Bulgarian Academy of Sciences

Acta cytobiologica et morphologica, 1

Sofia • 1989

Neuronal-glial cooperation in the production of myelin phospholipids

E. Zaprianova

Institute of Cell Biology and Morphology, Bulgarian Academy of Sciences, Sofia

The autor's experimental results and the data of other authors are summarized, showing convincingly the existence of cooperation between the neurons and the myelin-forming glial cells during the production of myelin phospholipids. The myelin alkenylacyl-glycerophosphoethanolamines, which correspond to ethanolamine plasmalogens, are synthesized predominantly in the neurons. A compact myelin membrane with the characteristic lipoprotein composition of the mature myelin can be formed only when there are myelin-forming glial cells and neurons.

Key words: myelination, neuron, myelin-forming glial cell, phospholipids.

Myelin is one of the lipid-richest membranes containing 70-80% of lipids by weight. Its main lipids are cholesterol, phospholipids and galactolipids. On a dry-weight basis, the total phospholipids constitute a larger percentage of the myelin lipids and 18% of the hydrated myelin [22].

It is already generally accepted that the principal cells which participate in the formation of the myelin membrane during myelination and remyelination are the Schwann cells in the peripheral nervous system and the oligodendrocytes in the central nervous system. Parallel with this, however, convincing data have been accumulated, which show the existences of varied and close interactions between these glial cells and the neurons during the different phases of myelinogenesis: multiplication and proliferation of the glial cells, wrapping of the glial plasma membrane around the axon, production of myelin components and formation of compact myelin [1, 6, 10, 14, 16, 21, 26, 28, 29, 30, 32]. For the first time on the basis of histochemical studies on the myelination in chick brain, we expressed the view about the existence of such interactions in the production of the myelin components, more specifically of phospholipids [33, 34]. Using histochemical, histoenzyme, electronmicroscopic and autoradiographic methods, in our subsequent experiments we traced the cellular localization of phospholipid synthesis during myelination in the brain of guinea pigs, mice, rats and rabbits [7, 9, 35, 36, 37, 38, 39, 40]. Our experiments were carried out on a precisely defined

myelinating system (nerve or pathway, the oligodendrocytes located along the fibres, and the neurons whose axons form the respective nerve or pathway). It was shown that before and during the period of initial myelination active phospholipid synthesis is observed in the oligodendrocytes situated along the nerve fibres. In the neurons such a synthesis is detected during active myelination. Positive histochemical reaction for phospholipids in the oligodendrocytes and in the neurons is revealed only during these periods. No lipid granules are found during the routine electronmicroscopic investigations, whereas the ultastructural features are these of a cell in which active lipoprotein synthesis takes place.

In our most recent studies we applied more effective techniques for the preservation of lipids and for enhancing their electronmicroscopic density [8, 20]. Under these conditions, lipid granules are discovered at the site of their synthesis and transport. We have established highly osmiophilic granules in the perikarya of the oligodenrocytes and the neurons during the above mentioned periods of myelinogenesis (in the cisterns of the granular endoplasmic reticulum, in the Golgi apparatus and in some vesicles), in the axons and in the myelin sheaths [41].

Naturally, the question arises which of the myelin phospholipids are synthesized in the neurons, and which in the myelin-forming glial cells?

The pathways of phospholipid biosynthesis in the brain do not differ much from well-known pathways of lipid synthesis in other organs. The cytidine pathway discovered by Kennedy [17] is the key pathway of lipid synthesis in the nerve tissue. Binaglia et al. [3] have studied *de novo* synthesis of phosphatidyl choline and phosphatidyl ethanolamine in isolated neurons and glial cells of rabbit cortex in vitro and have found much more active synthesis in the neurons. They concluded that the cytidine-dependent enzyme system is concentrated mainly in the neurons, as compared with the glial cells, and expressed the view that the lipid synthesis takes place mainly in the neurons and that the lipids synthesized in them are utilized by the glia.

