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Myelin Composition and Activities of CNPase and Na^+, K^+-ATPase in Hypomyelinated "pt" Mutant Rabbit

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Abstract: A disorder of CNS myelination was found in paralytic tremor ("pt") rabbits. The condition is inherited in a sex-linked recessive mode. Ultrastructurally, an obvious myelin deficiency with aberration of myelin sheath formation is observed. The yield of myelin isolation was reduced to 20–30% of control. Myelin isolated from 4-week-old "pt" rabbits contained reduced amounts of galactosphingolipids and of several myelin protein markers. Moreover, myelin basic protein, analyzed by two-dimensional gel electrophoresis, showed a deficit in its more basic components. All these facts suggest a delay in myelin maturation. Ganglioside content was increased as well as Na^+,K^+-ATPase specific activity. 2',3'-Cyclic nucleotide phosphodiesterase (CNPase) specific activity was the same in "pt" as in control myelin but differed by having greater sensitivity to detergent activation. **Key Words:** Myelin—CNPase—ATPase—"pt" rabbit. **Domańska-Janik K. et al.** Myelin composition and activities of CNPase and Na^+,K^+-ATPase in hypomyelinated "pt" mutant rabbit. *J. Neurochem.* **50,** 122–130 (1988).

Paralytic tremor ("pt") mutants with myelin deficiency in the CNS were reported among rabbits bred in the Department of Comparative Biology, Poland (Ossetowska and Leszowski, 1975).

Our previous investigations showed that in "pt" rabbit brain all the myelin lipids are reduced during development (Domańska-Janik et al., 1986). They were in agreement with the morphological studies of Taraszewska (1979) and Taraszewska and Zelman (1985) which showed that affected animals had numerous unmyelinated or thinly and defectively myelinated axons, indicating a prolonged and less active process of myelination.

Among the myelin-deficient mutants only Jimpy (jp) and msd in mice (Baumann, 1980), myelin-deficient (md) in rat (Yanagisawa et al., 1986), and "shaking pup" in dog (Inuzuka et al., 1986) have a mode of inheritance similar to that of "pt" rabbit (X-linked, recessive). In all these sex-linked mutations the myelin deficit is restricted to the CNS. However, by comparison to that in the species mentioned above, the clinical course of the disease is less severe in the "pt" rabbit although complete recovery has never been observed. The rabbit mutant differs also in the density of hypomyelination and oligodendroglial cell number, which is not reduced in "pt" rabbit (Taraszewska, 1983), but is in jp, msd, md, and "shaking pup" mutations.

In all dysmyelinating mutants so far reported (except the CBB hamster mutant, Kunishita et al., 1986) the myelin lamellar structure is abnormal (Matthieu, 1982). In the "pt" rabbit, partly underdeveloped intraperiodic lines and derangement of dense lines in myelin have been reported (Taraszewska, 1983; Taraszewska and Zelman, 1985). The structural anomalies in myelin in known dysmyelinating mutants are reflected by biochemical defects involving the metabolism of myelin lipids, proteins, and glycocompounds. The aim of the present work was to characterize biochemically the myelin fractions obtained from normal and "pt" rabbits.

MATERIALS AND METHODS

The "pt" rabbits were supplied from Department of Comparative Neurology, Polish Academy of Sciences

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Abbreviations used: CNPase, 2',3'-cyclic nucleotide phosphodiesterase; HFA, hydroxy fatty acids; LBP, large basic protein; MBP, myelin basic protein; NEPHGE, nonequilibrium pH gradient electrophoresis; NFA, nonhydroxy fatty acids; PAGE, polyacrylamide gel electrophoresis; PLP, proteolipid protein; PNS, peripheral nervous system; "pt", paralytic tremor; SBP, small basic protein; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid.

(Minsk Maz., Poland). Animals without the neurological symptoms of brain dysfunction served as controls. Animals had a strictly controlled genetic background. Rabbits were killed by intravenous air injection and simultaneously perfused through the heart with Ringer's solution. Brains (without cerebellum) were used to prepare myelin using a discontinuous sucrose gradient by the method of Norton and Poduslo (1973).

Analytical procedure

Lyophilized myelin samples were weighed and rehydrated with 10 volumes of H_2O (vol/vol). One volume of this mixture was sonicated with 12 volumes of chloroform/methanol (1:1 vol/vol). After centrifugation the pellet was reextracted with half of the volume of chloroform/methanol (1:1). Combined supernatants were brought to 2:1 (vol/vol) chloroform/methanol by addition of chloroform.

A sample of the first chloroform/methanol (1:1) myelin suspension was taken out, dried, and resuspended in 1% sodium dodecyl sulfate (SDS) for protein determination.

Determination of galactolipids

Extraction was performed according to Folch-Pi and Sloane-Stanley (1957) with three washes with theoretical upper phase, then taken to dryness under a stream of nitrogen. The lower phase lipid extract was separated further on a Florisil column according to the method of Kishimoto and Radin (1965). Lipids were dissolved in 1 ml of hexane and applied onto a hexane-equilibrated Florisil (200-mesh) column (0.7 cm internal diameter and 15 cm long) and eluted with 35 ml of hexane/ethyl ether (8:2) followed by 35 ml of chloroform/methanol (3:1).

