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The expression of B-50/GAP-43 in Schwann cells is upregulated in degenerating peripheral nerve stumps following nerve injury

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We have detected mRNA for B-50 (GAP-43, pp46, F1, neuromodulin), which was originally believed to be a neuron-specific protein, in non-neuronal cells in the rat sciatic nerve. In control rats, the level of B-50 mRNA in sciatic nerve tissue was much lower than in dorsal root ganglia. Following nerve crush or transection, the expression of B-50 mRNA in the distal nerve stump increased dramatically between 1 and 2 days post-injury. The B-50 mRNA levels in the distal stump of crushed nerves remained elevated for up to 4 weeks and subsequently returned to control levels after 7 weeks. In contrast, after nerve transection B-50 mRNA levels in the distal nerve portion continued to increase up to 7 weeks post-lesion. No changes in the levels of the B-50 transcript were observed in the proximal portion of either crush-lesioned or transected sciatic nerves. In situ hybridization demonstrated B-50 mRNA associated with Schwann cells in the distal nerve stump. The observation that Schwann cells are capable of producing B-50 mRNA was confirmed by Northern blot analysis of total RNA isolated from primary Schwann cell cultures. Taken together, these data show the expression of B-50 mRNA by Schwann cells and the up-regulation of B-50 mRNA in reactive Schwann cells upon loss of axonal contact.

INTRODUCTION

B-50, also designated GAP-43, pp46, F1 and neuromodulin, is a membrane-associated calmodulin-binding phosphoprotein present in the nervous system (reviewed by Skene[30]). Although the role of B-50 has not been clearly established, the expression of this protein is highly correlated with neuronal development and regeneration. It has been demonstrated that the protein is present in large quantities in the axonal growth cone[9,18,22], in outgrowing neurites in fetal and neonatal rat brain and spinal cord[13,17,25,26], and in outgrowing processes of nerve growth factor (NGF)-differentiated PC12 cells[38]. Furthermore, the protein is re-expressed in regenerating axons following mechanical damage[31,32,43] and the mRNA for B-50 increases about 10-fold in the dorsal root ganglia (DRG L_4–L_6) after sciatic nerve crush[15,37]. Taken together, these observations indicate that B-50 is associated with axonal outgrowth[30].

For many years it was suggested that B-50 is restricted in its expression to neurons[2,3,24]. However, these data have now been contradicted by several studies showing the expression of B-50 not only in neurons but also in various types of glial cells. B-50 immunoreactivity has been demonstrated in cultured astrocytes, oligodendrocytes[7,8,12,44,45], Schwann cells and satellite cells[6,47]. In agreement with these in vitro studies, in vivo studies have demonstrated B-50 immunoreactivity in non-neuronal components of the peripheral nervous system. Tetzlaff et al.[34] demonstrated B-50 immunoreactivity associated with regenerating axons but also with Schwann cell columns forming the bands of Bungner following sciatic nerve crush. We reported an unexpected increase in B-50 immunoreactivity at the degenerating neuromuscular junctions during the initial stages of Wallerian degeneration[41]. Curtis identified non-myelin forming Schwann cells and precursor Schwann cells as the major B-50 producing non-neuronal cell types of the peripheral nervous system[6]. More-

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over, co-localization of B-50 immunoreactivity to S-100-positive cells at the neuromuscular junction of the facial nerve has been demonstrated recently[36]. B-50 immunoreactivity in Schwann cells can derive either from phagocytosis of axonal debris or from endogenous B-50 synthesis. In order to discriminate between these two possibilities we studied the expression of B-50 mRNA in the sciatic nerve after injury using the polymerase chain reaction (PCR), Northern blot analysis and in situ hybridization. Furthermore, we analyzed temporal changes in the amount of B-50 mRNA following two types of lesion: (1) crush injury of the sciatic nerve resulting in the re-innervation of the target cells in muscle or skin within three weeks following crush, (2) transection of the nerve resulting in permanent separation of the proximal and distal nerve portion. Thus, these two types of nerve injury enable the comparison of B-50 mRNA expression in a nerve that does regenerate (nerve crush) and a nerve that fails to regenerate (nerve transection).