Freysz et al. [13] have shown that neuronal phospholipids have a much faster turnover than glial phospholipids. Goracci et al. [15] have traced the incorporation of intraventricularly introduced radioactive ethanolamine into phosphatidyl ethanolamine and ethanolamine plasmalogen in isolated neurons and glial cells of rabbit cortex. In the neurons, maximum labelling of the two phospholipids was observed on the 7th hour after the precursor was introduced, in the glial cells — between the 20th and the 36th hour. Much faster turnover was also observed for the phospholipids in the neurons, as compared with the glial cells. These data give grounds to the authors to assume that the neurons are the site of the most active synthesis of ethanolamine phospholipids, with their subsequent transfer to the glial compartment.

Interesting are the attempts to follow up the further fate of the newly-sythesized neuronal phospholipids. Their selective transferring to the growing neurite has been observed by Pfenniger and Johnson [23] who have investigated the incorporation of [^{3}H]-glycerol on explant cultures of rat superior cervical ganglion. After the pulse with the phospholipid precursor they separated perikarya and neurites microsurgically and extracted their phospholipids. The phospholipid extract from the perikarya exhibited a steep rise followed by a rapid decline in specific radioactivity. In the neurites an increase in specific radioactivity was observed only after a lag period of 60 min. The rapid and massive accumulation of radioactivity in the neuronal perikarya identifies them as the major sites of phospholipid synthesis. The proximodistal transfer of phospholipids could be blocked with the microtubule drug colchicin and the metabolic uncouper, 2,4-dinitrophenol. Pfenniger and Johnson concluded that the data obtained by them indicate preferential export of newly-synthesized phospholipids

from the perikarya into the growing neurites, most likely by rapid axoplasmic transport of formed elements.

The possibility that axonally transported lipids might undergo transaxonal migration and become incorporated into surrounding myelin lamellae was studied by isolating myelin from optic tracts of 22 to 25-day-old rabbits (period of active myelination) at various times, following intraocular injection of [3-^{14}C] serine and [2-^{3}H]glycerol [16]. These studies have revealed that a portion of radioactive material passing down the axon moved into the adjoing myelin sheath, resulting in radiolabelling of specific myelin lipids. Two simultaneous processes were proposed to account for this phenomenon: a) axon-myelin transfer of intact lipids; b) biosynthesis of myelin lipids by myelin-localized enzymes utilizing radiolabelled substrate originating in the axon (reutilization mechanism).

The subsequent investigations have demonstrated the metabolic dependence of myelin on axon in the central nervous system not only in the period of active myelinogenesis [19]. Axon-to-myelin transfer of lipids and lipid precursors have been studied by intraocular injection of [^{14}C]glycerol, [^{3}H]glycerol and [^{32}P]orthophosphate into rabbits, approximately 43-46 and 57 days of age. Choline and ethanolamine phosphoglycerides and myelin showed increasing [^{32}P]-radioactivity between the 7th and the 21st day following injection, while [^{3}H]- and [14C]-radioactivities remained relatively constant. The latter radioactivities decreased, however, in all the axon- and axolemma-enriched fractions during the same period. These results supported the concept of axon-myelin transfer of glycerol-labelled lipids during axonal transport. They showed that inorganic phosphate, possibly generated by catabolic activity within the axon, is able to enter myelin and participate in the reutilization mechanism previously described for serine, choline and acyl chains. The relative invariance of ^{3}H:^{14}C ratio suggested that the majority of glycerol is not reutilized in this manner but probably enters myelin through transfer of intact lipid.