The chloroform/methanol eluate containing galactolipids was analyzed further on silica gel G plates (Merck) developed in a solvent system containing chloroform/methanol/H_2O (70:30:4). The individual galactolipids were visualized by iodine vapor. Cerebrosides and sulfatides were scraped from the plates and eluted from the gel by the procedure described by Benjamins et al. (1976).

The galactolipid concentration was estimated as galactose content and then multiplied by a factor of 4.55 according to the method of Svennerholm (1956).

Determination of gangliosides

The gangliosides were separated from other lipids by the nonpartitioning method of Irvin and Irvin (1979). Lipids were applied onto a Unisil column (200-mesh/0.5 cm internal diameter and 2 cm long) that was equilibrated with chloroform/methanol (4:1). Lipids were eluted with 7 ml of chloroform/methanol (4:1) and then with 5 ml of chloroform/methanol/H_2O (50:50:15 by vol).

Ganglioside fractions obtained in the second eluate were evaporated under nitrogen, then resuspended in Folch theoretical upper phase. The gangliosides were separated from water-soluble substances on reversed-phase C_{18} Sep-Pak cartridges according to the method of Williams and McCluer (1980). Lipid-bound sialic acid was estimated by the method of Svennerholm (1957).

Enzymatic assays

2′,3′-Cyclic nucleotide phosphodiesterase (EC 3.1.4.37, CNPase). CNPase was assayed with 2′,3′-cyclic AMP, based on the method of Kurihara and Tsukada (1967). In brief, a solution of 0.03 *M* adenosine 2′,3′-cyclic phosphate (0.05 ml) was added to a mixture containing 0.05 ml of an enzyme suspension of protein (1–10 μg/sample) diluted in water or various Triton X-100 concentrations from 0.01 to 1%. The standard assay condition chosen was a 10 m*M* substrate concentration and a 15-min incubation time at 37°C as recommended by Tsukada and Suda (1980). As the enzyme is competitively inhibited by its reaction product, the rate of reaction was proportional to enzyme concentration up to 50% substrate hydrolysis. A sample containing the substrate but no enzyme served as control. The reaction was stopped by rapid chilling in an ice bath. The incubation mixture was immediately applied on TLC plates of DC-Alurolle Silica Gel F_{254} (Merck) in a 0.02 ml volume. Chromatograms were developed in saturated ethyl acetate/propanol/NH_3/H_2O (90:60:40:30 by vol). The spots of adenosine 2′-phosphate and adenosine 2′,3′-cyclic phosphate were located using a UV lamp, cut out, and eluted with 4 ml of 0.01 *M* HCl. The absorbance of the supernatant was read at 260 nm on a Kositron spectrophotometer.

Na^+,K^+-activated adenosine triphosphatase (EC 3.6.1.3, Na^+,K^+-ATPase). The enzyme activity of Na^+,K^+-ATPase was determined by measuring the inorganic phosphate liberated from the hydrolysis of ATP in the presence of cations or ouabain.

The assay mixture (0.5 ml final volume) contained brain homogenate or myelin fraction dissolved in water (about 50 μg of protein) and suspended in 0.05 *M* *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) containing 20 m*M* KCl, 150 m*M* NaCl, and 5 m*M* $MgCl_2$. The reaction was initiated by transferring the tubes from ice to a water bath at 37°C for 5 min, adding Tris-ATP to a concentration of 4 m*M*, and incubating for 15 min. The reaction was stopped by cooling the tubes in an ice bath followed by adding 0.5 ml of chilled 10% trichloroacetic acid (TCA) solution. The reagent blank (complete assay mixture with added TCA) was not incubated. After centrifugation, the solution was assayed for P_i by a procedure described by Lin and Way (1984). In brief, an aliquot was transferred to another tube in an ice bath and an equal volume of $FeSO_4$/ammonium molybdate reagent was added. The reagent was prepared just before use by dissolving 4 g of $FeSO_4 \cdot 7H_2O$ in 100 ml of a solution containing 1% ammonium molybdate in 0.6 *M* H_2SO_4. This mixture was kept in the ice bath for 1 h before the absorbance was read at 750 nm. The Na^+,K^+-ATPase activity represents the difference between the activity in the presence and that in the absence of 0.75 m*M* ouabain.

Electron microscopy

The material for electron microscopy was fixed in 1.5% glutaraldehyde in cacodylate buffer (pH 7.4) for 1.5 h and then in 1% OsO_4 in the same buffer, also for 1.5 h. After fixation the tissue was dehydrated in a mixture of ethanol and propylene oxide in an increasing concentration gradient. Then tissue blocks were embedded in Epon 812 and subjected to polymerization at 60°C for 21 days. The polymerized blocks were cut on an LKB-III ultramicrotome.