MATERIAL AND METHODS

Animals, surgery and tissue dissection

Male Wistar rats (120–140 g, TNO, Zeist, NL) were used in all experiments. Rats were anaesthetized with Hypnorm (Duphar, Weesp, The Netherlands) at a dose of 0.8 ml/kg b.wt. The right and the left sciatic nerves were either crushed or transected. The nerves were crushed 27 mm distal to the sciatic notch with haemostatic forceps as described in detail by De Koning[10]. The position of the distal border of the 2.0-mm long crush lesion was marked by placing an epineural suture (Ethicon 6.0) in the epineurium to identify the precise position of the crush site. For permanent transection of the sciatic nerve a piece of nerve tissue was removed and the proximal and distal nerve stumps were coagulated and ligated (Fig. 1). At various time-points after nerve damage the animals were sacrificed by decapitation. Different parts of the sciatic nerve were dissected. Crushed sciatic nerves were divided in a proximal (A), a peritraumatic (B), and a distal nerve portion (C) (Fig. 1). After transection, the proximal and distal nerve segments were dissected. Sciatic nerve pieces of 2 rats were pooled for RNA isolation. The left and right DRG (L_{4-6}) were dissected and the DRG of 3 animals were pooled for RNA isolation. Non-operated age-matched animals were used as controls. All tissues were stored at −80°C until further processing. Gloves were worn at all times and dissection instruments were sterilized before the dissection of each rat.

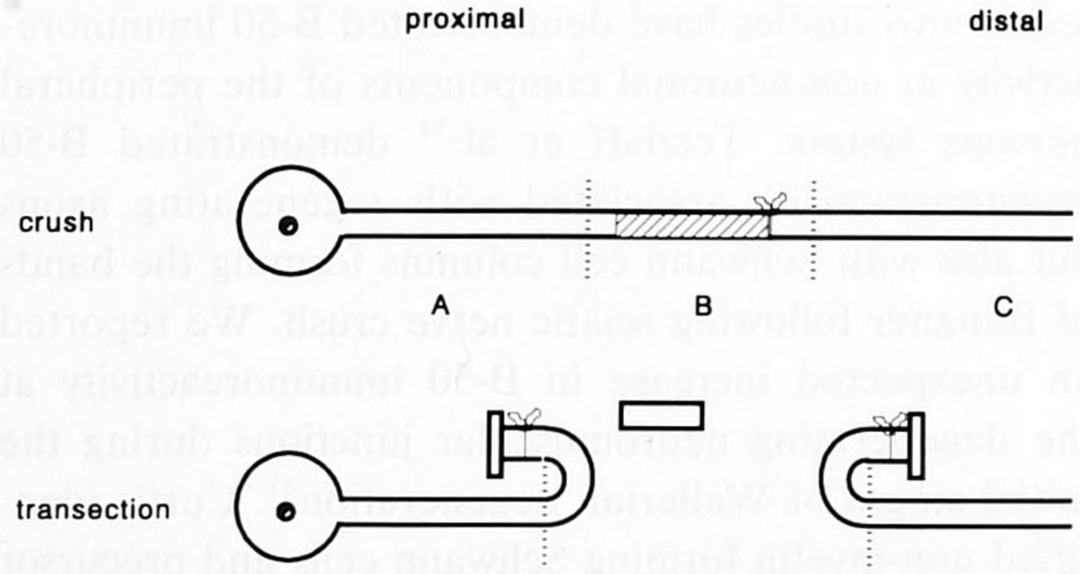

Fig. 1. Schematic illustration of a crushed and a transected sciatic nerve. The crushed nerve is divided into 3 parts: a proximal portion (A), a peritraumatic portion (B), and a distal nerve portion (C). The hatched portion of the nerve indicates the 2.0-mm long crush lesion the distal border of which is marked with an epineural suture. In the transected nerve, a piece of nerve is removed and both the proximal and the distal nerve portions are coagulated and ligated using surgical silk.

