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Axon–Myelin Transfer of Phospholipids and Phospholipid Precursors

Labeling of Myelin Phosphoinositides Through Axonal Transport

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Abstract

Previous studies have provided evidence for axon-to-myelin transfer of intact lipids and lipid precursors for reutilization by myelin enzymes. Several of the lipid constituents of myelin showed significant contralateral/ipsilateral ratios of incorporated radioactivity, indicative of axonal origin, whereas proteins and certain other lipids did not participate in this transfer-reutilization process. The present study will examine the labeling of myelin phosphoinositides by this pathway. Both $^{32}PO_4$ and [^{3}H]inositol were injected monocularly into 7–9-wk-old rabbits and myelin was isolated 7 or 21 days later from pooled optic tracts and superior colliculi. In total lipids ^{32}P counts of the isolated myelin samples showed significant contralateral/ipsilateral ratios as well as increasing magnitude of contralateral-ipsilateral

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differences during the time interval. Thin-layer chromatographic isolation of the myelin phosphoinositides revealed significant ^{32}P-labeling of these species, with PIP and PIP_2 showing time-related increases. This resembled the labeling pattern of the major phospholipids from rabbit optic system myelin in a previous study and suggested incorporation of axon-derived phosphate by myelin-associated enzymes. The ^{32}P label in PI, on the other hand, remained constant between 7 and 21 days, suggesting transfer of intact lipid. This was supported by the labeling pattern with [^{3}H]inositol, which also showed no increase over time for PI. These results suggest axon–myelin transfer of intact PI followed by myelin-localized incorporation of axon-derived phosphate groups into PIP and PIP_2. The general topic of axon–myelin transfer of phospholipids and phospholipid precursors is reviewed.

Index Entries: Myelin; myelin phospholipids: myelin phosphoinositides; axonal–transport; axon–myelin transfer.

Abbreviations: PI, phosphatidylinositol; PIP, phosphatidylinositol 4-monophosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; CL, contralateral; IL, ipsilateral; EM, electron microscope.

Introduction

Previous studies with radiolabeled lipids undergoing axonal transport revealed that a portion of radioactive material passing down the axon moved transcellularly into the adjoining myelin sheath, resulting in labeling of certain myelin lipids (Droz et al., 1978,1981; Haley and Ledeen, 1979; Toews and Morell, 1981; Alberghina et al., 1982a; Ledeen and Haley, 1983). In addition to intact lipid transfer, evidence was presented in those studies for translocation of precursor substances from axon to myelin followed by incorporation into specific myelin lipids. Phosphatidylcholine and phosphatidyl-ethanolamine were examples of lipids well-labeled by these mechanisms, whereas others (e.g., gangliosides and sulfatides) were not labeled in this manner.

These findings prompted the search for myelin-localized enzymes that would account for the observed labeling pattern. The first such activity to be detected was CDP-ethanolamine: 1,2-diacyl-*sn*-glycerol ethanolaminephosphotransferase, the enzyme that completes the synthesis of phosphatidylethanolamine (Wu and Ledeen, 1980). Two additional enzymes required for the synthesis of this phospholipid were subsequently shown to be present in purified myelin: CTP-phosphoethanolamine cytidylyltransferase (Kunishita and Ledeen, 1984) and ethanolamine kinase (Kunishita et al., 1987). Additional work has pointed to the occurrence of parallel enzymes that synthesize phosphatidylcholine (Ledeen, 1992). Thus, evidence has accumulated for the presence of myelin-intrinsic enzymes capable of converting diacylglycerol to the two major phospholipids of the myelin sheath. Concerning the origin of the hydrocarbon chains, axonal transport studies of the kind referred to above indicated transfer of acyl groups from the axon followed by incorporation into specific phospholipids of myelin (Toews and Morell, 1981; Alberghina et al., 1982a). This suggested the presence of acyl-utilizing enzymes involved in phospholipid synthesis, a number of which were subsequently found in purified myelin (Vaswani and Ledeen, 1987,1989a,b; Kahn and Morell, 1989). The absence of such enzymes as ganglioside sialyltransferase and cerebroside sulfotransferase (Wu and Ledeen, 1980) accorded well with the failure of those lipids to become labeled via the axon–myelin transfer pathway.