Ultrathin sections were contrasted on grids with 9% uranyl acetate (aqueous solution) for 12 min and lead citrate, also for 12 min. The microphotographs were taken using JEM 7A electron microscope.

Gel electrophoresis of myelin preparation

The protein content of each myelin preparation was determined according to the method of Lowry et al. (1951) using bovine serum albumin as the standard. Two aliquots of each preparation were taken. One was prepared for

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) by the addition of sample buffer (0.1 *M* Tris-HCl, pH 6.8; 2% SDS; 0.1 *M* dithiothreitol; 10% glycerol; 0.001% bromophenol blue) and heating at 100°C for 2 min. The other was prepared for nonequilibrium pH gradient electrophoresis (NEPHGE) by addition of the following mixture: 2% Nonidet P-40; 2% ampholytes LKB (pH range 3.5–10); 5% β mercaptoethanol; 9.5 *M* urea (O'Farrell, 1975).

SDS-PAGE. Discontinuous 1-mm thick slab gels were composed of a stacking gel (4.5% acrylamide; 0.12% bisacrylamide; 0.125 *M* Tris-HCl, pH 6.8; 0.1% SDS) and a separating gel (12% acrylamide; 0.32% bisacrylamide; 0.375 *M* Tris-HCl, pH 8.8; 0.1% SDS) as described by de Néchaud et al. (1983). The gels were calibrated with kits of molecular weight markers (Combithek-Boehringer Mannheim) and with preparations of mouse brain myelin.

Two-dimensional gel electrophoresis. The first dimension used NEPHGE (O'Farrell et al., 1977). The second dimension was as described above for SDS-PAGE.

Staining of the gels. The gels were stained with Coomassie Blue (0.25% wt/vol in 50% methanol, 7% acetic acid).

Immunoblots. The electrophoretograms were transferred according to Towbin et al. (1979) to nitrocellulose sheets at 0.5 A for 1 h. The sheets were then processed as described by de Néchaud et al. (1986), using as the first antibody a rabbit polyclonal antiserum against human myelin basic protein (MBP) (gift of Dr. C. Jacque) and as a second antibody, a peroxidase-labelled goat antibody directed against rabbit immunoglobulin G (IgG) (Institut Pasteur Production, France).

Statistical analysis

Student's *t* test was used to evaluate significant differences between control and "pt" rabbits.

RESULTS

Morphology

Figure 1 illustrates the differences between myelinating optic nerve of normal and "pt" rabbit. In the myelinating optic nerve of "pt" rabbit not only was the number of myelinated axons reduced but also abnormal lamellar structures were present. There was a decreased compaction of myelin lamellae. Similar morphological changes were reported previously by Taraszewska (1979, 1983).

Myelin composition

The myelin yield isolated from "pt" mutants was reduced, on the basis of its protein content, to 20–30% of control (Fig. 2).

The composition of myelin was evaluated in 4-week-old animals (a period of active myelination, Table 1). The protein content of myelin was normal, but the neutral galactolipids were reduced to 50% of control values.

The proportion of sulfatides to cerebrosides did not differ significantly in mutant myelin as compared to control. However, the levels of cerebrosides containing hydroxylated fatty acids (HFA) was reduced and that containing nonhydroxylated fatty acids (NFA) elevated in the mutant myelin.

The amount of ganglioside connected with "pt" myelin was increased significantly to about 150% of control values. The major ganglioside in myelin (GM1) represented >50% of total gangliosidic sialic acid in controls and was even elevated in "pt" mutants. The other reported myelin specific ganglioside (GM4) was not detected in the extract of rabbit myelin.

Myelin protein markers

The electrophoretic pattern obtained by SDS-PAGE with rabbit CNS myelin showed several protein markers: four forms of the protein tentatively named by analogy to mouse MBP (Barbarese et al., 1977), pre-large (pre-LBP), large (LBP), pre-small (pre-SBP), and small (SBP) basic proteins on the basis of their electrophoretic mobilities and their recognition by an antiserum against MBP—the proteolipid protein (PLP) and the Wolfgram doublet. There was comigration in our gel system between mouse and rabbit PLP, pre-LBP and LBP, but the Wolfgram doublet, the pre-SBP, and the SBP of the rabbit myelin had a lower apparent molecular weight than those of the mouse. All these markers were found at early stages of myelination (Fig. 3, myelin of 17-day-old rabbit) although the myelin fraction contained larger amounts at later stages (Fig. 3, myelin of adult rabbit). Furthermore, there was a slight decrease in their levels in comparing, at young ages, "pt" myelin to control myelin (Fig. 3, 17-day-old rabbit; Fig. 4, 4-week-old rabbit). When 4-week-old control rabbit myelin was analyzed by two-dimensional gel electrophoresis (Fig. 4A), each form of the MBP was resolved in a spot of a very basic isoelectric point (pH unit ⩾ 10) and, for the so-called pre-LBP and LBP, there were additional spots focusing in a less basic range (between 10 and 8 pH units) and yet recognized by an antiserum against MBP (Fig. 4C). Moreover, in 4-week-old "pt" rabbit myelin, the basic component of the pre-LBP was missing although its less basic component was present and still immunorevealed (large open arrow, Fig. 4B and D). These qualitative changes were less pronounced in older rabbits: at 6 weeks, the basic component of the pre-LBP had already appeared in "pt" CNS myelin and, either in normal or in "pt" adult, the level of the less basic component of the pre-LBP was the lowest.