The same two mechanisms were postulated to explain the axon-myelin transfer of phospholipids in the peripheral nervous system [10, 11]. The kinetics of phospholipid constituents transferred from the axon to the myelin sheath were studied in the oculomotor nerve and the ciliary ganglion of chicken after injection with [^{3}H]glycerol and methyl-[^{3}H] choline into the cerebral aqueduct. Quantitative electron microscopic radioautography revealed that labelled lipids were transported in the axons mainly associated with the smooth endoplasmic reticulum. Simultaneously, the labelling of the myelin sheath was found in the Schmidt-Lanterman clefts and the inner myelin layers. The outer Schwann cell cytoplasm and the outer myelin layers contained some label with methyl-[^{3}H]choline, but virtually none with [^{3}H]glycerol. Since [^{3}H]glycerol incorporated into phospholipids is practically not reutilised, the occurrence of label in myelin results from a translocation of entire phospholipid molecules and from their preferential insertion into Schmidt-Lantermann clefts. In this way, the axon-myelin transfer of phospholipids contributes rapidly to the renewal of a limited pool of phospholipids in the inner myelin layers. When methyl- [^{3}H] choline was used as precursor of phospholipids, the rapid appearance of the label in the inner myelin layers was interpreted also as an axon-myelin transfer of labelled phospholipids. However, the additional labelling of the outer Schwann cell cytoplasm adjacent to Schmidt-Lantermann clefts and of outer myelin layers reflects a local reincorporation of the bases released from the axon.

The studies on the axonal transport of ethanolamine glycerophospholipids have demonstrated the preferential accumulation of transported ethnolamine plasmalogen in myelin [6, 12]. Two-week-old chickens were injected into the

cerebral aqueduct with [1-^{3}H] ethanolamine, a suitable precursor to label the two classes of ethanolamine glycerophospholipids (diacyl-glycerophosphoethanolamine which corresponds to phosphatidyl ethanolamine, and alkenyl-glycerophosphoethanolamine which corresponds to ethanolamine plasmalogen). The axonally transported labelled lipids were identified by thin-layer chromatography and located in cell structures by light and electron microscopic autoradiography. The major part of axonally transported labelled lipids consisted of phosphatidyl ethanolamine and ethanolamine plasmalogen. The data obtained showed that axonal transport provides phosphatidyl ethanolamine to the axolemma and membranous elements of nerve endings. Part of them could be transferred from axon to myelin. In contrast, axonally transported ethanolamine plasmalogen could be preferentially and massively transferred to myelin.

Alkenylacyl-glycerophosphoethanolamine (ethanolamine plasmalogen) accounts for more than 30% of the phospholipid content in myelinated nerve fibre. Approximately 80% of the ethanolamine phospholipids of myelin are in plasmalogen form [22]. It was mentioned above that the neurons are the site of most active synthesis of ethanolamine phospholipids.

From all data presented so far it is evident that the existence of cooperation between the neurons and the myelin-forming glial cells for the production of myelin phospholipids has already been proved. This fact is very important and it should be borne in mind by the researchers of the processes of myelination and remyelination. Since the production of phospholipids of the myelin membrane requires the synthesis of part of them to take place in the neurons, then it is obvious that compact myelin cannot be produced by the oligodendrocytes or by the Schwann cells only. The results of all our comprehensive studies have shown that active phospholipid synthesis is observed in the neurons during the period of active myelination, i. e. when the compact myelin membrane is being formed. The transformation of the glial plasma membrane into a compact myelin membrane takes place through the incorporation of lipids and proteins during different periods of myelinogenesis. The term "myelin membrane" is understood to mean the compact myelin membrane which possesses the characteristic lipoprotein composition of the mature myelin, which differs both from the composition of the glial plasma membrane and from the loose myelin. The results of the studies on the formation of the myelin membrane in cell cultures are in support of this view.

P o d u s l o et al. [24] have observed the formation of membrane lamellae adjacent to the cell soma from oligodendrocytes obtained from bovine brain after their cultivation for 16 hours. When subcellular fractions were prepared from the cells in culture, three membrane fractions were obtained: a glial light fraction, an inermediate fraction that electron microscopically consists of whorls of membrane lamellae, and a plasma membrane fraction consisting primarily of small vesicles. In this way the authors were able to study the level of incorporation of the different precursors of the lipids and proteins into the three fractions. They have observed the highest level of incorporation for each lipid in the light glial fraction, and lower but parallel levels of incorporation in the membrane lamellae and plasma membrane fractions. Their incorporation study has shown that initially the cerebrosides, phosphatidyl choline and probably cholesterol were incorporated into these whorls of membrane lamellae. The authors concluded that these whorls of membrane may be the precursors of mature compact myelin.

S a r l i è v e et al. [27] have also obtained multilayer membranous material in dissociated cultures of mouse embryo brain, which they have called "myelin-like or premyelin structures".

