Water Translocation from the Axial Cylinder to Myelin Sheath Structures of the Nerve Fiber

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We studied isolated myelinated nerve fibers from frog sciatic nerve surviving in Ringer solution or in water-free liquid perfluorodecalin immiscible with water or mineral oil. Swelling of incisures and perikaryon, loosening of myelin in the node, and formation of the axial cylinder varicosities were found in the fibers surviving in Ringer solution after 5-7 h. The same process, swelling of Schmidt–Lantermann myelin incisures, Schwann cell perikaryon, and loosening of myelin lamellae in the Ranvier nodes was found in water-free perfluorodecalin medium. However, swelling of the perikaryon and incisures spread along the axial cylinder and the reaction of the fiber developed in perfluorodecalin much later and unfolded slower than in the control. These changes developed much sooner and progressed much more rapidly than in perfluorodecalin in fibers surviving in mineral oil. Swelling of the myelin sheath structures in water-free medium indicated an uncommon new form of the neuron-glia relationships: water translocation from the axial cylinder to Schwann cell under unfavorable conditions.

Key Words: *myelin nerve fibers; neuron-glia relationships; perfluorodecalin*

Intravital microscopy is the most adequate method for studies of the structural processes in myelinated nerve fibers (MNF). Using this method, it is possible to trace the dynamics and reversible nature of morphological processes in the neuron determining its functional potentialities [4]. All the known modes of restructuring in living MNF can be described as a universal complex of reactive changes consisting of loosening of the myelin lamellae in the incisures and node with seeming widening of the intersegmental fissure, enlargement of Schwann cell (SC) perikaryon with simultaneous thinning of the axial cylinder in the perikaryon, node, incisures and the relevant formation of varicosities [4]. These changes are unspecific and are observed in many pathological processes: ischemia, mechanical injury, neuritis, allergic demyelinizing process, *etc.* [7,10]. Despite varicose deformations of the axial cylinder, the outer contour of the MNF and its diameter do not change. Swelling of the incisures and other myelin structures are paralleled by the corresponding local decrease of the axial cylinder diameter in these sites. It remains unclear whether the myelin structures swell at the expense of external water (as usual) or all these changes are caused by an uncommon process – water translocation from the axial cylinder into the myelin sheath. We studied this problem.

MATERIALS AND METHODS

In order to solve this problem, aqueous medium in Ringer solution had to be replaced with some other fluid chemically inert medium not containing water and not mixing with water. In our experiments these were liquid perfluorodecalin (PFD) and mineral oil.

Perfluorocarbon compounds are used as blood substitutes with gas transporting function. They are

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capable of tissue oxygenation, are characterized by membrane-stabilizing, cardioprotective, diuretic, and antioxidant effects [2,11] and are used to compensate for massive blood loss, for therapy of critical ischemias of various tissues, of acute and chronic hypovolemia, and in traumatic, hemorrhagic, burn, and infectious toxic shock [1,3,5]. We used PFD as a chemically inert water-free fluid.

Surviving isolated sciatic MNF from 24 frogs were studied. The preparations were plunged in Ringer solution (control), PFD, or mineral oil. In order to obtain isolated fibers, the sciatic nerves were treated with 0.4% pronase (Serva) in Ringer solution for 40 min. The nerve was then accurately laminated, Ringer solution was removed with filter paper, and the prepared nerve was plunged in water-free medium. The absence of water along the nerve fibers was verified under microscope (Ringer solution did not mix with PFD or mineral oil and formed clearly seen contrast droplets). The preparations with residual Ringer solution droplets in the medium were not used in experiments. Intact normal fibers were selected and their fragments at a distance of more than two nodes of Ranvier from the site of the fiber rupture were studied. Control experiments showed that fiber reaction to mechanical injury was usually confined to one marginal internodal segment of the fiber. Local retraction by terminals was seen at the site of fiber rupture, but no axoplasm leakage was seen in any case. The site of injury was spontaneously sealed with myelin.