Electrophoretograms of sciatic nerve homogenates from 4- and 6-week-old "pt" rabbits did not differ from controls (results not shown). They displayed the same electrophoretic pattern of MBP as in the CNS myelin, indicating that the electrophoretic features of the pre-LBP are not artifactually obtained after myelin isolation. Moreover, since there is no difference between "pt" and control for the PNS myelin, either in the morphology or in the electrophoretic pattern, the "pt" mutation seems expressed only in the CNS.

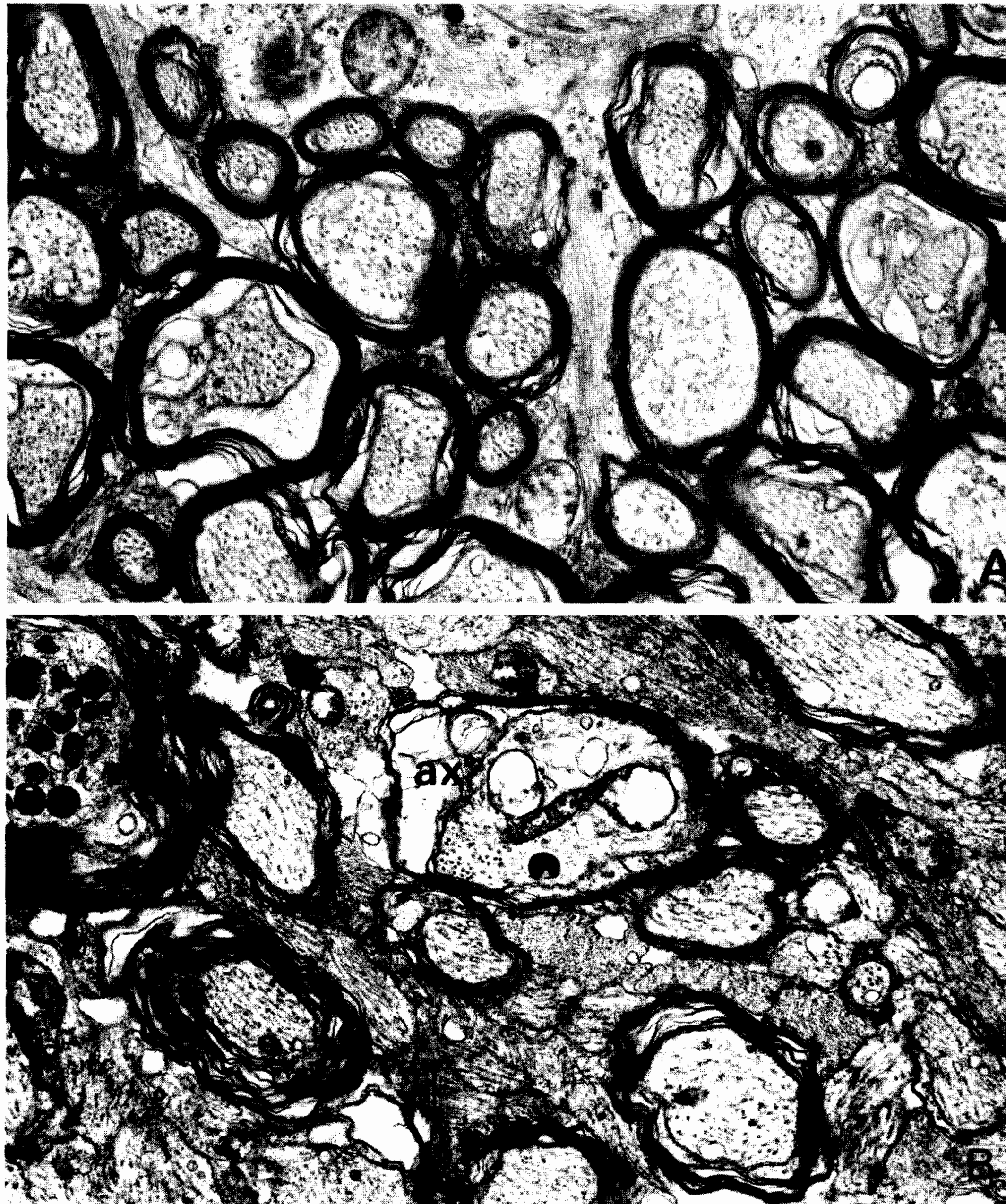

FIG. 1. Ultrastructure of the optic nerve of 4-week-old rabbits. **A:** Normal myelination in control rabbit. **B:** Obvious myelin deficiency from mutant rabbit. Among visible unmyelinated axons an irregular pattern of myelin sheaths with splitting of myelin lamellae or concentrically rolled (redundant) myelin sheaths (arrow) is present. Enlarged periaxonal space and swollen mitochondria are noticed in the axon (ax). ×12,750.