The production of extensive myelin-like membranes by highly purified oligodendrocytes in culture was demonstrated also in the study of R o m e et al. [25]. The formation of membranous material around a carbon fibre placed in cultures of isolated oligodendrocytes has been observed [2]. After cultivation for about four weeks, the whole fibre was enwrapped by the processes of the oligodendrocytes. The sucrose gradient centrifugation of these cultures results in the separation of a fraction composed electron microscopically of lamellae and some unidentified debris. The authors also concluded that the membrane fragments might resemble premature myelin.

However, there are some researchers of the myelin formation in cell cultures [5], who state that "cultured neonatal rat oligodendrocytes elaborate the myelin membrane in the absence of neurons". They isolated oligodendrocytes from the brains of 24-hour-old rats, maintained them in culture and performed transmission electron microscopic observations on 14- and 26-day-old cultures. In the 26-day-old cultures they have established the existence of intracellular membranes which were shown to be continuous with the oligodendrocyte processes. These membranes tended to form multilaminar whorls and apparent cylinders with "open" lumen. Some of these membranes were localized within indentations of the cell membrane. They often exhibited the appearance of loose myelin. The researchers have not studied the composition of the membranous fraction, nor the synthetic activity of the oligodendrocytes. They do not rule out the possibility of the existence of a small number of neurons in the cultures. Besides, they have demonstrated the presence of a large amount of membranous material in the cytoplasm of the oligodendrocytes, most of which is found to be in various stages of degeneration.

The tubular myelin in the pulmonary alveoles is also known to possess external features of the myelin membrane [31], as well as "myelin figures" resulting from poor fixation of the lipids.

K n a p p et al. [18] suggested that "axons may play a vital role in orchestrating certain aspects of membrane production by oligodendrocytes." Evidence for this idea stems from both qualitative and quantitative differences between oligodendrocytes in vivo and in vitro. They have maintained primary cultures of neonatal mouse cerebra for up to 4 weeks in the absence of neurons. Oligodendrocytes in these cultures pass through a sequence of cytoarchitectural changes and antigen expression which "mimics" the differentiation of oligodendrocytes in vivo. The majority of them elaborate large sheets of membranous material from the tips and lengths of cell processes. These membranous sheets, which contain galactocerebroside and myelin basic protein, are reminiscent of unwrapped myelin profiles in vivo. They suggested that oligodendrocyte shape and membrane production are in part regulated from within the oligodendrocyte itself.

A role for neurons in regulating the production of myelin components is suggested by the investigations showing that the appearance of galactocerebroside and myelin basic protein are accelerated in neuron-rich embryonic cultures [4].

In conclusion, it can be pointed out that: 1) the hypothesis about neuronal-glial interactions during production of myelin phospholipids, put froward for the first time by us in 1966, has been successfully proved by studies on axonal lipid transport; 2) the existence of neurons and glial cells is necessary for the formation of a compact myelin membrane with the characteristic lipoprotein composition of mature myelin.