Enzyme activities related to phosphoinositide metabolism are of special interest owing to the high concentration of polyphosphoinositides in myelin and the rapid turnover of their respective phosphomonoester groups (Ledeen, 1992). Moreover, it was recently shown that a muscarinic agonist and a nonmetabolizable analog of GTP stimulate breakdown of myelin phospho-

inositides, suggesting participation in second messenger pathways within the myelin membrane (Larocca et al., 1987a; Golly et al., 1990). This was consonant with the presence of muscarinic receptors in myelin (Larocca et al., 1987b) along with PIP_2 phosphodiesterase (Keough and Thompson, 1970; Deshmukh et al., 1982; Palmer, 1990) and GTP-binding proteins (Braun et al., 1990; Larocca et al., 1990,1991). PI kinase and PIP kinase have been detected in myelin (Eichberg and Dawson, 1965; Iacobelli, 1969; Schacht, 1976) and in myelin subfractions (Deshmukh et al., 1978), while PI kinase was recently purified from bovine brain myelin (Saltiel et al., 1987). Phosphomonoesterase activities, hydrolyzing PIP_2 to PIP and the latter to PI, are also prominent in myelin and its subfractions (Deshmukh et al., 1982).

In view of this evidence for myelin localization of enzymes catalyzing synthesis and breakdown of myelin phosphoinositides, it becomes of interest to determine the source of the various substrates whose utilization is implied. The myelin-forming glial cell must be viewed as one possible source, considering that this is very likely the origin of the enzymes themselves. This would, however, require efficient transport of small molecules with high turnover rates over rather long distances from cell body to compacted and adaxonal regions of the sheath. The axon, situated much closer to those regions, seems a more likely source for at least some of the required substrates in view of evidence in support of this process for other phospholipids *(see above)*. A combination of axonal and glial contributions is of course a possibility. As in previous studies (Haley and Ledeen, 1979; Ledeen and Haley, 1983) the optic system of the rabbit has been utilized, employing time-related increase in contralateral-ipsilateral difference as the criterion for utilization of axonally-derived substrate. The results demonstrate that two of the phosphoinositides of myelin become labeled through incorporation of axonally derived phosphate, while the third showed evidence of intact lipid transfer. Preliminary results of this study have been presented (Haley et al., 1991).

Methods

Animal Injection

Four groups of 8–9 New Zealand white rabbits, 6–7 wk-old, were injected in the vitreous of the right eye with 25 µL containing [2-^{3}H]myo-inositol (250 µCi) and [^{32}P]orthophosphate (250 µCi), dissolved in saline. The animals had been previously anesthetized with Innovar-Vet. Two groups were sacrificed after 7 d and the other two after 21 d by cardiac puncture following anesthesia. The left and right superior colliculi and optic tracts (distal to the optic chiasm) were dissected out, separately pooled, and kept on ice until processing for myelin isolation a few hours later.

Myelin Isolation and Extraction

Myelin was isolated (Norton and Poduslo, 1973), and modified (Haley et al., 1981), use of a third "floating up" sucrose gradient reduced contaminants to a very low level. Following the final water washes the myelin was lyophilized to dryness. Weighed portions of about 3–8 mg were placed in siliconized glass tubes and extracted (Bell et al., 1982). Siliconized glassware was used throughout. The myelin was extracted with 1 mL of chloroform–methanol (1/1, vol/vol) and then twice with 1 mL of chloroform–methanol (2/1, vol/vol) containing one part in 400 of 12*M* HCl for 20 min at 37°C. After centrifugation the residue was washed with 1 mL of the latter solvent and 0.5 mL chloroform was added to the combined chloroform–methanol extracts. Folch partitioning was carried out by adding 0.2 vol of 100 m*M* HCl. The upper phase was discarded and the lower phase reextracted twice with 0.66 vol chloroform–methanol–100 m*M* HCl (3/48/47). The lower phase was evaporated repeatedly to near dryness (to precipitate solubilized proteins) while adding enough methanol to maintain a single phase. The dried residue was agitated with 2 mL of chloroform–methanol–water (5/5/1) containing 20 µL conc HCl and centrifuged. An aliquot of the supernatant was taken for

counting ("whole lipid extract") and the remaining supernatant was applied to a column of Sephadex LH-20.