Enzymatic activities

CNPase activity was reduced in homogenates from "pt" rabbit brain to about 70% of control values (Table 2). In myelin, however, the specific activity of the enzyme was normal. The maximal activity of CNPase in myelin could be assayed only after its activation by detergent. However, a substantial part of the enzyme activity can be measured also in untreated, native membrane preparation. This "overt" part of the enzyme was significantly (about two times) increased in "pt" myelin (Fig. 5) in all studied developmental groups. The CNPase in "pt" mutant myelin preparations was found to differ from control also with respect to its sensitivity to Triton X-100 (Fig. 6); the full activation of CNPase could be achieved in a detergent concentration 10 times lower than in myelin obtained from control animals. The detergent treatment caused an increase only in the

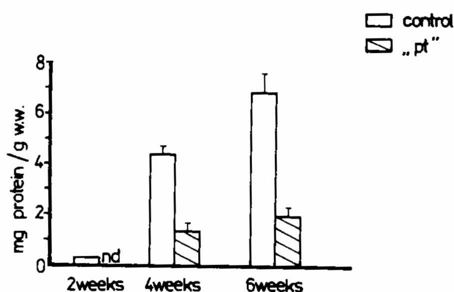

FIG. 2. Amount of myelin fraction recovered from control and "pt" rabbit brains at different ages. The values are means ± SD of three or four myelin separation experiments. nd, not detected.

V_{max} of the enzyme without any effect on the affinity of the enzyme to substrate. K_m was on the level of 5.15 ± 0.64 mM for activated and 5.2 ± 0.65 mM for unactivated enzyme forms.

Na^+,K^+-ATPase specific activity of total brain homogenates was reduced in "pt" rabbits to 70–80% of control values (Table 3). However, in myelin fractions, specific activity of this enzyme increased significantly in "pt" mutants to 171% and 192% of control in 4- and 6-week-old animals, respectively. Specific activity of Na^+,K^+-ATPase was slightly lower in myelin than in homogenates of control rabbits whereas in mutant myelin-connected activity significantly exceeded that of homogenates.

DISCUSSION

Myelin yield in "pt" rabbit brain was reduced to 20–30% of control during development. Abnormalities in myelin composition in "pt" rabbit CNS included a severe reduction in galactolipids, an age-dependent decrease in some myelin protein markers, an increase in gangliosides, and changes in myelin-related enzyme activities (CNPase and Na^+,K^+-ATPase).

TABLE 1. *Myelin composition from 4-week-old control and "pt" rabbits*

	Control	"pt"
Myelin dry weight (mg/g wet weight of tissue)	30.2 ± 2.4	5.0 ± 0.66[b]
Proteins as percent of myelin dry weight	26.3 ± 1.99	29.5 ± 2.66
Neutral galactolipids (μg/mg proteins)	727 ± 53.2	355 ± 57.9[b]
HFA cerebroside	53%	41%
NFA cerebroside	29%	44%
Sulfatide HFA + NFA	17%	15%
Gangliosides (μg *N*-acetylneuraminic acid/mg protein)	2.73 ± 0.31	4.07 ± 0.39[a]
Percent of GM1	51%	68%

The values represent means ± SD from eight experiments except for the values for gangliosides, which are the results from three experiments.

Statistically significant differences: [a]p < 0.01; [b]p < 0.001.

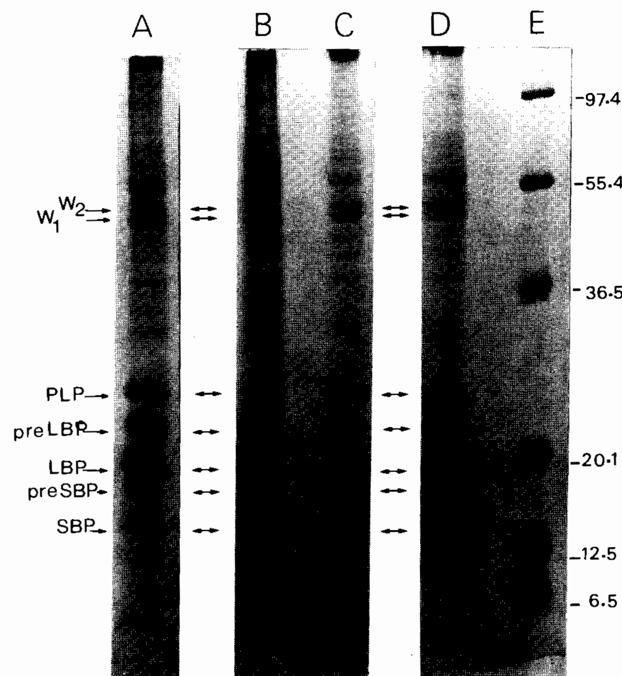

FIG. 3. SDS-PAGE pattern of CNS myelin proteins of control and "pt" mutant rabbits. Coomassie Blue stain. The numbers on the right are the molecular masses (in kilodaltons) of commercial markers (track E). Tracks A, B, C, and D contain myelin preparations of rabbit at different ages (A and B, 17-day-old; C and D, adult). The total protein content loaded in each track is adjusted to 30 μg. Myelin in B and D is prepared from "pt" and in A and C from control animals. W_1, W_2, Wolfgram protein doublet.