References

1. Aguayo, A., I. Charron, G. M. Bray. Potential of Schwann cell from unmyelinated nerves to produce myelin. A quantitative ultrastructural and radioautographic study. — J. Neurocytol., **5**, 1976, 565-573.
2. Althaus, H. H., H. Montz, V. Neuhoff. Isolation and cultivation of mature oligodendroglial cells. — Naturwissenschaften, **71**, 1984, 309-315.
3. Binaglia, L., G. Goracci, G. Porcellati, R. Robertis, H. Woelk. The synthesis of choline and ethanolamine phosphoglycerides in neuronal and glial cells of rabbit in vitro. — J. Neurochem., **21**, 1973, 1068-1076.
4. Bologa, I., J. C. Bisconte, R. Joubert, P. J. Marangos, C. Derbin, F. Rioux, N. Herschkowitz. Accelerated differentiation of oligodendrocytes in neuronal-rich embryonic mouse brain cell cultures. — Brain Res., **252**, 1982, 1283-1287.
5. Bradel, E. J., F. P. Prince. Cultured neonatal rat oligodendrocytes elaborate myelin membrane in the absence of neurons. — J. Neurosci. Res., **9**, 1983, 381-392.
6. Brunetti, M., B. Droz, L. di Giamberardino, H. L. Koenig, F. Carretero, G. Porcellati. Axonal transport of ethanolamine glycerophospholipids. Preferential accumulation of transported ethanolamine plasmalogen in myelin. — Neurochem. Pathol., **1**, 1983, 59-80.
7. Christova, M., E. Zaprianova. Histochemical study on myelinogenesis in guinea-pig brain. — Abstr. 5th Intern. Congr. Histochem. Cytochem., Bucharest, 1976, p. 76.
8. Claude, A. Growth and differentiation of cytoplasmic membranes in the course of lipoprotein granules synthesis in hepatic cells. I. Elaboration of elements of the Golgi complex. — J. Cell Biol., **47**, 1970, 745-766.
9. Deleva, D., E. Zeprianova. Histochemical investigation on the myelination of rabbit hypoglossal and oculomotor nerves. — C. R. Acad. Bulg. Sci., **38**, 1985, 129-132.
10. Droz, B., L. di Giamberardino, H. L. Koenig, J. Boyenval, R. Hassig. Axon-myelin transfer of phospholipid components in the course of their axonal transport as visualized by radioautography. — Brain Res., **155**, 1978, 347-353.
11. Droz, B., L. di Giamberardino, H. L. Koenig. Contribution of axonal transport to the renewal of myelin phospholipids in peripheral nerves. I. Quantitative radioautographic study. — Brain Res., **219**, 1981, 57-72.
12. Droz, B., M. Brunetti, L. di Giamberardino, H. L. Keonig, G. Porcellati. Selective distribution of axonally transported phospholipids to nerve endings and/or to myelin: The case of ethanolamine glycerophospholipids. — In: Phospholipids in the Nervous System. (Ed. L. A. Horroks), N. Y., Raven Press, **2**, 1985, 315-327.
13. Freysz, L., R. Bieth, P. Mandel. Kinetics of the biosynthesis of phospholipids in neurons and glial cells isolated from rat brain cortex. — J. Neurochem., **16**, 1969, 1417-1424.
14. Fulcrand, J., A. Privat. Neuroglial reaction secondary to Wallerian degeneration in the optic nerve of the postnatal rat: Ultrastructural and quantitative study. — J. Comp. Neurol., **176**, 1977, 189-224.
15. Goracci, G., E. Francescangeli, C. Piccinim, L. Binaglia, N. Woelk, G. Porcellati. The metabolism of labelled ethanolamine in neuronal and glial cells of the rabbit in vivo. — J. Neurochem., **24**, 1975, 1181-1186.
16. Haley, J. E., R. W. Leeden. Incorporation of axonally transported substances into myelin lipids. — J. Neurochem., **32**, 1979, 735-742.
17. Kennedy, E. P. Synthesis of phosphatides in isolated mitochondria. — J. Biol. Chem., **291**, 1953, 399-409.
18. Knapp, P. E., W. P. Bartlett, R. P. Skoff. Cultured oligodendrocytes mimics in vivo phenotypic characteristics: cell shape, expression of myelin-specific antigens and membrane production. — Develop. Biol., **120**, 1987, 356-365.
19. Ledeen, R. W., J. E. Haley. Axonal-myelin transfer of glycerol-labeled lipids and inorganic phosphate during axonal transport. — Brain Res., **269**, 1983, 267-275.
20. Ledingham, J. M., P. O. Simpson. The use of p-phenylendyamine in the block to enhance osmium staining for electron microscopy. — Stain. Techn., **47**, 1972, 239-243.
21. Mirsky, R., J. Winter, E. R. Abney, R. M. Pruss, J. Gavrilovic, M. C. Raff. Myelin-specific proteins and glycolipids in rat Schwann cells and oligodendrocytes. — J. Cell Biol., **84**, 1980, 483-494.
22. Norton, W. T. Isolation and characterization of myelin. — In: Myelin (Ed. P. Morell), N. Y., London, Plenum Press, 1977, 161-199.
23. Pfenniger, K. H., M. P. Johnson. Membrane biogenesis in the sprouting neuron. I. Selective transfer of newly synthesized phospholipid into the growing neurite. — J. Cell Biol., **97**, 1983, 1038-1042.