Phosphoinositide Isolation

The above Sephadex LH-20 column (1.65 cm × 30 cm), packed in chloroform–methanol–water (5/5/1), was eluted with the latter solvent as previously described (Byrne et al., 1985). Prior to the run the column was calibrated with [^{3}H]phosphatidylcholine. The first 18 mL were discarded and the labeled phospholipids collected in the next 13 mL. This was evaporated, redissolved in a small vol of the same solvent and divided into one-third and two-thirds portions for thin-layer chromatography. Each sample was applied as a 6 cm streak, with adjacent standards, to silica gel 60 plates (20 × 20 cm). The two-thirds portion was chromatographed in the solvent system chloroform–methanol–water–ammonia (90/90/22/7) for isolation of PIP and PIP_2, while the one-third portion was run in the system chloroform–methanol–acetic acid–formic acid–water (140/60/24/8/4) for isolation of PI and other phospholipids. The dried plates were placed in an iodine chamber for band detection and the marked zones were scraped into scintillation vials. Then, 3 mL water were added, the samples sonicated briefly, and then shaken with 10 mL Hydrofluor (National Diagnostics, Somerville, NJ) to form a gel for counting. They were counted as doubly labeled samples with a liquid scintillation spectrometer, and then as singly labeled for ^{3}H after decay of ^{32}P. With double labeling, corrections were made for loss of ^{32}P with time. Except where individual values are shown (Table 1), most data are presented as the average of two values.

Results

Determination of ^{32}P label in total lipid extracts of myelin from both optic tract and superior colliculus revealed significant contralateral/ipsilateral (CL/IL) ratios in all samples (Table 1), indicating the bulk of label to have entered myelin via the axon rather than the circulation. Absolute amounts of transported/transferred radiolabel, indicated by CL–IL differences, showed significant increases between 7 and 21 d. This observation for total phospholipids of myelin accords with a previous study (Ledeen and Haley, 1983), which demonstrated similar behavior for the major phospholipids of myelin under similar experimental conditions. The increase between 7 and 21 d, observed for both optic tract and superior colliculus, is interpreted to indicate a contribution from axonally derived substrate ($^{32}PO_4$) incorporated by myelin localized enzymes.

Table 1
Incorporation of $^{32}PO_4$ into Total Myelin Lipids[a]

	Optic tract		Superior colliculus	
Days	CL - IL	CL/IL	CL - IL	CL/IL
7	21,900	14	12,400	5.8
7	15,600	13	5,520	14
21	35,200	13	17,700	16
21	35,400	11	18,900	13

[a]Groups of rabbits given a monocular injection of $^{32}PO_4$ (and [^{3}H]inositol—*see* Table 2) were sacrificed after 7 or 21 d and myelin isolated from pooled optic tracts and superior colliculi. ^{32}P radioactivity was determined in each whole lipid extract. The amount of ^{32}P transferred to myelin is indicated by the contralateral (CL)–ipsilateral (IL) difference, whereas the ratio CL/IL indicates the relative contributions from axon and circulation. Results are expressed as DPM/10 mg myelin, actual counts in any given sample being approx 30–70% of those shown. The time-related increases in CL–IL differences were 88% for optic tract and 204% for superior colliculus.

Results in this study for individual phosphoinositides indicated significant ^{32}P labeling in all three molecular species, this occuring in myelin from both optic tract and superior colliculus (Fig. 1). In optic tract myelin, PIP and PIP_2 were more heavily labeled than PI and showed increases over time. In superior colliculus the most heavily labeled phosphoinositide was PIP, whose radioactivity also increased over time. PI did not show time-related label increase in myelin from either tissue. Ratios of CL/IL label in all these