Cell culture

Primary Schwann cell cultures were prepared essentially according to Brockes et al.[5]. In short, sciatic nerves from 5–10 5-day-old rat pups were dissected, pooled and treated with collagenase and trypsin. The nerves were passed through a 0.8 mm needle followed by a 50 μm filter to retain cell debris. Schwann cells were collected by centrifugation, resuspended and plated in DMEM containing 10% fetal calf serum and 6 g/l glucose. Schwann cell cultures were treated with 10 μM Ara-C for 48 h.

Primary fibroblasts were obtained by cutting the skin of the animals used for the Schwann cell preparation described above into small (0.5 mm) pieces. The pieces were transferred to a 25 cm^2 culture flask, the medium was aspirated allowing the pieces to adhere. After 10 min 2 ml DMEM with 10% fetal calf serum was added. When cell outgrowth was initiated, 5 ml of the same medium was added. Cells were harvested by trypsination as soon as the culture bottle was covered for more than 50%.

After 9 days in culture, Schwann cells and primary fibroblasts were triturated, collected by centrifugation and subsequently frozen in liquid N_2 and stored at −80°C until further processing.

RNA isolation

Total RNA was prepared by a single-step method using RNAzol (Cinna/Biotecx, Friendswood, USA), a guanidinium isothiocyanate/phenol-based extraction solution according to the manufacturers instructions. In short, the tissue samples were homogenized in RNAzol (2 ml/100 mg tissue) in a glass tube with a tightly fitting teflon pestle by 10 strokes up and down. For each 2 ml of homogenate 0.2 ml chloroform was added, shaken vigorously and put on ice. After centrifugation (12,000 × g, 15 min, 4°C), the upper aqueous phase containing the RNA was transferred to a fresh tube and an equal volume of isopropanol was added. The RNA was allowed to precipitate for at least 45 min at −20°C. RNA was pelleted by centrifugation (12,000 × g, 20 min, 4°C) and washed twice with 70% ethanol. The pellet was briefly dried in vacuo and dissolved in sterilized water. The amount of RNA was determined spectrophotometrically by UV absorption at A_{260}, the purity was assessed via the A_{260}/A_{280} ratio and the integrity was examined on a 2% agarose gel stained with ethidium bromide. The RNA was used for Northern blots or served as templates for the production of cDNA for PCR. Because the PCR method is very sensitive to cross-contamination, precautions to avoid false positives were taken during the preparation of the RNA. A fresh sterilized pestle was used for each sample.

cDNA synthesis

Single-stranded cDNA was synthesized from 10 μg total RNA per sample. The RNA was denatured at 65°C for 5 min and put on ice. cDNA synthesis was carried out in 20 μl of 1× first strand buffer (50 mM Tris-HCl, pH 7.6, 70 mM KCl, 10 mM $MgCl_2$, 4 mM dithiothreitol), 1 mM of each dNTP, 3 μM random primers, 20 U RNAsin and 40 U Moloney Murine Leukaemia Virus (MMLV) reverse transcriptase (Pharmacia, Uppsala, Sweden) for 2 h at 37°C.

Polymerase chain reaction

Both the sense and the antisense primers for B-50 amplification were 24-mers. The sequence of the 5′ B-50 primer complementary to a sequence in exon 1 starting at position 41 is 5′-AGCTGTGCTG-TATGAGAAGAACC-3′ and the sequence of the 3′ B-50 primer complementary to a sequence in exon 2 starting at position 265 is 5′-CCCTCCTTCTTCTCCACACCATCA-3′. The expected size of the amplification product is 247 bp[24]. The cDNA equivalent to 1 μg total RNA was amplified by PCR in a final volume of 50 μl. The PCR mixture contained 1×PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin), 0.2 mM of each dNTP

