The Fine Structure of the Node of Ranvier in the Rat Cerebellar Cortex

Fumio NASU and Kenichirou INOMATA
Department of Anatomy (Prof. K. INOMATA), Toho University School of Medicine, Tokyo, Japan

Received June 18, 1987

Summary. The transitional zone between the myelinated and the terminal portions of nerve fibers was electron microscopically investigated in the cerebellar cortex of normal rats. A noteworthy finding in the present study is the existence of a “heminode” consisting of the myelinated and non-myelinated portions, as demonstrated within a single section. The heminode seems to be a characteristic structure in the granular layer of the rat cerebellar cortex.

The node of Ranvier is a specialized structure composed of a paranode and node, a series of pockets along the length of myelinated fibers, in both the peripheral and central nervous system (PETERS et al., 1976). As the myelin sheath approaches a node, the myelin sheath on either side diminishes by layers, leaving the nodal axolemma completely uncovered by any continuous external membrane. This gradually diminishing region is called the paranode. Functionally, the node of Ranvier is important for rapid conduction (LILLIE, 1925; TASAKI, 1939; HODGKIN, 1966; STÄMPFLI, 1981). It has recently been said that this structure is also essential for mediating synaptic transmission (FUNCH and FABER, 1982). In other terms, the node of Ranvier is the effector center for saltatory conduction (CAHALAN, 1978).

The purpose of the present study is to observe the fine structure of the preterminal and terminal portions of axons with special reference to changes in the axoplasm and myelin sheath over the most peripheral node of Ranvier. Direct visualization of those structural changes was attained by obtaining longitudinally cut preterminal and terminal regions of the same axons within single thin sections of the brain.

MATERIALS AND METHODS

Adult male Wistar rats, weighing approximately 300g, were anesthetized by an intraperitoneal injection of sodium pentobarbital and were then perfused through the heart with a mixture of 0.2% picric acid, 2% glutaraldehyde and 3% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3 (1330 mOsm). After several minutes, the cerebellum was removed and immersed in the same fixative for 12 hrs at room temperature (22°C). The material was rinsed with cold 0.1 M phosphate buffer containing 8% sucrose, pH 7.3 (439 mOsm), and then sectioned with a Microslicer (Dosaka EM, Kyoto, Japan) at approximately 500 μm. The sections were exposed to cold 2% osmium-tetroxide in 30
mM PIPES buffer (pH 7.3), dehydrated in a graded series of ethanol and propylene oxide and embedded in Spurr's medium. Ultrathin sections were prepared with a Reichert-Jung Ultracut E, stained with uranyl-acetate and lead citrate, and examined with a JEM 2000 EX electron microscope at 80 kV.

RESULTS

At the transitional zone between the myelinated and terminal regions of the axons, the myelin lamellae terminated layer by layer, the innermost layer of myelin ending first, i.e., farthest from the node. The myelin sheath terminated in a configuration corre-

Fig. 1 and 2. The transitional zone between the myelinated axon and axon terminal in the granular layer of the cerebellar cortex. Synaptic vesicles are evident in the axon terminal. Fig. 1: ×32,000, Fig. 2: ×18,000.
sponding to half of a typical node of Ranvier (Fig. 1, 2).

The axolemma at the node contained mitochondria, tubular or vesicular elements of the endoplasmic reticulum, and microtubules. An electron dense undercoating was present immediately beneath the axon membrane at the terminal node as is the case with typical nodes. The undercoating did not extend beneath the axon membrane of the myelinated portion. Neither did it extend towards the terminal, being interrupted.

Fig. 3 and 4. The transitional zone between the myelinated and non-myelinated nerve fibers in the granular layer of the cerebellar cortex. At the paranode (★), the myelin sheath is completely interrupted. These fibers indicate the heminode. Fig. 3: ×17,000, Fig. 4: ×23,000.
at the initial segment of the terminal portion. The axon slightly bulged outward and formed a bulbous terminal swelling. The axoplasm in the terminal portion contained numerous synaptic vesicles and mitochondria. The former reached the neck of the bulbous swelling, making a distinct border between the myelinated and terminal portions of the axon. These vesicles were round, clear, agranular ones, varying in diameter from 30 to 70 nm. Occasional tubular elements of the endoplasmic reticulum were found in the peripheral area of the axonal ending.

The most striking feature was the existence of nerve fibers consisting of myelinated and non-myelinated portions (Fig. 3, 4). The initial segment of the paranode was the contact between the innermost layer of the myelin sheath and the axon surface membrane. The lamellae of the myelin sheath terminated in an overhanging pattern by which each of the myelin layers came into contact with the axon surface membrane forming terminal loops of the myelin lamellae. At the paranode, the myelin sheath was completely interrupted, thereby making it impossible to detect the myelin sheath. The major dense line of the myelin sheath, together with the appearance of the paranode, open to accommodate the cytoplasm. Within the paranodal cytoplasm, microtubules appeared as circular profiles. Additionally, the axon became thinner from the paranode to the non-myelinated portion. Within the axoplasm from the myelinated to the non-myelinated portion, mitochondria were found to be decreased, while the tubular profiles of the endoplasmic reticulum were increased. At the paranode, the mitochondria changed into a thin, elongated type. At the transitional zone, an electron dense undercoating was present subjacent to the axon membranes but reaching the non-myelinated portion.