The preparations were examined under an inverted phase contrast microscope MBI-13 (LOMO) by serial microphotography and subsequent computer processing of the images. A "microbox" was formed with mineral on the slide and covered with a small slide. The data of observations of MNF survival in Ringer solution served as the control. Those preparations (12 nerves) were examined throughout 25 h in Ringer solution. Sixteen experiments with MNF in PFD and 24 experiments with fibers surviving in mineral oil were carried out.

RESULTS

All components of the complex of reactive changes developed in the control MNF preparations surviving in Ringer solution (Fig. 1). Schmidt–Lantermann incisures were swollen and impressed into the axial cylinder. Local loosening of the myelin sheath into several lamellar complexes was seen in the swelling incisures (Fig. 1, *a*). Schwann cells perikaryons were also swollen and impressed into the axial cylinder (Fig. 1, *b*). This local compressure of the incisures and perikaryon into the axial cylinder modified the configuration of the axial cylinder. It acquired a necklace-like shape, the axon compressions alternating with varicosities, the diameter of the compressed segment of the fiber under all incisures remaining strictly the same, equal to approximately $^1/_3$ of its initial diameter. Myelin loosening was seen in the nodes, leading to loss of contrast in the node cone region, as a result of which the node fissure looked dilated (Fig. 1, *c*). Large myelin lamellae cleaved and separated to the side of the node, often forming a structure resembling a secondary cone distant from the fissure. The first changes manifested 30 min after the beginning of experiment. The diameters of these fibers were almost unchanged (by 4.1%, which, in fact, is comparable with the error of measurements).

All components of the complex of reactive changes, found in the control, developed in the preparations surviving in PFD medium (Fig. 2). The perikaryon volume increased and myelin was loosened in sites of Schmidt–Lantermann incisures and in the cones and bulbs of the node of Ranvier (Fig. 3). The perikaryon glioplasm and swelling incisures were impressed into the axial cylinder, forming its varicose deformation. However, swelling of the perikaryon was paralleled by not only its local compression against the axial

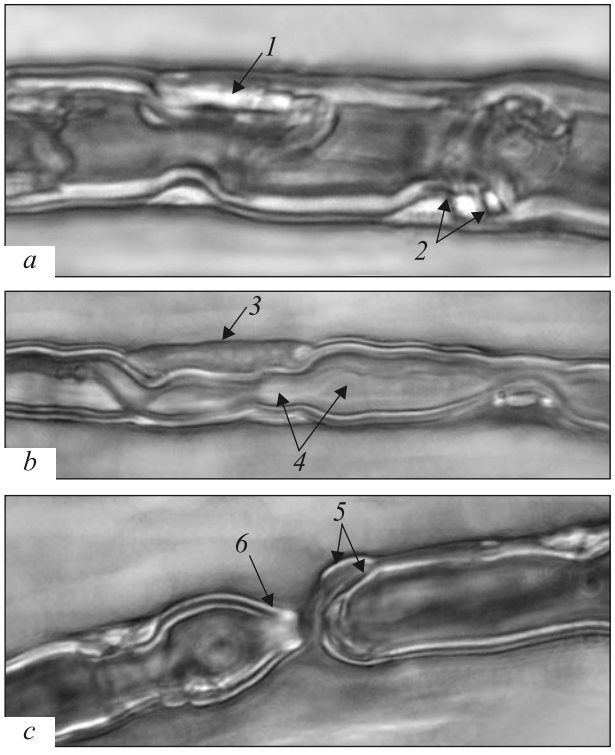

Fig. 1. Complex of reactive changes in MNF surviving in Ringer solution. *a*) swelling of Schmidt–Lantermann myelin incisures (*1*) with loosening of myelin sheath (*2*); *b*) swelling of SC perikaryon (*3*) and compression of axial cylinder by it (*4*); *c*) myelin loosening in the right bulb (*5*) and disappearance of the node cone in the right segment; retained node cone (*6*). Here and in Figs. 2, 3: phase contrast. ×400.