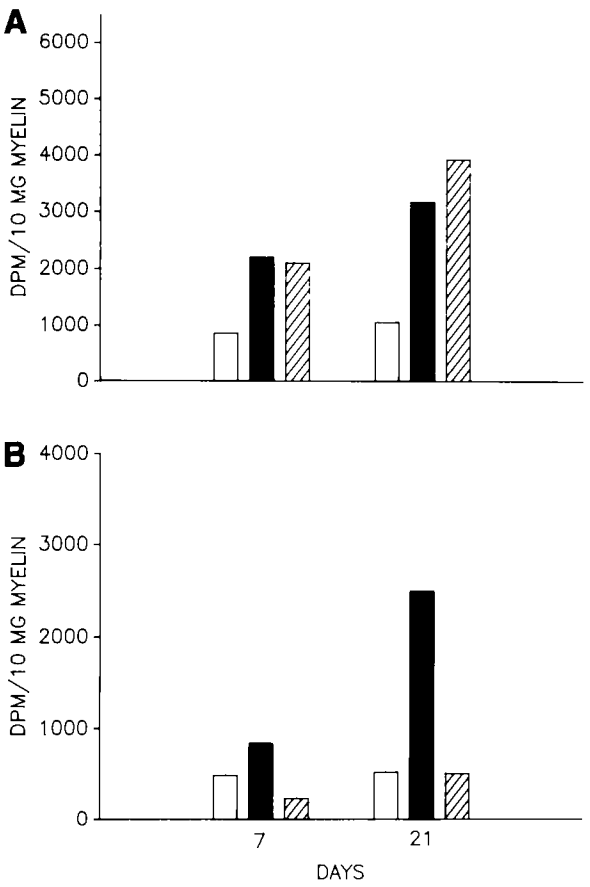

Fig. 1. Amount of axonally derived ^{32}P translocated to phosphoinositides from myelin of optic tract and superior colliculus. Data represent contralateral–ipsilateral differences, expressed as DPM/10 mg myelin. Actual counts in any given sample were approximately 35–75% of those shown. Values shown are the average of two independent determinations. Ratios of contralateral-to-ipsilateral label (not shown) were >7 in all cases and 10 in most. □ = PI; ■ = PIP; ▨ = PIP_2.

phosphoinositides were sufficiently high (>7 in all cases and 10 in most) to preclude systemic incorporation as a major source. Comparing the results in Fig. 1 and Table 1, incorporation of axonally derived ^{32}P into the three phosphoinositides was approx 25% of that going into total phospholipid for optic tract, whereas the corresponding figure for superior colliculus was 17%. These values represent labeling enrichment since phosphoinositides comprise only 5–10% of myelin phospholipids.

Table 2
Incorporation of [^{3}H]inositol into Myelin Phosphoinositides

	7 d		21 d	
	CL–IL	CL/IL	CL–IL	CL/IL
	Optic tract			
PI	2070 sss 14		1090	12
PIP	834 ssss 7.6		571	6.9
PIP_2	234 ssss 4.1		744	7.8
	Superior colliculus			
PI	125 ssss 3.1		176	2.9
PIP	122 ssss 3.9		296	7.0
PIP_2	243 sss 13		200	4.6

[a]These data were derived from the same experiment depicted in Fig. 1 following isolation of doubly labeled phosphoinositides. ^{3}H counts are expressed as DPM/10 mg myelin, actual counts (CL–IL) in any given sample being approx 35–75% of those shown. Values shown are the average of two independent determinations.

The situation was less clear with respect to ^{3}H labeling of myelin phosphoinositides, arising from simultaneous injection of [^{3}H]inositol. Both CL/IL ratios and CL–IL differences were significantly lower than for ^{32}P in the same samples, indicating smaller contributions from the axon (Table 2). Nevertheless, there appeared to be measurable incorporation of [^{3}H]inositol into phosphoinositides of optic tract myelin, although time-related increases between 7 and 21 d were not observed for PI and PIP. These two phospholipids showed even fewer ^{3}H counts in the superior colliculus, as did PIP_2. Taken together, these results suggest that reutilization of axonally derived inositol occurred to a very limited extent if at all. A possible exception was PIP_2 in the optic tract, but the apparent time-dependent increase in ^{3}H labeling must be viewed with caution owing to the technical difficulties inherent in the experiment (e.g., the problem of quantitative isolation of phosphoinositides in high purity by thin-layer chromatography), which are magnified when the level of incorporated counts is low.

Discussion

These experiments have demonstrated that phosphoinositides of optic system myelin become labeled transcellularly by substances undergoing axon-to-myelin transfer during the course of their migration down the axon. All three phosphoinositides isolated from myelin of both optic tract and superior colliculus showed significant incorporation of ^{32}P, with high CL/IL ratios as evidence for axonal origin. In the case of PIP and PIP_2, the fact that ^{32}P increased between 7 and 21 d indicated that these two species were labeled at least in part by myelin-localized enzymes incorporating $^{32}PO_4$ from the axon. As previously mentioned, kinases capable of catalyzing such reactions have been detected in purified CNS myelin. This time-related increase resembles that previously observed for ^{32}P incorporation into myelin phosphatidylcholine and phosphatidylethanolamine (Ledeen and Haley, 1983) and is consistent with the changes of ^{32}P over time in total myelin lipids found in this experiment (Table 1). Similar results were obtained in previous studies employing [^{14}C]serine (Haley and Ledeen, 1979) and [^{14}C]palmitate (Alberghina et al., 1982a) as intravitreally injected precursors in the rabbit optic system; in each case ^{14}C label in myelin lipids increased over several days, whereas that arising from simultaneously injected [^{3}H]glycerol slowly decreased during the same time period. The behavior of the latter label was believed to be characteristic of those substances entering myelin from the axon exclusively in the form of intact lipid.

