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Postmicrosomal protein fractions from short-time-predegenerated rat sciatic nerve

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Abstract. The postmicrosomal protein fraction obtained from distal stumps of rat sciatic nerves at 0-6 days following transection were investigated by means of one- and two-dimensional electrophoresis. In all experimental groups, total amount of protein was significantly higher than in the control group. Proteins were resolved into 27 bands after SDS-PAGE. Their molecular weights ranged between 16.2 and 335.4 kDa. Eleven fractions displayed significant quantitative differences. After 2-D-electrophoresis, the pI of the proteins ranged from 4.2 to 7.4. They were resolved to 28 molecular masses from 13.5 kDa to 335.4 kDa. The greatest numbers of fractions (90-109) were observed on the 3rd, 4th, 5th and 6th day after nerve transection. Thus, during first 6 days after transection intensive changes in protein fraction content and composition take place in the distal stump of peripheral nerve. These processes seem to be most prominent on the 4th day after lesion. Results confirm our earlier *in vivo* findings.

Key words: degeneration, electrophoresis, postmicrosomal fractions, proteins

INTRODUCTION

Transection of peripheral nerve induces, distally to the lesion, dramatic changes characteristic of Wallerian degeneration (Aguayo 1985). The proximal axonal portion is able to regenerate across the distal part of the nerve and may reinnervate the target organs, restoring their morphology and function. Cellular, acellular and neurotrophic factors act in concert to support axonal regeneration in the peripheral nervous system (PNS). On the other hand in the central nervous system (CNS) regenerative responses of injured axons are abortive and restoration of nervous tracts does not occur.

The effective nerve regeneration occurring after injury probably depends on biochemical changes following the lesion. There is some evidence that the distal stump of a damaged mammalian peripheral nerve contains some soluble factors which can promote axonal regrowth in the PNS as well as in the CNS (Windebank and Poduslo 1986, Singer et al. 1988, Blexrud et al. 1990).

Previous studies performed at our department have revealed that in the postmicrosomal fractions from nerves injured and predegenerated for 7, 14, 21 and 35 days quantitative and qualitative changes in soluble proteins appeared (Sieroń et al. 1991). Some of these fractions revealed neurotrophic activity in respect to injured hippocampal neurites (Lewin-Kowalik et al. 1992a,b). *In vitro* studies showed two peaks of neurotrophic activity of cut peripheral nerves - the first one appeared in the first ten days and the second was observed after the 28-th day following transection. In our *in vivo* experiments the greatest neurotrophic activity toward injured CNS neurites has been demonstrated by 7-day predegenerated peripheral nerve grafts as well as by purified extracts obtained from them. Thus the first phase of distal stump neurotrophic activity seems to be particularly intensive.

The aim of this study was to examine by means of one- and two-dimensional gel electrophoresis the time--dependent dynamic changes in protein content and composition of postmicrosomal fractions obtained from distal stumps of rat sciatic nerves 1 to 6 days after transection.

METHODS

Experiments were carried out on 70 male Wistar C rats (body weight 150-200 g). All animals were kept on a standard laboratory diet and water *ad libitum*. All experiments were conducted in accordance with Polish Animal Protection Law, and the procedures used were approved by the local Ethics Committee. The animals were divided into seven equal groups - one control (C) and six experimental (D1, D2, D3, D4, D5, D6 - according to the time-lapse of predegeneration). After chloral hydrate intraperitoneal anesthesia (420 mg/kg b. w.) both sciatic nerves were cut near the hip joint. In the control group a sham operation was performed. 1, 2, 3, 4, 5 or 6 days following transection the animals were killed by decapitation. The distal stumps of cut sciatic nerves in the experimental groups or the whole sciatic nerves in the control group were dissected and stored in cold Ringer solution for mammals. In each group, nerves from all 10 animals were pooled. Subsequently the nerves were homogenized using an Ultrasonic Processor GE 50 (ALDRICH, USA) with a buffer with pH 6.4 [0,1 M morpholinoethane sulfonic acid (MES); 1mM ethylene glycol bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA); 0,1 mM ethylenediaminetetraacetic acid (EDTA) (all SERVA, Germany)] (2 ml per 1 g of nerve tissue). The homogenates were filtered through cotton gaze and filtrates were centrifuged at 600 x g for 10 min. In order to obtain postmicrosomal fractions the supernatants were centrifuged at 20,000 x g for 10 min and thereafter at 105,000 x g for 120 min. All procedures were carried out at 0°C in the presence of proteinase inhibitor 1mM phenyl-methyl-sulfonate-fluoride (PMSF) (SIGMA, USA). Total protein concentration was determined by the Bradford method (Bradford 1976).