(Pharmacia Inc., Pitscataway, NJ), 1 μM of each primer, and 2.5 U of *Taq* polymerase (Pharmacia). An intelligent heating block (Hybaid Ltd., Teddington, UK) was used to cycle the temperature of the samples. Samples were denatured for 10 min at 100°C and quickly cooled on ice prior to the addition of *Taq* polymerase and dNTP. Samples were overlaid with 50 μl mineral oil and incubated for 30 cycles as follows: 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. After the last cycle the samples were incubated at 72°C for 10 min and stored at −20°C until analysis. To avoid cross-contamination the following measures were taken[46]: (1) positive displacement pipettes were used throughout the RNA isolation, the cDNA synthesis and PCR sample preparation, (2) preparation of PCR reaction mixtures, sample preparation and analysis of amplified products were each performed in separate rooms, (3) negative control samples contained water instead of template cDNA were pipetted during each experiment.

Analysis of the PCR products

On a 2% agarose gel prepared in TAE buffer (40 mM Tris, 20 mM acetate, 2 mM EDTA, pH 7.0) containing ethidium bromide (0.4 μg/ml) 10 μl of the PCR samples was loaded. After electrophoresis and inspection of the gel under UV light, the agarose gel was placed in 0.25 M HCl for 30 min and rinsed in distilled water for 30 min. Subsequently the PCR products were transferred to a nylon membrane (Zeta probe, Bio-Rad Laboratories, Richman, CA, USA) by capillary blotting in 0.4 N NaOH overnight. The membrane was rinsed in 5×SSC and prehybridized in 1.5×SSPE (0.18 M sodium chloride, 10 mM sodium phosphate, pH 7.0, 1 mM EDTA), 1% SDS, 0.5% defatted milk powder (Refit, Campina, Eindhoven, The Netherlands) and 0.5 mg/ml denatured herring sperm DNA for 3 h at 70°C. Hybridization was performed overnight at 70°C overnight in the same solution with the addition of 10% dextran sulphate and 1×10^6 cpm/ml of a 1,130 bp B-50 cDNA isolated from clone pGB0[24] ^{32}P-labeled by random priming. The membranes were washed in 2×SSC, 0.1% SDS for 20 min at 50°C and finally in 0.1×SSC, 1% SDS, 5 mM EDTA for 10 min at 65°C. The membranes were exposed to X-ray film (Kodak, X-OMAT) overnight.

Northern blot analysis

For Northern blot analysis, total RNA was denatured with glyoxal and DMSO in 10 mM phosphate buffer at 50°C for 60 min[21]. Subsequently samples were cooled on ice and run on a 1% agarose gel in 10 mM sodium phosphate, pH 7.0, followed by capillary blotting to Nylon transfer membranes (Hybond N, Amersham UK) in 20×SSC. The membranes were exposed to UV light (2 min) and baked at 80°C for 2 h. Membranes were prehybridized for 3 h at 42°C in hybridization solution containing 50 mM Tris-HCl, pH 7.5, 50% formamide, 1 M NaCl, 10% dextran sulphate, 0.1 mg/ml denatured herring sperm DNA, 0.2% BSA, 0.2% polyvinylpyrrolidon, 0.2% ficoll, 0.1% sodium pyrophosphate, 1% SDS. Hybridization was performed in the same buffer containing 1×10^6 cpm/ml of B-50 cDNA probe as described for the Southern blot hybridization or a rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe. Labeling of the probes was performed with [^{32}P]dCTP using a random primed DNA labeling kit (Boehringer, Mannheim) according to the protocol supplied with the kit. The washing procedure included the following steps: 5×SSC/0.1% SDS, 2×SSC/0.1% SDS, 1×SSC/0.1% SDS, 0.2×/SSC 0.1% and 0.1×SSC/0.1% SDS. Each wash step was performed for 20 min at 42°C. Northern blots hybridized with a B-50 cDNA probe were exposed for 8 days using Kodak X-OMAT film with an intensifying screen (Amersham, UK), whereas hybridization with a GAPDH probe was followed by an exposure time of 3 days. Prior to reprobing, membranes were stripped by boiling in 0.01×SSC, 0.01% SDS for 20 min.