The labeling pattern of PI, in which ^{32}P remained fairly constant over time (Fig. 1), appears to conform to the latter pattern. The same conclusion is suggested by the behavior of [^{3}H]-inositollabel in PI of optic tract myelin, which decreased between 7 and 21 d (Table 2). The fact that the ^{3}H and ^{32}P radiolabels in PI did not show identical changes over time could reflect differences in the kinetics of biosynthetic incorporation of the two precursors. PIP from the same source showed a similar pattern with respect to ^{3}H (in contrast to time-related increase in ^{32}P) whereas PIP_2 appeared to be differ in that ^{3}H rose over time. However, the latter conclusion is necessarily tentative in view of the low level of counts and resulting potential for error. This also applies to all ^{3}H-labeled samples of phosphoinositides from superior colliculus myelin. A previous study on axonal transport of phosphoinositides in the rabbit optic system reported that only low levels of these substances reach the superior colliculus (Alberghina et al., 1982b). Another report of a preliminary nature suggested that axonally transported phosphoinositides, labeled with [^{3}H]-inositol, did not pass into myelin in appreciable amounts (Droz et al., 1979), consistent with the proposed single pathway mechanism involving transfer of whole lipid only (principally PI).

The question of myelin purity is critical in establishing that the reported radioactivities of isolated lipids represent true myelin constituents rather than contaminants. Comparing the data (Table 2) with ^{3}H radioactivities of whole lipid extracts (not shown) revealed isolated phosphoinositides in toto to contain approximately 20–50% of the latter, depending on time and tissue source. This range of values is well above the likely level of contamination of the isolated myelin. As discussed in recent reviews (Ledeen, 1984,1992), analysis of "microsomal" marker enzymes, representing a mixture of plasmalemmal and intracellular membranes, indicated extremely low levels of contamination in myelin isolated by the method employed here. Adhering axonal fragments, the most likely source of potential contamination, were shown to comprise less than 2% of total protein in such myelin (Haley et al., 1981).

The tentative picture that emerges from this and previous studies is that of myelin phosphoinositides arising at least in part from axonally transported materials, the transfer occurring by both of the proposed mechanisms. PI is suggested to transfer as intact lipid, whereas the phosphate groups subsequently attached to the 4- and 5-positions of inositol derive from the axon and are incorporated by myelin-associated enzymes. This

does not preclude some portion of myelin PIP and PIP_2 arising through transfer of the intact lipids, although there is no direct evidence as yet to support that. The relative ^3H-labeling of optic tract phosphoinositides (Table 2) is consistent with PI being the main (and possibly only) phosphoinositide to be translocated intact. Among the 40 or more myelin localized enzymes reported to date (Ledeen, 1992), there is no indication of any involved in de novo PI synthesis, although it is not known whether a systematic search has been carried out. On the other hand, phospholipid exchange activity capable of transferring PI between myelin and other membranes was reported to be present in the nervous system (Wirtz et al., 1976; Brammer, 1978; Ruenwongsa et al., 1979; Ledeen, 1985).

The above model presumes phosphoinositide synthesis to occur in neuronal perikarya, but the general picture would not change appreciably if synthesis were to occur partially (or even wholly) in the axon prior to transfer. This possibility deserves consideration in view of evidence for intraaxonal synthesis of PI in mouse sciatic nerve (Gould, 1976; Gould et al., 1987; Padilla and Pope, 1991) and the presence of phosphoinositide-synthesizing enzymes in axoplasm (Kumara-Siri and Gould, 1980; Gould and Alberghina, 1990). The above mouse study (Gould, 1976), in which [^3H]-inositol was directly injected into sciatic nerve, revealed progressive increase of radioautographic grains over myelin, coincident with decline of axon-localized grains. Most of the radiolabel was shown to reside in PI and the results, although not definitive, were consistent with axon–myelin transfer of this phosphoinositide.