The obtained postmicrosomal fractions were subjected to electrophoretic analysis, initially in one and thereafter two dimensions. The first step of the electrophoresis of postmicrosomal protein fractions was carried out on SDS-polyacrylamide slab gels (one dimension), according to Laemmli (1970). The protein samples were mixed with half volumes of standard buffer (0.02 M tris(hydroxymethyl)aminomethane (Trizma base) (SIGMA, USA) pH 8,8; 0,002 M ethylenediaminetetraacetic acid (EDTA) (SERVA, Germany); 5% Sodium dodecyl sulfate (SDS) (SIGMA,USA); 10% β-mercaptoethanol (SIGMA, USA); 0,2% Bromophenol Blue (SERVA, Germany)) and denatured at 100°C for 5 min. Each sample well was loaded with 60 µl of protein solution containing 40 µg of protein. Following the electrophoresis, gels were fixed with 20% trichloroacetic acid (TCA) (ROTH, Germany), stained at room temperature in 0.25% Coomasie Brillant Blue R-250 (SERVA, Germany) solution, and subsequently destained (50%

methanol, 10% acetic acid) and photographed. Molecular weights of proteins were calculated by approximation from a standard curve 2 x 3 points according to the Southern method (Southern 1970). Quantification of proteins along each lane was achieved by scanning and continuous plotting of the absorbance at 595 nm on a Beckman R-112 densitometer. Areas under individual absorbance peaks were measured from the scan and expressed as percentages of the total area (Davis 1987).

The same fractions were also analyzed by means of two-dimensional electrophoresis according to O'Farrel (1975). This process consists of two steps: isoelectric focusing and standard SDS-PAGE-electrophoresis. The isoelectric focusing was performed in glass tubes filled with urea gel with a pH gradient of 4.2 to 7.4 (achieved using of 3-10 Pharmalyte (PHARMACIA, Sweden)). One "empty" (without protein fractions) tube was used for preparing the standard curve for the pH estimation. After focusing the gel "noodles" were removed and, by means of 1% agarose, joined with polyacrylamide gels used for two dimensional analysis. After electrophoretic resolution the gels were fixed and silvered (Wray et al. 1981). The molecular weights of the proteins were calculated by the method described above. Stained gels were analyzed on a densitometer and subsequently by means of a computer program (One-Discan; Scanalytics USA). Statistical analysis was performed using the non-parametric Wilcoxon test and one-way Anova (Łomnicki 1995).

RESULTS

In all experimental groups (D1-D6) the total protein concentration was significantly higher ($P<0.05$) than in the control one (Table I). The concetration of proteins one day after nerve transection (group D1) was nearly twice as great as control values, and revealed a tendency to rise on the following days (groups D2 and D3) and then stabilize.

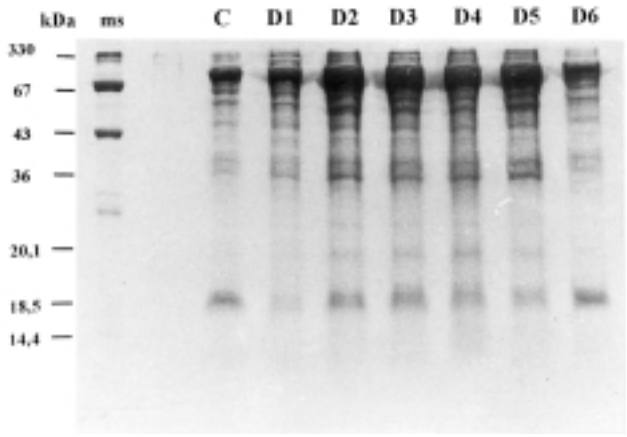

Fig. 1. Electrophoretogram (one dimension) of postmicrosomal fractions from the distal stumps of rat sciatic nerves obtained 1-6 days following transection (D1-D6, respectively) and from non-transected nerves (control, C). ms, molecular weight standards.

The analyzed protein mixtures were resolved into 27 bands (Fig. 1), whose apparent molecular weight ranged from 16.2 to 335.4 kDa in one way SDS-PAGE electrophoresis (Table II). All 27 fractions were present only in the control group and in experimental group D5. The greatest number of protein percentage changes was observed on the 4th, 5th and 6th day after the nerve transection. Six fractions (38.4, 42.4, 54.0, 64.9, 68.5, 75.8) kDa displayed significant changes in their concentrations expressed as a percent of the total as compared to the control. Five fractions (22.5, 24.0, 25.5, 31.6 and 50.4 kDa) disappeared transiently during nerve degeneration. Fraction 22.5 kDa was absent on D1; 24.0 kDa at D1, D2 and D3; 25.5 kDa at D1, D2, D3 and D4; 31.6

Table I

Total protein concentration (mg/g tissue) in postmicrosomal fractions from the distal stumps of non-transected (C) and pre-transected (1-6 days after transection, groups D1-D6, respectively) rat sciatic nerves

C (n = 10)	D1 (n = 10)	D2 (n = 10)	D3 (n = 10)	D4 (n = 10)	D5 (n = 10)	D6 (n = 10)
1.4 ± 0.32	**2.43 ± 0.81**	**3.17 ± 0.44**	**3.45 ± 0.56**	**3.34 ± 0.47**	**3.42 ± 0.50**	**3.30 ± 0.44**

Bold, significantly different from the control (C), $P<0.05$.