DISCUSSION

According to ROSENBLUTH (1984), asymmetry of paranodal structures occurs commonly; i.e., the myelin may appear relatively normal on one side of a node and form typical paranodal structures while being immature on the opposite side of the node or even absent, resulting in the formation of a heminode. He noted that the heminode in the Tremble mouse peripheral nerve was a congenital abnormal myelin. As SPENCER and SCHAUMBURG (1978) speculated, the sequence of events occurring close to the affected nodes of Ranvier appears to hold the key to the process of fiber degeneration. HIRANO et al. (1966) noted cytoplasmic organelles such as dense bodies, fibrils and mitochondria in the lateral loops. According to HIRANO (1984), elongation of the node is apparently accompanied by retraction of the lateral loops. Our findings suggested the presence of a degenerative-like substance at the paranode, yet the lateral loops were intact. Consequently, it is difficult to accept these observations as representative of degeneration.

The contours of the axolemma at the node are usually irregular; beneath is a layer of dense, granular materials (PETERS, 1966). At the non-myelinated portion, we noted the existence of an electron dense undercoating just subjacent to the axonal membrane. In regard to this WAXMAN (1974) reported in his observation of the monkey oculo-motor nucleus that there was no dense undercoating at the synaptic node of Ranvier, and the cytoplasmic undercoating did not extend more than than 2 or 3 μm along the surface of colaterals arising at the nodes (WAXMAN, 1974). A heminode in the from of a useful non-pathological model has so far escaped detection in the central nervous system. At sites of transition between non-myelinated and myelinated axon segments in the retina-optic nerve junction, some non-myelinated axons have exhibited patches of axolemmal undercoating with externally associated astrocytic process (HILDE-
BRAND et al., 1985). Consequently, our observations indicate that the non-myelinated portion may be a part of the node.

In conclusion, the heminode seems to be a fascinating structure in the granular layer of the rat cerebellar cortex.

Acknowledgements. We thank Ms. M. OHARA, Kyushu University, for a critical reading of the manuscript.

REFERENCES

Cahalan, M.: Voltage clamp studies on the node of Ranvier. In: (ed. by) S. G. Waxman: Physiology and pathobiology of axons. Raven Press, New York, 1978 (p. 155-168).

Funch, P. G. and D. S. Faber: Action-potential propagation and orthodromic impulse initiation in Mauthner axon. J. Neurophysiol. 47 : 1214-1231 (1982).

Hildebrand, C., S. Remahi and S. G. Waxman: Axo-oglial relations in the retina-optic nerve junction of the adult rat: electron-microscopic observations. J. Neurocytol. 14 : 597-617 (1985).

Hirano, A.: Nodes of Ranvier in pathological conditions. In: (ed. by) J. C. Zagoren and S. Fedoroff: The node of Ranvier. Academic Press, London, 1984 (p. 213-243).

Hirano, A., H. M. Zimmerman and S. Levine: Myelin in the central nervous system as observed in experimentally induced edema in the rat. J. Cell Biol. 31 : 397-411 (1966).

Hodgkin, A. L.: The conduction of the nervous impulse. Liverpool Univ. Press, Liverpool, 1966.

Lillie, R. S.: Factors affecting transmission and recovery in passive iron nerve model. J. gen. Physiol. 7 : 473-507 (1925).

Peters, A.: The node of Ranvier in the central nervous system. Quart. J. exp. Physiol. 51 : 229-236 (1966).

Peters, A., S. L. Palay and H. deF. Webster: The fine structure of the nervous system. W. B. Saunders, Philadelphia, 1976.

Rosenbluth, J.: Membrane specializations at the nodes of Ranvier and paranodal and juxtaparanodal regions of myelinated central and peripheral nerve fibers. In: (ed. by) J. C. Zagoren and S. Fedoroff: The node of Ranvier. Academic Press, London, 1984 (p. 31-67).

Spencer, P. S. and H. H. Schaumburg: Pathobiology of neurotoxic axonal degeneration. In: (ed. by) S. G. Waxman: Physiology and pathobiology of axons. Raven Press, New York, 1978 (p. 265-282).

Stämpfli, R.: Overview of studies on the physiology of conduction in myelinated nerve fibers. In: (ed. by) S. G. Waxman and J. M. Ritchie: Demyelinating diseases, basic and clinical electrophysiology. Raven Press, New York, 1981 (p. 11-23).

Tasaki, I.: Electro-saltatory transmission of nerve impulse and effect of narcosis upon nerve fiber. Amer. J. Physiol. 127 : 211-227 (1939).

Waxman, S. G.: Ultrastructural differentiation of the axon membrane at synaptic and non-synaptic central nodes of Ranvier. Brain Res. 65 : 338-342 (1974).