cylinder, but mainly by dissemination of the axial cylinder compression along the fiber (Fig. 3, *a*). However, compressions of the axial cylinder in the perikaryon region were significant in some preparations (Fig. 3, *a*). The diameter of compressed axial cylinder was $^1/_3$-$^1/_5$ of the outer diameter of the fiber. This diameter could be the same over the entire length of swollen perikaryon and its contour was uniform. Despite these pecularities, the varicosities did form between the incisures (Fig. 2). However, they were shorter than in the control and hence, the bridges between them were longer. The nodes of Ranvier in the neighboring myelin segments changed asymmetrically. In case of significant cleavage of myelin lamellae in one cone or in case of its complete disappearance the second cone remained normal for a long time (Fig. 3, *b*). The main distinction of MNF in PFD medium in comparison with control fibers surviving in Ringer solution was a much later and slower development of the structural reaction (sometimes after 20 h). The MNF structure remained normal for a long time. The diameter of the fiber varied between 1.5 and 5.3%, similarly as in the control.

The MNF reaction in fibers surviving in mineral oil developed early (30-40 min after the beginning of experiment). It developed more rapidly than in PFD and was not associated with increase of MNF diameter. The fiber was destroyed after 120-150 min. Local swelling of the myelin sheath in sites of incisures, nodes, and perikaryon resulted in the formation of the axial cylinder varicosities. In contrast to the fibers surviving in PFD, these ones were longer, as the swelling of incisures almost did not disseminate along the fiber.

Since the outer diameter of MNF did not increase much in all three series of experiments, while the myelin sheath structural elements were swollen, we concluded that the axoplasm water fraction was partially translocated from the axial cylinder to the myelin sheath structures. The incisures were swollen, while the axon thinned in the same site and by about the same volume, and only the structures containing the SC glioplasm underwent swelling. The swelling structures did not protrude outside but were depressed inside the axial cylinder. A sort of mass exchange (water exchange) between axons and SC was seen [4].

The study has shown that the complex of nonspecific reactive changes, including swelling of the incisures and perikaryon, myelin loosening in the nodes, and formation of the axial cylinder varicosities, presumably due to aqueous fraction translocation from the axial cylinder to the SC cytoplasm, located mainly in the incisures, node, and perikaryon. This process presumably indicated early colloid changes in the axial cylinder and release of the aqueous fraction from the

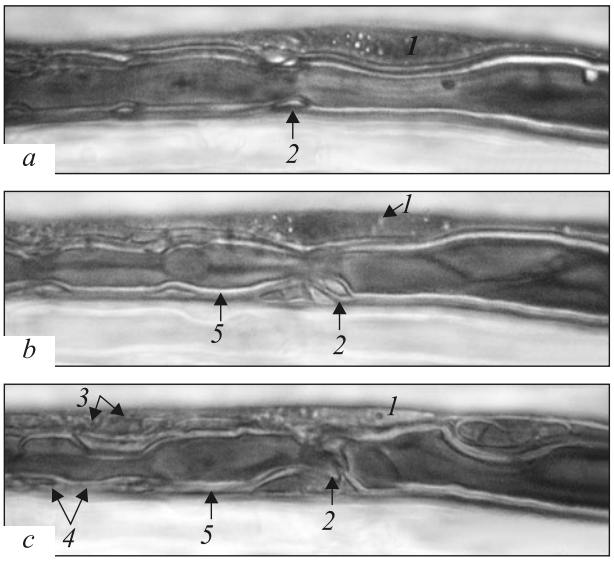

Fig. 2. Simultaneous swelling of Schmidt–Lantermann myelin incisures and SC perikaryon in MNF after 6 h of survival in PFD. *a-c*) dynamics of survival of the same fiber. *1*) SC perikaryon; *2*) myelin swelling and cleavage in Schmidt–Lantermann incisure; *3*) SC cytoplasm, swelling along the fiber; *4*) compact axial structure of the neuroplasm, resistant to compression by incisures; *5*) forming varicosity of axial cylinder.