Table II

Composition of proteins (% of total) in electrophoretograms of postmicrosomal fractions from distal stumps of the rat sciatic nerves non-transected (C) and at 1st, 2nd, 3rd , 4th, 5th and 6th day following transection (D1-D6)

	Molecular mass kDa (mean ± SD)	Experimental group C (n = 10)	D1 (n = 10)	D2 (n = 10)	D3 (n = 10)	D4 (n = 10)	D5 (n = 10)	D6 (n = 10)
	335.439 ± 1.650	1.24 ± 0.63	1.82 ± 0.47	1.50 ± 0.67	1.60 ± 0.50	1.97 ± 0.52	1.48 ± 0.39	2.16 ± 0.93
	236.059 ± 1.046	0.31 ± 0.24	0.43 ± 0.44	0.42 ± 0.25	0.35 ± 0.27	0.43 ± 0.22	0.37 ± 0.29	0.25 ± 0.15
	182.899 ± 5.976	0.42 ± 0.41	0.37 ± 0.27	0.47 ± 0.40	0.38 ± 0.26	0.37 ± 0.31	0.33 ± 0.26	0.28 ± 0.19
	160.803 ± 5.037	0.32 ± 0.34	0.39 ± 0.27	0.42 ± 0.26	0.32 ± 0.21	0.47 ± 0.26	0.22 ± 0.16	0.32 ± 0.26
	138.942 ± 5.560	0.63 ± 0.54	0.68 ± 0.22	0.97 ± 0.28	0.80 ± 0.37	0.67 ± 0.22	0.33 ± 0.26	0.33 ± 0.23
	119.513 ± 4.654	0.91 ± 0.84	0.55 ± 0.35	0.48 ± 0.30	0.88 ± 0.33	0.80 ± 0.18	0.85 ± 0.27	0.82 ± 0.32
	99.134 ± 4.211	0.97 ± 0.53	0.92 ± 0.30	1.15 ± 0.39	0.90 ± 0.35	0.95 ± 0.31	0.82 ± 0.42	0.72 ± 0.41
	80.312 ± 1.621	0.63 ± 0.54	0.82 ± 0.19	0.85 ± 0.30	0.63 ± 0.25	0.83 ± 0.38	0.92 ± 0.35	0.90 ± 0.32
↑	**75.778 ± 2.495**	1.73 ± 1.12	1.37 ± 0.48	1.27 ± 0.45	**6.72 ± 0.82**	**5.47 ± 0.94**	1.23 ± 0.42	1.17 ± 0.50
↓	**68.510 ± 1.725**	9.15 ± 3.64	9.02 ± 0.71	8.38 ± 0.69	9.72 ± 0.83	**2.10 ± 0.60**	**2.65 ± 0.70**	**1.92 ± 0.48**
↑	**64.889 ± 0.956**	3.10 ± 1.70	2.57 ± 0.56	2.50 ± 0.46	2.48 ± 0.52	**7.80 ± 1.56**	**9.42 ± 0.47**	**10.35 ± 1.25**
	59.966 ± 1.809	2.68 ± 1.74	2.50 ± 0.43	2.25 ± 0.77	2.10 ± 0.27	2.18 ± 0.60	2.08 ± 0.50	1.98 ± 0.56
↑	**53.966 ± 1.174**	2.43 ± 1.40	**5.23 ± 0.31**	**5.45 ± 1.24**	3.03 ± 1.22	2.03 ± 0.62	1.93 ± 0.89	2.82 ± 0.49
	50.371 ± 0.961	2.60 ± 0.84	1.84 ± 0.27	1.87 ± 0.41	5.60 ± 1.16	2.30 ± 0.51	3.05 ± 0.58	*
	46.644 ± 0.883	3.10 ± 1.63	2.76 ± 0.53	2.78 ± 0.48	2.82 ± 0.48	2.65 ± 0.42	2.30 ± 0.89	6.72 ± 0.94
↑	**42.391 ± 0.756**	3.91 ± 2.67	3.68 ± 0.76	5.18 ± 1.54	4.15 ± 0.30	3.03 ± 0.56	**7.40 ± 0.49**	**8.30 ± 0.43**
↑	**38.365 ± 0.764**	1.95 ± 1.11	2.12 ± 0.29	3.15 ± 0.57	2.43 ± 0.50	2.27 ± 0.78	2.45 ± 0.74	**7.20 ± 0.34**
	35.951 ± 0.438	2.98 ± 1.64	3.00 ± 0.40	3.47 ± 0.59	3.15 ± 0.65	2.75 ± 0.55	2.97 ± 0.82	3.35 ± 0.81
	31.588 ± 0.664	5.8 ± 4.64	4.94 ± 0.67	5.37 ± 0.49	5.30 ± 0.60	*	4.65 ± 0.77	5.42 ± 1.55
	25.465 ± 0.418	12.1 ± 6.43	*	*	*	*	10.02 ± 0.92	10.37 ± 0.060
	24.031 ± 0.374	3.10 ± 1.42	*	*	*	2.82 ± 0.41	2.68 ± 0.58	2.65 ± 0.38
	22.506 ± 0.566	3.78 ± 1.54	*	3.60 ± 0.47	3.73 ± 0.51	2.97 ± 0.78	3.15 ± 0.74	2.93 ± 0.69
	21.088 ± 0.126	1.83 ± 1.64	2.02 ± 0.48	2.88 ± 1.34	1.33 ± 0.62	1.90 ± 0.57	1.77 ± 0.57	1.82 ± 0.73
	20.015 ± 0.298	2.24 ± 1.32	1.94 ± 0.29	1.85 ± 0.30	1.72 ± 0.31	1.85 ± 0.60	1.68 ± 0.50	1.75 ± 0.41
	18.620 ± 0.374	1.49 ± 1.32	1.44 ± 0.30	1.50 ± 0.57	1.27 ± 0.50	1.50 ± 0.49	1.38 ± 0.44	1.51 ± 0.19
	17.205 ± 0.192	1.45 ± 1.28	1.22 ± 0.50	1.64 ± 0.56	1.30 ± 0.43	1.42 ± 0.57	1.84 ± 0.90	1.64 ± 0.66
	16.248 ± 0.292	2.15 ± 1.43	2.00 ± 0.68	1.80 ± 0.80	1.80 ± 0.91	1.63 ± 0.78	1.64 ± 0.64	1.72 ± 0.47