Neutral glycosphingolipids

The lipid changes in the whole "pt" rabbit brain during development showed a marked reduction of all the lipids destined for myelin. Among cerebrosides, there was a reduction of the HFA content in isolated myelin as well as in whole brain (Domańska-Janik et al., 1986). The equal distribution of HFA and NFA cerebrosides characteristic for "pt" myelin was reported previously in immature optic nerve (Tennekoon et al., 1980) with a subsequent developmental evolution toward the pattern found in normal control rabbits.

The molar ratio of galactolipids to phospholipids was lower in "pt" rabbits (Domańska-Janik et al., 1986). This ratio is thought to reflect myelin maturation (Baumann et al., 1968).

Acidic glycosphingolipids (gangliosides)

In our study, gangliosides and Na^+,K^+-ATPase were increased in "pt" myelin but their association with myelin is still controversial. The ganglioside increase was due mostly to GM1, the only ganglioside found in rabbit brain which has been suggested previously to be specific for myelin (Norton, 1977; Domańska-Janik et al., 1986). Also the ganglioside

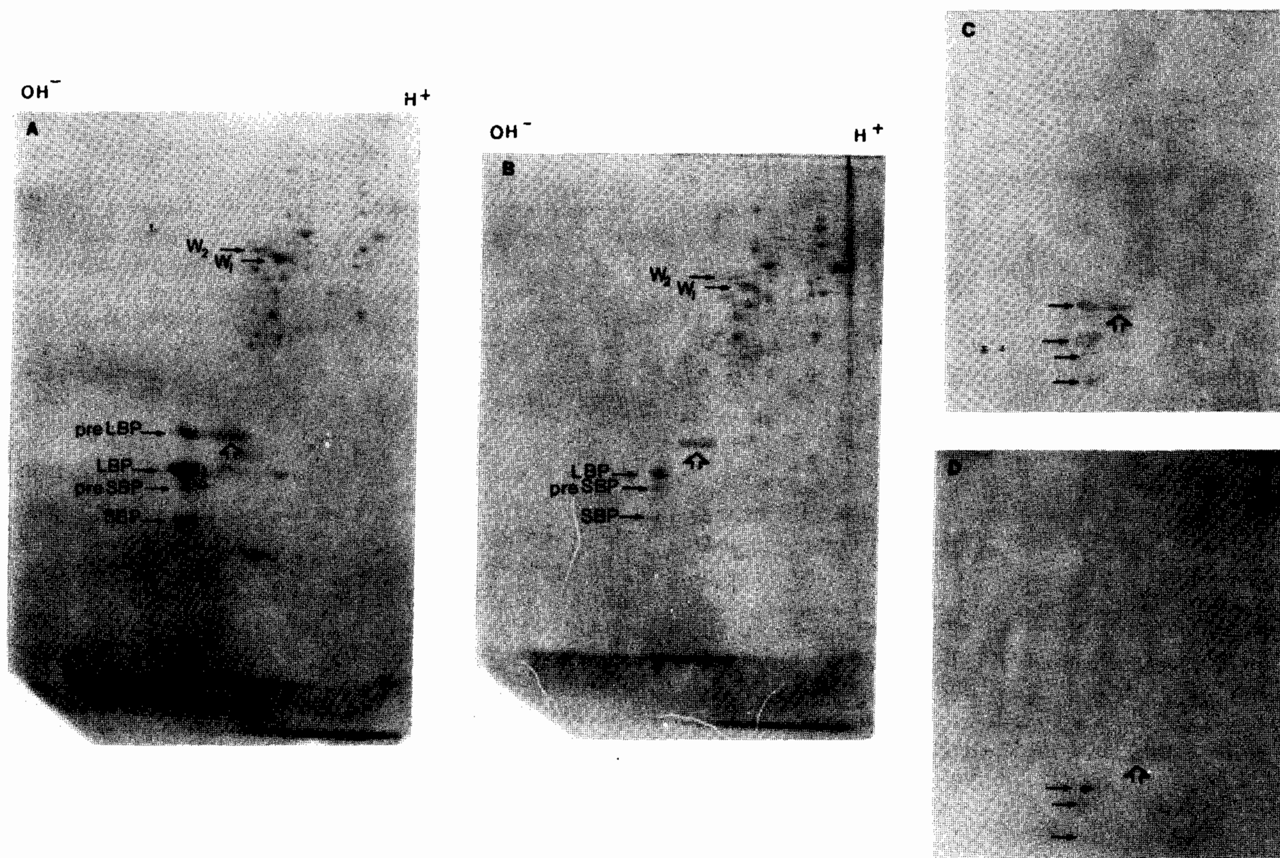

FIG. 4. Two-dimensional gel electrophoretograms of 4-week-old control (**A**) and "pt" (**B**) rabbit myelin preparation. Coomassie Blue stain. The NEPHGE gels were loaded with about 1,000 μg of protein. Immunoblots of similar electrophoregrams (C, control; D, "pt") revealed by an antiserum against MBP. W_2, W_1, Wolfgram protein doublet. PLP does not enter the NEPHGE gel.

pattern obtained in our myelin preparation—consisting principally of GM1 species—differs markedly from that typical for brain tissue as well as for other membraneous subfractions.