In situ hybridization

The preparation of radiolabeled antisense B-50 RNA probe and the procedures used for in situ hybridization were performed as described in detail by Verhaagen[42]. In short, 2 days following unilateral sciatic nerve crush the nerve portion distal to the nerve crush was dissected. The contralateral sciatic nerve was used as the control nerve. Tissues were quickly frozen in liquid N_2-cooled isopentane. 6-μm thick cryostat sections were mounted on poly-L-lysine-coated microscope slides, fixed in 2% paraformaldehyde in 100 mM phosphate buffer, pH 7.4, for 30 min, rinsed in PBS and kept in 70% ethanol overnight. The next day slides were rinsed in PBS followed by 2×SSC and subsequently acetylated in 0.25% acetic anhydride/0.1M triethanolamine. After additional washes in 2×SSC and PBS, sections were incubated in 0.1 M glycin/0.1 M Tris-HCl, pH 8.0, rinsed briefly in 2×SSC and dehydrated. The hybridization was performed at 62°C for 5 h in hybridization solution (50% formamide, 10% dextran sulfate, 250 μg/ml denatured salmon sperm DNA, 1 mg/ml tRNA, 10 mM dithiothreitol, 4×SSC) containing the [^{35}S]αUTP radiolabeled B-50 probe 7×10^5 cpm/slide. Following hybridization, sections were rinsed in 2×SSC/50% formamide at 50°C followed by 0.1×SSC/20 mM β-mercaptoethanol at 62°C. Sections were dehydrated, rinsed in xylene and 100% ethanol. The air-dried sections were coated with NTB-2 liquid emulsion (Kodak) and stored at 4°C and developed after an exposure time of 10 days. Cells were counterstained with haematoxylin and observed with bright field illumination.