Bold, statistically different from the control, $P<0.05$; *, fraction not found; ↑, transient increase; ↓, transient decrease.

kDa at D4 and 50.4 kDa on D6. The 54.0 kDa fraction reached its maximal level on D1 and D2, and returned to control level in the next three days. The 75,8 kDa fraction reached the maximum in the D3 and D4 groups. The concentration of the 68.5 kDa fraction decreased on the 4th to 6th day after nerve transection. At the same time the 64.9 kDa fraction reached its maximum. In groups D5 and D6, the 42.4 kDa fraction showed a significant increase, as did proteins of 38.4 kDa fraction in group D6.

Protein fractions were resolved by isoelectric focusing within pH range of 4.2 to 7.4. After two dimensional analysis of all examined groups, 28 bands with molecular masses ranging from 13.5 kDa to 335.4 kDa were found. This range of molecular masses was very similar to that found in the one-dimensional electrophoresis. However, each band visualized in one dimension was in fact a mixture of molecules with different pI, which gave us over 100 “dots” in 2-D resolution on day 3 and 4 postinjury (Table III).

Table III

Number of bands after one-dimensional SDS-PAGE, pH range and dot number after two-dimensional SDS-PAGE of postmicrosomal fractions from non-transected rat sciatic nerves (C) and 1-6 days after transection (D1-D6, respectively). Total number of molecular masses was 28, however none of examined groups contained all of them

	Number of bands (SDS-PAGE) $n = 10$	pH range (isoelectic focusing) $n = 10$	Number of dots (2D-SDS-PAGE) $n = 10$
C	27	4.2-7.0	62
D1	24	4.2-7.0	65
D2	25	4.4-7.0	68
D3	25	4.2-7.4	102
D4	25	4.2-7.4	109
D5	27	4.2-7.0	90
D6	26	4.4-7.3	99

In the control group, two-dimensional electrophoresis revealed a relatively narrow range of pI and a low number of fractions (Fig. 2A). The greatest number of fractions was observed on the 3rd, 4th, 5th and 6th day following nerve transection.

Proteins obtained from the non-transected nerves contained 62 fractions with pI within the range of 4.2 to 7.0. The widest range applied to the 68.5 kDa fraction, with a pI range of 4.4-7.0. In the D1 group we found 65 fractions in with a pI range from 4.2 to 7.0. The 68.5 kDa fraction here showed a wide range as well (4.6-7,0). The pI of the remaining fractions was from 4.2 to 6.3. The 13.5 kDa fraction was totally absent. Proteins in the D2 group were resolved into 68 fractions. The pH ranged from 4.4 to 7.4. Most abundant were fractions of 80.3, 54.0, 46.6, 42.4 and 18.6 kDa. In the D3 group, 102 frac-