Myelin protein markers

There was a general decrease of several protein markers in young "pt" myelin. However, it never reached the amount observed in drastic dysmyelinations such as jp (Sorg et al., 1986; Yanagisawa and Quarles, 1986), md (Yanagisawa et al., 1986), or "shaking pup" (Inuzuka et al., 1986) where the PLP level was very low, or as in shi (Sorg et al., 1986), where virtually no MBP was detected. Moreover, with aging, the deficit in myelin markers seemed to be compensated in "pt" rabbit (Fig. 3).

Some alterations found in the MBP of 4-week-old "pt" rabbit myelin suggested a delayed maturation, e.g., the absence of the more basic component of the pre-LBP. This component seemed to appear later in the "pt" than in the control myelin. The two components of pre-LBP may arise from differences either in

TABLE 2. *Protein content and CNPase activity in brain homogenates and myelin fractions from control and "pt" rabbit brain*

Age of animals		Homogenate Protein (mg/g wet weight)	Homogenate CNPase (μmol/min/mg protein)	Myelin Protein (mg/g wet weight)	Myelin CNPase (μmol/min/mg protein)
4 weeks	Control	111 ± 2.0	5.9 ± 0.22	4.4 ± 0.34	31.8 ± 5.0
	"pt"	108 ± 1.8	4.2 ± 0.85[a]	1.33 ± 0.25[b]	35.1 ± 5.5
6 weeks	Control	94 ± 1.6	8.6 ± 0.83	6.9 ± 0.69	42.3 ± 7.1
	"pt"	96 ± 1.9	6.3 ± 1.24[a]	1.97 ± 0.21[b]	40.0 ± 5.3

The values represent the means ± SD of five experiments.
Significantly different from controls: [a]$p < 0.01$; [b]$p < 0.001$.

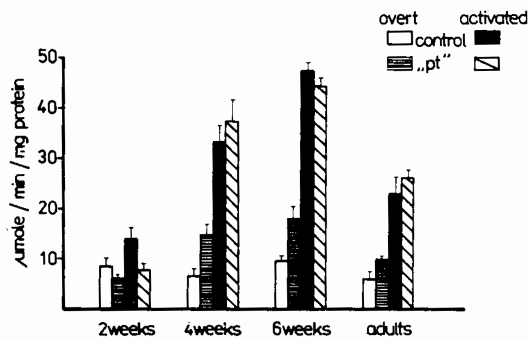

FIG. 5. Overt and Triton X-100-activated CNPase in control and "pt" myelin fractions. The values are means ± SD of four or five estimations.

TABLE 3. *Na^+, K^+-ATPase specific activity in homogenates and myelin fractions of control and "pt" rabbit brain*

Age of animals		µmol P_i/h/mg protein	
		Homogenate	Myelin
4 weeks	Control	13.3 ± 1.40	8.13 ± 1.24
	"pt"	9.6 ± 1.90[a]	13.9 ± 1.85[b]
6 weeks	Control	9.4 ± 1.18	7.12 ± 0.77
	"pt"	7.4 ± 0.80[a]	13.7 ± 1.62[b]

The values represent the means ± SD from six to eight experiments. Statistically significant differences from control: [a]$p < 0.01$; [b]$p < 0.001$.

the sequence or in posttranslational modifications. With regard to the former, although to date data are not available for the rabbit species, there is evidence of alternative splicing of the MBP gene in human and in mouse (de Ferra et al., 1985; Takahashi et al., 1985; Roth et al., 1986) and information about developmental control on this event (Newman et al., 1987). With regard to the latter, Agrawal et al. (1981) have purified two in vivo phosphorylated forms of rabbit MBP—precisely the two called in the present report pre-LBP and LBP and shown to be heterogeneous by two-dimensional electrophoresis. Ulmer and Braun (1983–1984) have described phosphorylated forms of MBP in immature myelin at early developmental stages in mouse brain. But phosphorylation alone, even at five different sites per molecule (Martenson et al., 1983), would not account for the isoelectric focusing over a broad pH range of the two well-separated components of pre-LBP (Fig. 4). The net charge of MBP would also be affected by other reported posttranslational modifications such as loss of COOH-terminal arginine (Deibler et al., 1975), deamidation of glutamine residues (Chou et al., 1976), and glycosylation at one or several sites (Hagopian et al., 1971; Cruz and Moscarello, 1983; Cruz et al., 1984). It could then be supposed that several steps of MBP processing occur from its site of synthesis to its site of insertion in the myelin, leading to a high final ratio of basic MBP components entering into the composition of a well compacted myelin.