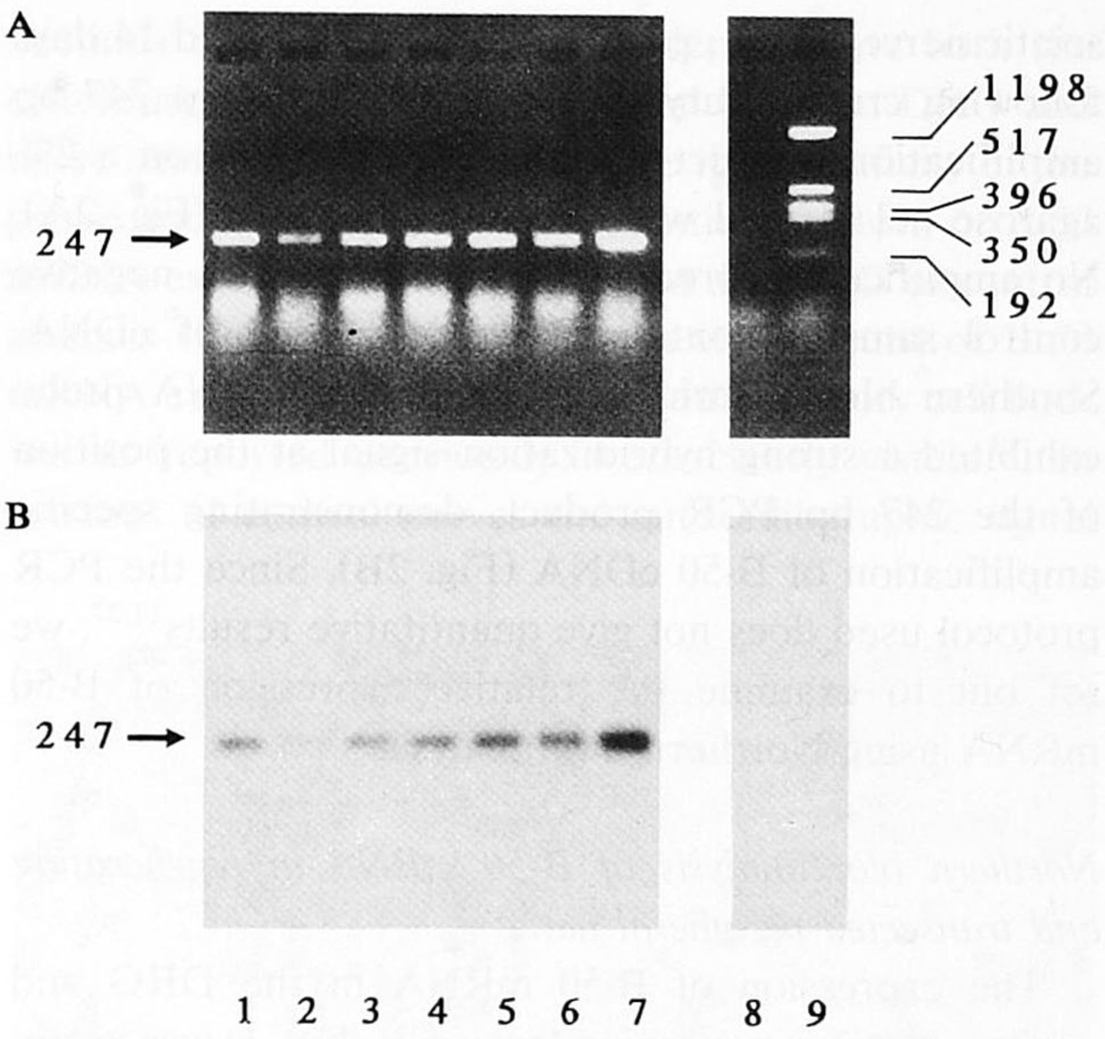

Fig. 2. PCR amplification of B-50 cDNA obtained from peritraumatic nerve portions at different time-points following nerve crush. Agarose gel electrophoresis revealed amplification products of 247 bp in all samples except in the negative control sample (panel A). Primer dimers are visible in all samples in the lower part of the gel. One band of 247 bp is present in all samples after Southern blot analysis hybridized with a B-50 cDNA probe (panel B). No signal was detectable in the negative control sample nor in the lane containing the molecular weight marker. Lane 1, control sciatic nerve; lanes 2–7, sciatic nerve samples 0.5 and 6 h, and 1, 2, 4 and 14 days following sciatic nerve crush, respectively; lane 8, negative control; lane 9, molecular size marker (500 μg pGEM4 cut by *Hin*f1).

RESULTS

Detection of B-50 mRNA in the sciatic nerve by the polymerase chain reaction

The presence of B-50 mRNA in the sciatic nerve was investigated by PCR using specific primers located in exon 1 and exon 2 of the B-50 gene. Fig. 2 shows the analysis of PCR products amplified from cDNA obtained from the peritraumatic nerve region of the

sciatic nerve (Fig. 1, part B) between 0.5 h and 14 days following crush injury. In all nerve samples a 247 bp amplification product was readily detectable on a 2% agarose gel stained with ethidium bromide (Fig. 2A). No amplification products were observed in negative control samples containing water instead of cDNA. Southern blots hybridized with a B-50 cDNA probe exhibited a strong hybridization signal at the position of the 247 bp PCR product, demonstrating specific amplification of B-50 cDNA (Fig. 2B). Since the PCR protocol used does not give quantitative results[11,27], we set out to examine the relative expression of B-50 mRNA using Northern blot analysis.

Northern blot analysis of B-50 mRNA in regenerating and transected peripheral nerve

The expression of B-50 mRNA in the DRG and peritraumatic nerve region (region B, Fig. 1) was examined at a number of time-points ranging from 0.5 h to 14 days following sciatic nerve crush using Northern blot analysis. Fig. 3A shows a Northern blot hybridized with a B-50 cDNA probe. After stripping, the blot was hybridized with a GAPDH probe in order to control for the amount of RNA present in each lane (Fig. 3B). In control rats, the expression of B-50 mRNA in the sciatic nerve was very low in comparison with levels in DRG. In the lesioned rats, an increase in the expression of B-50 mRNA in the peritraumatic region of the sciatic nerve was observed between 1 and 2 days following crush. B-50 mRNA levels remained elevated for