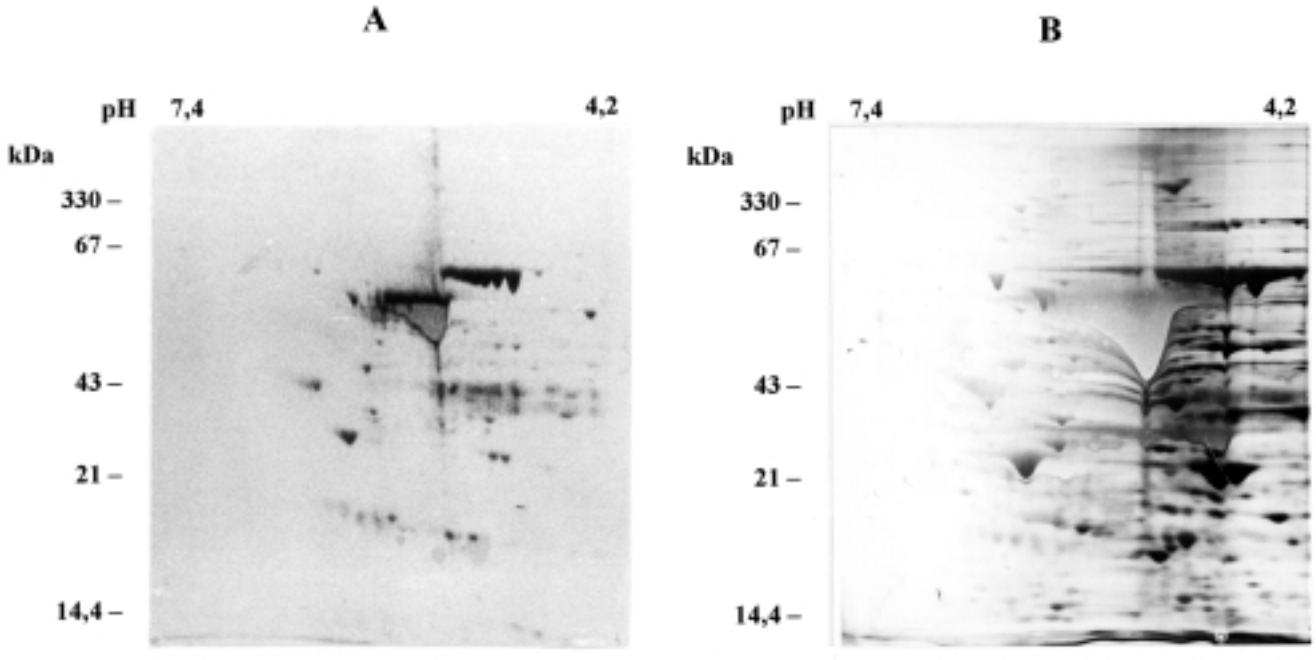

Fig. 2. Electrophoretograms after 2-D-PAGE in groups: A, C: control, non-transected nerves. B, D6: six days after nerve transection.

tions exhibited pH from 4.2 to 7.4 and two of them (80.3 and 75.8 kDa) were present in the highest percentages. In the D4 group we found 109 fractions with pH ranging from 4.2 to 7.4. Most abundant were the fractions of 75.8 and 64.9 kDa as well as fractions from 42.4 to 13.5 kDa. Protein with the highest molecular mass, 335.4 kDa, was present only in the 4.2 pI. Proteins in the D5 group were resolved into 90 fractions with a pH range from 4.2 to 7.0. High molecular weight fractions (335.4 to 182.9 kDa) were present in a narrow range of pH from 4.2 to 5.8 only. In the D6 group, 99 fractions ranged with a pH from 4.4 to 7.3. The biggest changes in the presence as well as the abundance of sub-fractions were related to proteins of 75.8, 68.5, 64.9, 54.0, 42.4 and 38.4 kDa (Fig. 2B).

On the basis of the above findings it is concluded that the most intensive changes in protein composition of extracts obtained from short-time-predegenerated rats' nerves took place 4, 5 and 6 days following nerve transection.

DISCUSSION

On the basis of results presented one may conclude that in postmicrosomal fractions obtained from rat sciatic nerves pre-degenerated for a short time (1-6 days) qualitative and quantitative changes of soluble proteins appeared. These changes concerned protein concentration and composition and depended on the time from the nerve injury.

These results indicate that a short time after nerve transection, protein metabolism is activated in the distal part of degenerating peripheral nerve. Thus, one should wonder in what kind of cell populations would proteins be synthesized. Nerve cells can be excluded as a potential source, because this part of the nerve has no connection with the cell body, and the integrity of its structure is destroyed, however with different intensity depending on the place of nerve transection (Lubińska 1982). And what, if any, role does the distal part of a transected nerve play? Contrary to the prevailing view that no synthesis is present in the axon, recent papers have shown that translation processes do take place there. Some proteins are modified and some are synthesized in the cortical zone of the axon (Koenig and Giuditta 1999). However, these mechanisms were revealed in undamaged neurons, so we do not know whether they had any influence on protein metabolism in the distal part of transected neurites.