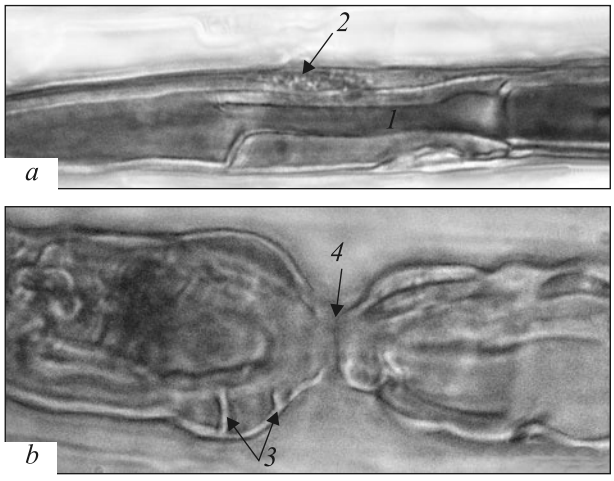

Fig. 3. Reactive changes in the axial cylinder and the node of Ranvier in MNF surviving in PFD medium. *a*) axial cylinder compression and formation of compact axial structure (*1*) in swelling of SC cytoplasm (*2*); *b*) loosening of myelin lamellar complexes (*3*) in the left bulb of the node and "disappearance" of the left cone (*4*) of the node.

axoplasm; changes of this kind develop in various functional treatments and in nervous system diseases. Aggregation of the axon cytoskeleton proteins in disease has been shown by electron microscopy [8,9]. "Separation" of the liquid fraction in the submembrane peripheral compartments of the axon is paralleled by the formation of compact aggregation of filamentous tubular proteins in the axial region of the axial cylinder [4]. The forming compact axial cord cannot be

compressed by the swelling perikaryon and incisures. Aqueous fraction of the neuroplasm is translocated to the cytoplasmatic structures of the myelin sheath. The fact that the reaction of the fiber surviving in PFD is delayed and develops much slower is presumably due to saturation of this fluid by oxygen [3]. This fact can bring about a protective effect, observed in many other cases of PFD use [6].

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REFERENCES

1. L. A. Bogdanova, E. I. Maevskii, R. Ya. Senina, *et al.*, *Ros. Biomed. Zh.*, **2**, 30-36 (2007).
2. G. R. Ivanitskii, *Biofizika*, No. 1, 5-33 (2001).
3. I. N. Kuznetsova, *Obshchaya Reanimatologiya*, **3**, No. 3/1, 17-34 (2007).
4. O. S. Sotnikov, *Statics and Structural Kinetics of Living Asynaptic Dendrites* [in Russian], St. Petersburg (2008).
5. L. V. Usenko, E. N. Kligunenko, and A. A. Krishtafor, *Ukr. Med. Zh.*, **3**, No. 29, 117-120 (2002).
6. N. V. Shevlyakova, A. Ya. Chizhov, E. I. Maevskii, *et al.*, *Ros. Biomed. Zh.*, **52**, 246-247 (2004).
7. G. Acar, G. Tanriover, N. Demirb, *et al.*, *Acta Histochem.*, **106**, No. 5, 363-371 (2004).
8. Z. Sahenk and L. Chen, *J. Neurosc. Res.*, **51**, No. 2, 174-184 (1998).
9. K. D. Tanner, J. D. Levine, and K. S. Topp, *J. Comp. Neurol.*, **395**, No. 4, 481-492 (1998).
10. C. Wang, B. G. Gold, L. J. Kaler, *et al.*, *J. Neurochem.*, **98**, No. 6, 1817-1827 (2006).
11. B. Wiryawan, M. S. Dowhy, and B. P. Fuhrman, *Crit. Care Med.*, **31**, 42-44 (2003).