A recent study demonstrated purified myelin to contain a high level of IP_3-phosphatase but no activity for further degradation (e.g., IP_2-phosphatase) (Larocca et al., 1988; full report in preparation). The results of the present study indicating absence of *de novo* synthesis of PI in myelin would correlate with the failure of IP3 to undergo complete hydrolysis to a (presumably nonexistent) pool of free inositol in myelin. One might speculate that the product of IP_3-phosphatase, IP_2, leaves myelin to be further degraded and recycled in another compartment, possibly the axon, which was shown to be a site of active phosphoinositide synthesis. In view of the second messenger-forming role of myelin phosphoinositides (*see* Introduction), an understanding of this mechanism may require consideration of the axon and myelin sheath together as a functional unit (Fig. 2).

Our results and those of others employing the optic system have their counterpart in studies utilizing various components of the peripheral nervous system. Droz et al. (1978,1979,1981) applied a combination of biochemical and EM radioautographic techniques to chicken ciliary ganglion, radiolabeled glycerol and choline being injected into the cerebral aqueduct to label phospholipids that subsequently migrated into the oculomotor axons and calciform nerve endings. Significant numbers of silver grains were observed over the myelin sheath for both precursors (appreciably more with choline). The rapid appearance (6 h) of grains over myelin following [^3H]glycerol injection and the subsequent 3-d buildup was attributed to transfer of intact phospholipid, whereas the [^3H]choline pattern suggested that in addition to phospholipid transfer, free choline diffused from the axon into adjacent myelin and Schwann cell cytoplasm to become incorporated into myelin lipids. The free choline was believed to be released from transported choline-containing phosphoglycerides, either by base-exchange reaction or the action of phospholipase D. That suggestion accorded with the demonstration of phospholipase D activity in an axolemma-enriched fraction from brain (DeVries et al., 1983). Quantification of EM radioautograms revealed that the label moved from inner to outer layers of myelin (Droz et al., 1981). Gould et al. (1982) obtained similar results with [^3H]choline in rat sciatic nerve, grains appearing over myelin as early as 6 h following [^3H]choline injection into the lumbar spinal cord; labeling of myelin relative to axon increased with time and by 12 d most of the label was localized in myelin.

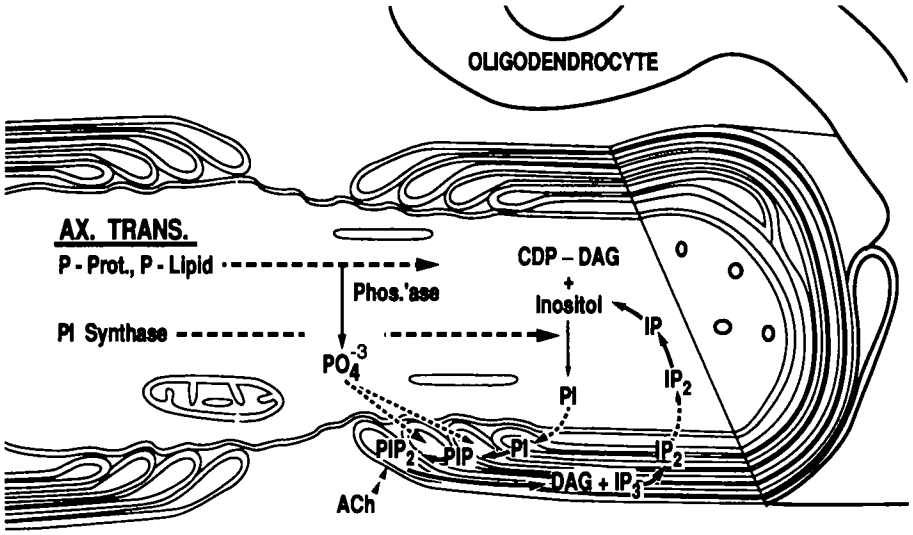

Fig. 2. Proposed mechanism of phoshoinositide cycle involving concerted action of axon and adjacent myelin sheath. Solid arrows depict chemical reactions, dotted arrows transfer between myelin and axon, dashed arrows axonal transport, and arrowhead activation of cholinergic synapse. PI synthase, previously shown to arrive in the axon through axonal transport (Kumara-Siri and Gould, 1980), catalyzes synthesis of PI that would enter myelin and be converted to PIP and PIP_2; the incorporated phosphate is postulated to derive from the axon, following phosphatase hydrolysis of axonally transported phosphoproteins and/or phospholipids. Upon activation of the cholingergic receptor, shown to be present in myelin (Larocca et al., 1987b), PIP_2 is broken down to the second messengers DAG and IP_3. The latter is hydrolyzed in myelin to IP_2, which transfers to the axon for completion of hydrolysis to inositol and recycling into PI. The receptor and myelin-localized enzymes are thought to occur in the paranodal loops and possibly regions of compacted myelin as well.