In the distal segment after the peripheral nerve cut Schwann cells are particularly active metabolically. Wallerian degeneration, which takes place in the injured peripheral nerve, includes stimulation of Schwann cells division (Lunn et al. 1989, Macklin 1992). According to Aguayo (1985) the period of these divisions lasts for two weeks with highest intensity on the 2nd and 3rd day after cutting. Salonen et al. (1988) demonstrated that the number of cells in the distal stump is four times higher than that in control nerves and some 80% of them were Schwann cells. Looking for a mechanism of this stimulation Baichwal and his collaborators claim that there is a protein that evokes the cells divisions (Baichwal et al. 1988, Baichwal and DeVries 1989). They have shown that in the myelin-containing medium of macrophage cell culture a protein which stimulated Schwann cells mitosis occurred. This factor is produced by macrophages as a metabolite of myelin which is modified in lysosomes. It may be a myelin-derived factor and macrophages release it from myelin membranes. According to other studies, mitogenic factor can be synthesized by macrophages themselves (Unanue 1976, Page et al. 1978). Recent evidence also suggests that myelin basic protein (MBP) associated with ganglioside GM1 and basic fibroblast growth factor (bFGF) receptor is also able to stimulate Schwann cell mitosis (Tzeng et al. 1999). Thus, the increase in Schwann cells division depends on the presence of different protein factors and this division probably results in vigorous synthesis of proteins.

We estimated the amount of proteins per one gram of the nervous tissue and showed the increase of protein contents. We noticed the highest protein concentrations on the 3rd, 5th and 6th day after the nerve injury. This increase could be caused by protein synthesis in denervated Schwann cells and other cellular components of the degenerating distal stump or it could be a result of proteins leaking from the neighboring proximal stump (Zhao et al. 1993) or surrounding blood vessels (Weerasuriya et al. 1990). The ending of the injured nerve also releases a liquid containing some components of extracellular fluid and axoplasm (Carbonetto and Cochard 1987). Our analysis of postmicrosomal fractions originating from the sciatic nerve showed that the total protein content in all examined groups was much higher than in the control group. The postmicrosomal fractions consist of water soluble compounds released during homogenization from axoplasm, fibroblasts and macrophages, as well as the cytoplasm of Schwann cells and components of blood plasma. Poduslo et al. (1985) revealed that the intercellular fluid of injured nerves contained more proteins than the liquid originating from un-

injured rat sciatic nerves. This effect was particularly distinct on the 4th and 7th day after nerve transection. They explained the increase of protein concentration in extracellular fluid by secretion of proteins by non-neuronal cells into the extracellular space. Therefore, we suggest that a similar mechanism may be involved in the increase of total protein concentration demonstrated in our experiment.

Morphological changes during Wallerian degeneration, apart from Schwann cell division, consist of the deterioration of nerve fiber structure, myelin sheet cleavage and the absorption of products of this cleavage. Apart from the above-described morphological changes there are also numerous biochemical alterations present. The distal part of sciatic nerve is the place where calcium ion dependent protease activity is increased (de Nechaud et al. 1986). This causes the destruction of myelin proteins. In 1-D-electrophoresis we showed quantitative changes of eleven protein fractions. It is very difficult to point to the putative background of these changes. However the initial absence and the later appearance of fractions of 25.5, 24 and 22.5 kDa was more or less parallel to the myelin decay. Thus, we may suppose that the appearance of fraction with molecular mass similar to the myelin protein PLP (about 23 000 Da) is concerned with myelin decay after the nerve injury (Alberghina et al. 1985, Poduslo et al. 1985, de Neuchaud et al. 1986, Davis 1987). These fractions were present on the 4th, 5th and 6th day after transection and this could suggest that they also contained protein products of the cleavage of structural elements of degenerating nerve fibers. Distinctive changes refer to proteins with molecular mass between 75.0 to 22.7 kDa. Bigger proteins, probably derived from nerve structural elements, do not show such distinctive statistical differences. The increased percentage of fraction 42.4 kDa occurring on the 5th and 6th day may result from an increase of contents of some group of microtubule associated proteins. It likely that protein S-100 (21 kDa) as well as MAP1 (30-35 kDa), MAP2 (35-40 kDa), MAP3 (18 kDa) and MAP4 (22-24 kDa) are a component of the fractions we have found (Binder et al. 1983, Burgoyne and Cumming 1984, Riederer and Matus 1985, Olmsted 1986). Such proteins are necessary for microtubule and neurofilament building in the regenerating neurites and proteins related to microtubule form a connection between the microtubule and neurofilament fibers in neuron outgrowths. They appear in fetal development (MAP4) or on the first day after birth (MAP2). Some of them occur in nerve growth cones (MAP3) when growth begins but later they disappear. This means that they become undetectable in cerebral cells of adult animals. So we may suppose that some proteins we detected originate from the gradual microtubule decomposition.

After 2-D electrophoresis we have determined that there are about 100 proteins with different isoelectrical points contained in 28 fractions visualized in the one dimensional SDS-PAGE. The spectrum of sub-fractions and their pH ranges changed according to the time after the nerve injury.

