept has not been commonly accepted. In this regard, it is a usual experience to see nerve fascicles soon after being excised and placed in the physiological saline exhibit a wavy motion suggestive of shortening and lengthening. Woodhall (1974) has described the observation that the ends of excised human nerve retract by some 4% of their in situ length. A similar observation has also been reported by Thomas and Olsson (1984). Thus, there is some basis for suspecting that the perineurial cell have contractile properties, even though direct evidence is lacking. With regard to this issue, it seems worthwhile to discuss the similarities between the perineurial cells of nerve fascicles and the myoid cells of the limiting lamina of the seminiferous tubules, in various animals (Murakami et al. 1979; Matsufuji, 1990). The TEM and SEM appearance of both cell types has several common features;

- (1) Attenuated cells with polygonal outline are held together by junctional structures, forming a continuous tube.
- (2) The continuous cellular tubes are arranged in lamellae the number of which varied depending on the size of the tube and the species.
- (3) The individual cells contain closely aggregated bundles of microfilaments and dense bodies along the inner aspect of the plasmalemma.
- (4) The plasmalemma of the individual cells is studded with numerous vesicular

invaginations and also invested by the basal lamina which in some regions may be lacking.

As the myoid cells around the seminiferous tubule are thought to contract (Clermont, 1958), the perineurial cell may also possess some kind of contractile ability.

A long-standing question is the cellular source of the perineurium; ectodermal or mesodermal. Although several candidates have been proposed (Thomas and Olsson, 1984), the leading contenders have been Schwann cells and fibroblasts. Not-with-standing the well-known theory of ectodermal origin by Shanthaveerappa and Bourne (1984), recent studies attribute the source of the perineurial cell to the mesenchym (Scaravilli, 1984; Radek et al. 1986; Bunge et al. 1989). According to Thomas and Jones (1967), Radek et al. (1986) and Scaravilli (1984), the bundles of regenerating nerve fibers after nerve trauma were surrounded by cells which were at first lacking the basal lamina and morphologically resembled fibroblasts, but gradually during several weeks they became covered with the basal lamina and showed characteristics of perineurial cells. Bunge et al. (1989) have demonstrated in culture that perineurial cells were only labelled with retroviral marker when the fibroblast had been previously infected but not when Schwann cells had been infected. The morphological similarities between the perineurial cells around the nerve fascicles and the myoid cells around the seminiferous tubules in the rats suggest a common origin for the both cell types.

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