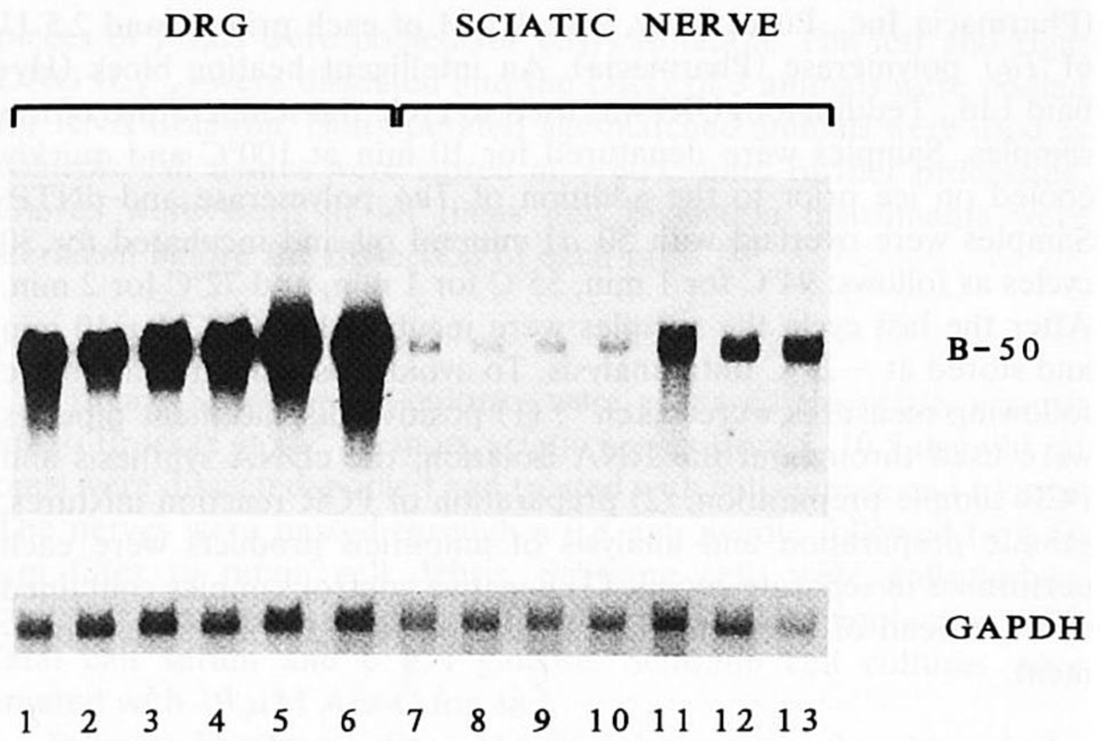

Fig. 3. Northern blot analysis of DRG samples and peritraumatic nerve regions of the sciatic nerve after bilateral nerve crush. The blot was initially hybridized with a B-50 probe (upper panel), subsequently stripped and reprobed with GAPDH (lower panel). Lane 1, control DRG; lanes 2–6, DRG 0.5 and 6 h, and 1, 2, and 14 days following sciatic nerve crush, respectively; lane 7, control sciatic nerve; lanes 7–13 peritraumatic region of the sciatic nerve 0.5 and 6 h, and 1, 2, 4 and 14 days following nerve crush, respectively. Each lane contains 20 μg of total RNA. The difference in the level of B-50 mRNA expression between DRG and sciatic nerve samples is clearly visible. B-50 mRNA expression in the sciatic nerve is increased between days 1 and 2 following injury.

at least two weeks post-lesion, the longest time-point examined in this experiment.