This may be explained by the post-translational modification of proteins which takes place in the distal part of the nerve. This process is particularly active as far as proteins with molecular masses of 80-90 kDa, 53-66 kDa, 22-46 kDa and 17 kDa are concerned. Their isoelectric points ranged from 5.0 to 7.9 (Chakraborty et al. 1987). According to Ingoglia et al. (1991), proteins that are the subjects of post-translational modification may form aggregates of higher molecular masses and may also bind to substances that caused their degradation. Changes of protein fraction contents prove that there are dynamic fluctuations of parallel processes of degradation and synthesis. Increases in the percentage of individual protein fractions, found in our work, are the result of an increased number of proteins of the same molecular weight but different pI building this fraction. This rise of protein number may be the result of modification processes described above or of the synthesis of the proteins *de novo*.

Fluctuation of quantitative and qualitative protein contents are relevant to the results of the *in vivo* experiments performed by our group. Predegenerated peripheral nerve implants were used to promote the regeneration of injured hippocampal neurites and retinal ganglion cells (Lewin-Kowalik et al. 1992a,b, Gołka et al. 1998). Purified fractions obtained from the distal part of predegenerated nerves were also used for the same purpose. It was ascertained that neurotrophic activity in the distal segment of injured peripheral nerve fluctuated in the first week after transection. As far as implants are concerned, we observed a biphasic increase of growth promoting activity on the 4th and 7th day (Lewin-Kowalik et al. 1997, Lewin-Kowalik et al. 1998). Thus *in vivo* neurotrophic activity during first 6 days after transection is simultaneous with intensive changes in protein content and composition in the distal stump. Processes taking place on the 4th day seem to be especially interesting. The neurotrophic activity of predegenerated

distal stumps can originate from newly synthesized proteins or may be the result of a new role played by proteins already known. In order to solve this problem, identification of these proteins in Western blot and their isolation by means of HPLC is necessary. This could be followed by the application of individual fractions in the *in vivo* experiment to check their neurotrophic abilities.

REFERENCES

Aguayo A.J. (1985) Axonal regeneration from injured neurons in the adult mammalian central nervous system. In: Synaptic plasticity (Ed. C.W. Cotman). The Guilford Press, New York, p. 457-483.

Alberghina M., Bernocchi G., Biggiogera M., Viola M., Karlson J.O., Manfredi-Romanini R., Giuffrida A.M. (1985) Synthesis of myelin proteins and ultrastructural investigations in regenerating rat sciatic nerve. J. Neurosci. Res. 13: 551-562.

Baichwal R.R., Bigbe J.W., DeVries G.H. (1988) Macrophage-mediated myelin-related mitogenic factor for cultured Schwann cells. Proc. Natl. Acad. Sci. USA 85: 1701-1705.

Baichwal R.R., DeVries G.H. (1989) A mitogen for Schwann cells is derived from myelin basic protein. Biochem. Biophys. Res. Com. 164: 883-888.

Binder L.I., Frankfurter A., Rebhun L.I., Kim H., Caceres A., Payne M.R., Rebhun L.I. (1984) Heterogenity of microtubule-associated protein 2 during rat brain development. Proc. Natl. Acad. Sci. USA 81: 5613-5617.

Blexrud M.D., Lee D.A., Windebank A.J., Brunden K.R. (1990) Kinetics of production of a novel growth factor after peripheral nerve injury. J. Neurol. Sci. 98: 287-299.

Bradford M.M. (1976) A rapid and sensitive method for the quantation of microgram quantities of protein utilizing the principle of protein-dye binding. Annal. Biochem. 72: 248-254.

Burgoyne R.D., Cumming R. (1984) Ontogeny of microtubule-associated protein 2 in rat cerebellum: Differential expression of the doublet polypeptides. Neuroscience 11: 156-167.

Carbonetto S., Cochard P. (1987) In vitro studies on the control of nerve fiber growth by the extracellular matrix of the nervous system. J. Physiol. 82: 258-270

Chakraborty G., Leach T., Zanakis M.F., Sturman J.A., Ingoglia N.A. (1987) Posttranslational protein modification by polyamines in intact and regenerating nerves. J. Neurochem. 48: 669-675.

Davis H.L. (1987) Sciatic nerve protein composition in normal and dystrophic C57BL/6J mice. Neurosci. Lett. 75: 95-100.

de Nechaud B., Gumpel M., Bourre J. M. (1987) Changes in some myelin protein markers and in cytoskeletal components during Wallerian degeneration of mouse sciatic nerve. J. Neurochem. 46: 708-716

Gołka B., Lewin-Kowalik J., Święch-Sabuda E., Larysz-Brysz M., Górka D. (1998) Regrowth of the optic nerve fibres and survival of retinal ganglion cells after predegenerated nerve grafts implantation. Eur. J. Neurosci. 10: (Suppl.) p. 333.

Ingoglia N. A., Chakraborty G., Yu M., Luo D., Sturman J.A. (1991) Proteins isolated from regenerating sciatic nerves of rats form aggregates following posttraslational amino acid modification. J. Mol. Neurosci. 2: 185-192.