24. Poduslo, S. E., K. Miller, J. S. Wolinsky. The production of a membrane by purified oligodendroglia maintained in culture. — Exper. Cell Res., **137**, 1982, 203-215.
25. Rome, L. H., P. N. Bullock, M. Cardwell, A. M. Adinolfi, D. Swanson. Synthesis of a myelin-like membrane by oligodendrocytes in culture. — J. Neurosci. Res., **15**, 1986, 49-65.
26. Salzer, J. L., A. K. Williams, L. Glaser, R. P. Bungie. Studies of Schwann cell proliferation. II. Characterization of the stimulation and specificity of the response to a neurite membrane fraction. — J. Cell Biol., **84**, 1980, 753-766.
27. Sarliève, L. L., M. Fabre, J. Susz, J. M. Matthieu. Investigation on myelination in vitro: IV. "Myelin-like" or premyelin structures in cultures of dissociated brain cells from 14 to 15-day-old embryonic mice. — J. Neurosci. Res., **10**, 1983, 191-210.
28. Spencer, P. S., H. J. Weinberg. Axonal specification of Scwann cell expression and myelination. — In: Physiology and Pathobiology of Axons. (Ed. S. G. Waxmann), N. Y., Raven Press, 1978, 389-405.
29. Weinberg, H., P. S. Spencer. Studies on the control of myelinogenesis. I. Myelination of regenerating axons after entry into a foreign unmyelinated nerve. — J. Neurocytol., **4**, 1975, 385-418.
30. Weinberg, H., P. S. Spencer. Studies on the control of myelinogenesis. II. Evidence for neuronal regulation of myelination. — Brain Res., **113**, 1976, 363-378.
31. Williams, M. C. Conversion of lamellar body membranes into tubular myelin in alveoli of foetal rat lungs. — J. Cell Biol., **72**, 1977, 260-271.
32. Wood, P. M., R. P. Bunge. Evidence that sensory axons are mitogenic for Schwann cells. — Nature (Lond.) **256**, 1975, 662-664.
33. Zaprianova, E. Histochemical studies on lipid metabolism in the central nervous system in relation to myelination. — 9hD Dissertation, Sofia, 1966.
34. Zaprianova, E. Histochemistry and morphological metabolism of lipids in the chicken brain in relation to myelination. — Acta anat., **75**, 1970, 276-300.
35. Zaprianova, E. Etudes des lipides et des enzymes des noyaux du nerf oculomoteur au cours de sa myélinisation chez le poulet. — Bull. Assoc. Anat., **146**, 1971, 675-678.
36. Zaprianova, E. The activity of the nerve cells at the time of myelination in the central nervous system. — Proceed. VII Intern. Congr. Neuropath., Amsterdam, Excerpta Medica, 1975, 729-732.
37. Zaprianova, E. Myelination in the Central Nervous System. S., BAS, 1980, 124 p.
38. Zaprianova, E. Intercellular interactions between neurones and glial cells in the CNS myelinogenesis. — Folia morph., XIX, 1981, 163-165.
39. Zaprianova, E., J. Taxi. Electron microscopic radioautographic study of phospholipid synthesis in developing brain. — Abstr. 6th Congr. Bulg. Anat. Histol. Embryol., Plovdiv, 1975, p. 109.
40. Zaprianova, E., D. Kadiysky, M. Christova, M. Svetoslavova. Histochemical investigations on the lipid metabolism in the brain of mice during myelination. — C. R. Acad. Bulg. Sci., **35**, 1982, 1279-1282.
41. Zaprianova, E., M. Christova, D. Kadiysky, D. Deleva, E. Katzarova, M. Svetoslavova. Neuron-glia ineractions in the production of CNS myelin phospholipids (morphological data). — Abstr. 3rd Intern. Symp. Myel. Demyel., Varna, 1982, 7-8.