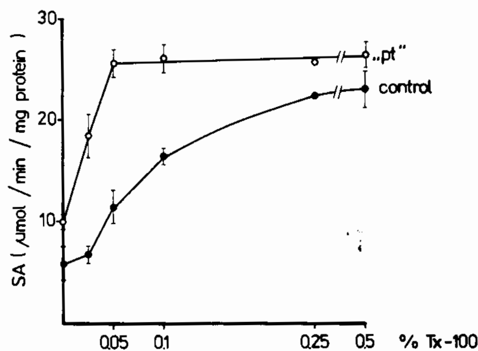

FIG. 6. Activation of adult rabbit myelin CNPase by different concentrations of Triton X-100. Each point represents a mean ± SD of three estimations.

Among several signs of delay in myelin maturation reported here, the recovery of more immature MBP components in the myelin of "pt" rabbit offers then an opportunity to study if it is due to an extension of the availability of mRNAs encoding developmentally controlled MBP variants, to a slowing down of the intracellular processing of MBP forms, or to both.

Na^+,K^+-ATPase

Na^+,K^+-ATPase activity measured in homogenates and myelin fractions of control animals was close to that reported previously for the mouse and rat brain (Bourre et al., 1982; Reiss et al., 1981). The reduced activity of Na^+,K^+-ATPase, similar to that found in "pt" brain homogenates, was reported for all dysmyelinating murine mutants (Bourre et al., 1982).

It is possible that one of the consequences of the structural and functional abnormality in "pt" myelin is the activation of the ion transport system. The recently suggested role for myelin-connected Na^+,K^+-ATPase is the translocation of K^+ released by the axon during the period of neuronal activity (Reiss et al., 1981). The enzyme activity also could be activated by modifications in the myelin fluidity, as suggested previously (Bourre et al., 1982).

The other possible source of Na^+,K^+-ATPase in the myelin preparation could be its contamination by axolemma, which has been reported to contain considerable Na^+,K^+-ATPase activity (De Vries et al., 1978). However, on the basis of unchanged CNPase specific activity in "pt" myelin (see the next paragraph), significantly increased Na^+,K^+-ATPase in the same preparation should be considered as a mutation-specific feature.

CNPase

In spite of the reduction of the above-mentioned myelin components (MBP and galactolipids) CNPase specific activity was very close to control values in

"pt" myelin. Moreover, galactolipid content in total "pt" brain (Domańska-Janik et al., 1986) exceeded markedly the yield of myelin. These findings suggest not only delayed but also changed myelogenesis in "pt" mutant rabbits. The total CNPase activity in "pt" mutant brain was only slightly (70%) reduced, although myelin yield showed severe reduction to 25% of control. A similar dissociation has been observed in other mutants. In shiverer mice, the myelin content is extremely reduced but CNPase in brain homogenate is not affected (Mikoshiba et al., 1980). The same discrepancy has been found in the case of CNPase and galactolipids for heterozygotes affected by the shiverer mutation (Cammer et al., 1984). On the contrary, in jimpy and quaking mice, CNPase decreased in parallel with myelin reduction (Kurihara et al., 1970); however, galactolipid yield was relatively high (Baumann et al., 1968). The reason for these discrepancies is not known but one of possibilities is the insufficient utilization of already synthetised premyelin components for the formation of myelin. A similar conclusion was drawn from the recent histochemical studies on "pt" rabbit (Zelman and Taraszewska, 1984). There was also morphological evidence for a normal or even an elevated number of oligodendrocytes in "pt" mutants (Taraszewska, 1983). The oligodendroglia may be the locus for the deposition of the myelin-related components.

As mentioned before, CNPase specific activity in "pt" myelin was close to control values. After treatment of the membranes with detergents, there was a fourfold increase in CNPase activity in control myelin preparations, indicating that the majority of the enzyme is present in the latent form in native membranes. The remaining, "overt" activity presumably represents the part of the enzyme localized more superficially on the multilamellar myelin membrane. This overt, unactivated CNPase was two times higher in "pt" myelin than in control.

The full activation of the enzyme in mutant myelin was achieved by a 10 times lower Triton X-100 concentration. This enzyme behavior may explain the inability of "pt" mutants to build the proper multilamellar myelin structure. The defectively composed membranes would disintegrate more readily in the presence of detergents. A deficit in MBP and especially in some of its electrophoretic components observed during the period of active myelination could result in defective protein–lipid interactions and thereby affect myelin compaction. Recently we have shown a decrease in the amount of chloroform/methanol-extractable protein–lipid complex in "pt" rabbit myelin (Wikieł et al., 1987). MBP has a particular capacity to form such lipid–protein complexes with acidic phospholipids (Jones and Rumsby, 1977).

The study on heterozygotes affected by the shiverer mutation (Cammer et al., 1984) implies that the deficit in MBP during myelination can affect the synthesis of other myelin constituents. Whether a similar error disturbs myelogenesis in "pt" rabbits is a matter for further study.

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