Koenig E., Giuditta A. (1999) Protein-synthesizing machinery in the axon compartment. Neuroscience 89: 5-15.

Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 222: 680-685.

Lewin-Kowalik J., Górka D., Larysz-Brysz M., Gołka B., Święch-Sabuda E., Małecka-Tendera E., Krause M. (1997/1998) Short-time-predegenerated peripheral nerve grafts promote regrowth of injured hippocampal neurites. Acta Physiol. Hung. 85: 259-268.

Lewin- Kowalik J., Górka D., Larysz-Brysz M., Gołka B., Święch-Sabuda E., Małecka-Tendera E., Krause M. (1998) Purified extracts from short-time-predegenerated rats sciatic nerves promote the regrowth of injured hippocampal neurites. Acta Physiol. Hung. 85: 325-334.

Lewin- Kowalik J., Sieroń A.L., Krause M., Barski J.J., Górka D. (1992a) The influence of peripheral nerve grafts predegeneration stage on the regrowth of hippocampal injured neurites and concomitant changes in postmicrosomal fractions proteins of grafts. Acta Physiol. Hung. 79: 219-231.

Lewin-Kowalik J., Sieroń A., Krause M., Barski J.J., Górka D. (1992b) Time-dependent regenerative influence of predegenerated nerve grafts on hippocampus. Brain Res. Bull. 29: 831-835.

Lubińska L. (1982) Patterns of Wallerian degeneration of myelinated fibres in short and long peripheral stumps and in isolated segments of rat phrenic nerve. Interpretation of the role of axoplasmic flow of the trophic factor. Brain Res. 233: 227-240.

Lunn E.R., Perry V.H., Brown M.C., Rosen H., Gordon S. (1989) Absence of Wallerian degeneration does not hinder regeneration in peripheral nerve. Eur. J. Neurosci. 1: 27-33.

Łomnicki A. (1995) Wprowadzenie do statystyki dla przyrodników. Wydawnictwo Naukowe PWN, 248 p.

Macklin W.B. (1992) The myelin proteolipid protein gene and its expression. In: Myelin: biology and chemistry (Ed. R.E. Martenson and E. Russel). CRC Press, Boca Raton, FL, p. 257-276.

O'Farell P. H. (1975) High resolution two dimensional electrophoresis of proteins. J. Biol. Chem. 250: 4007-4021.

Olmsted J.B. (1986) Microtubule associated proteins. Ann. Rev. Cell Biol. 2: 421-457.

Page R.C., Davies P., Allison A.C. (1978) The macrophage as a secretory cell. Int. Rev. Cytol. 52: 119-157.

Poduslo J., Low P., Nickander K.N., Dyck P.J. (1985) Mammalian endoneurial fluid: collection and protein analysis from normal and crushed nerves. Brain Res. 332: 91-102.

Riederer B., Matus A.: Differential expression of distinct microtubule-associated proteins during brain development. Proc. Natl. Acad. Sci. USA 82: 6006-6009.

Salonen V., Aho H., Roytta M., Peltonen J. (1988) Quantitation of Schwann cells and endoneurial fibroblast--like cells after experimental nerve trauma. Acta Neuropathol. 75: 331-336.

Sieroń A., Lewin-Kowalik J., Krause M., Fertała A. (1991) Time dependent dynamic changes of protein content and composition in the submicrosomal fraction from the rat sciatic nerve following transection. Ann. Acad. Med. Siles. 23: 81-89.

Singer P.A., Mehler S., Fernandes H. L. (1988) Effects of extracts of injured nerve on initiating the regenerative response in the hypoglossal nucleus in the rat. Neurosci. Lett. 84: 155-162.

Southern E.M. (1975) Long range periodocities in mouse satellite DNA. J. Mol. Biol. 94: 51-69.

Tzeng S.-F., Deibler G. E., De Vries G. H. (1999) Myelin basic protein and myelin basic protein peptides induce the proliferation of Schwann cells via ganglioside GM1 and the FGF receptor. Neurochem. Res. 24: 255-260.

Unanue E.R. (1976) Macrophage dependent factor that stimulates the proliferation of fibroblasts in vitro. Am. J. Pathol. 84: 501- 514.

Weerasuriya A., Curran G.L., Poduslo J.F. (1990) Developmental changes in blood-nerve transfer of albumin and endoneurial albumin content in rat sciatic nerve. Brain Res. 521: 40-46.

Windebank A.J., Poduslo J.F. (1986) Neuronal growth factors produced by adult peripheral nerve after injury. Brain Res. 385: 197-200.

Wray W., Boulikas T., Wray U. P., Hanock R. (1981) Silver staining of proteins in polyacrylamide gels. Anal. Biochem. 118: 197.

Zhao Q., Dahlin L.B., Kanje M., Lundborg G. (1993) Repair of the transected rat sciatic nerve: matrix formation within implanted silicone tubes. Restor. Neurol. Neurosci. 5: 197-204.

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