Droz and coworkers (Brunetti et al., 1983) employed their PNS system to demonstrate that [1-^{3}H]ethanolamine is a useful precursor for tracking axonal and transaxonal movement of both phosphatidylethanolamine and ethanolamine plasmalogen. An interesting selectivity was observed in that the diacyl form migrated predominantly to the axolemma and membranous elements of the nerve endings, whereas the plasmalogen form was preferentially transferred to myelin. Utilizing the rat optic system it was discovered that following intraocular injection of [2-^{14}C]ethanolamine, labeled phosphoethanolamine and CDP-ethanolamine could be detected in extracts of optic nerve and optic tract (Ledeen et al., 1985).

Neither the mechanism of the transfer nor its physiological significance are well understood at present; Brunetti et al. (1981) proposed that axon–myelin transfer of phospholipid could correspond to a series of equilibration processes with reversible exchanges occurring between various membrane structures:

Axonal smooth endoplasmic reticulum ↔ axolemma ↔
glial plasmalemma ↔
loose myelin of the Schmidt-Lanterman clefts ↔
compact myelin

They pointed out that exchange with the inner myelin layers would permit rapid equilibration of phospholipids in that structure, a considerably faster result than arrival of new lipid synthesized by the Schwann cell that requires a number of days (Gould and Dawson, 1976).

The flow of axonal lipids and lipid precursors into the myelin–glial compartment may represent a form of metabolic dependence on the neuron, and possibly interdependence of the two cell types. In 1966, Singer and Salpeter pictured the axon as an open system, permitting flow to and

from adjacent glia, and proposed that the facts of Wallerian degeneration reflected a trophic contribution of the axon to the integrity of the myelin sheath. As early as 1928, Ramon y Cajal had attributed Wallerian degeneration to loss of trophic substances produced by catabolism within the axon. The model of an open system is supported by the recent observation that axonal transport of a fluorescent dye in rat optic nerve resulted in abundant transfer of fluorescent label into adjacent oligodendroglia (Wiggins, 1988), analogous to that previously seen in crayfish giant axons (Viancour et al., 1981). Several other findings have been cited as evidence of axon–glial translocation in both directions (Lasek et al., 1977; Gainer, 1978; Goldstein et al., 1982; Berkeley and Contos, 1897; Grossfield et al., 1988). These examples of material transfer may be viewed as one aspect of the more general phenomenon of neuron–glia communication (Barres, 1991), which includes various forms of signal transduction as well as ionically-mediated membrane modulation (Lev-Ram and Grinvald, 1986).

The transfer of choline to Schwann cells has been specifically cited (Brunetti et al., 1981) as a potentially important contribution of the neuron to myelin maintenance, considering that the entry of choline into peripheral nerve is limited by the blood–nerve barrier and the perineurial sheath (Hendelman and Bunge, 1969). However, such contribution need not be restricted to choline and could well encompass a variety of substances required for renewal of specific myelin components. In addition to the above cited examples, there is evidence that polyamines and nucleosides undergo such translocation (Ingoglia et al., 1982; Lindquist et al., 1985). It is conceivable that axon–myelin transfer could serve a maintenance-repair role during and after myelination, although glia would also be expected to contribute since the axon does not provide substrate for the myelin-localized synthesis of all lipids. Furthermore, there is evidence for continuous flow of lipids from glial cell bodies into the lamellae, even for mature myelin (Hendelman and Bunge, 1969; Gould and Dawson, 1976). Although axon–myelin transfer may be quantitatively less important than the glial contribution, it could have significant influence on the maintenance and functioning of certain specialized structures essential for saltatory conduction at the axon–myelin junction. The relative contribution from each source is among the many questions remaining to be elucidated concerning the nature and significance of the axon–myelin and axon–glia modes of intercellular communication.

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