In order to examine the changes in B-50 mRNA more thoroughly, total RNA was extracted from the proximal and distal regions of crushed or transected

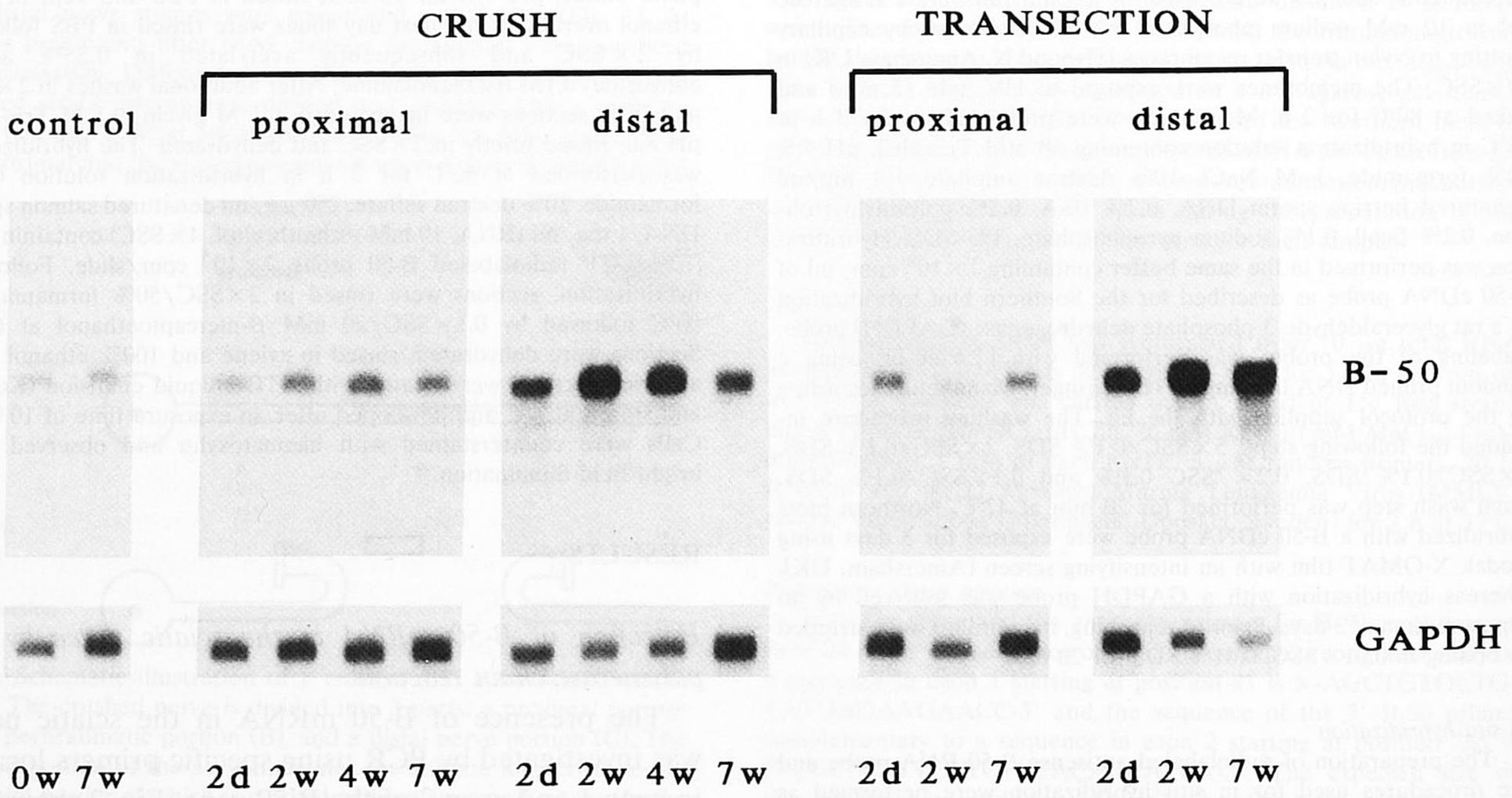

Fig. 4. Comparison of B-50 mRNA expression in proximal and distal sciatic nerve portions isolated at different time-points after nerve crush or nerve transection. The upper panel shows the autoradiogram obtained after B-50 hybridization and in the lower panel the GAPDH signal is depicted. Each lane contains 20 μg of total RNA.

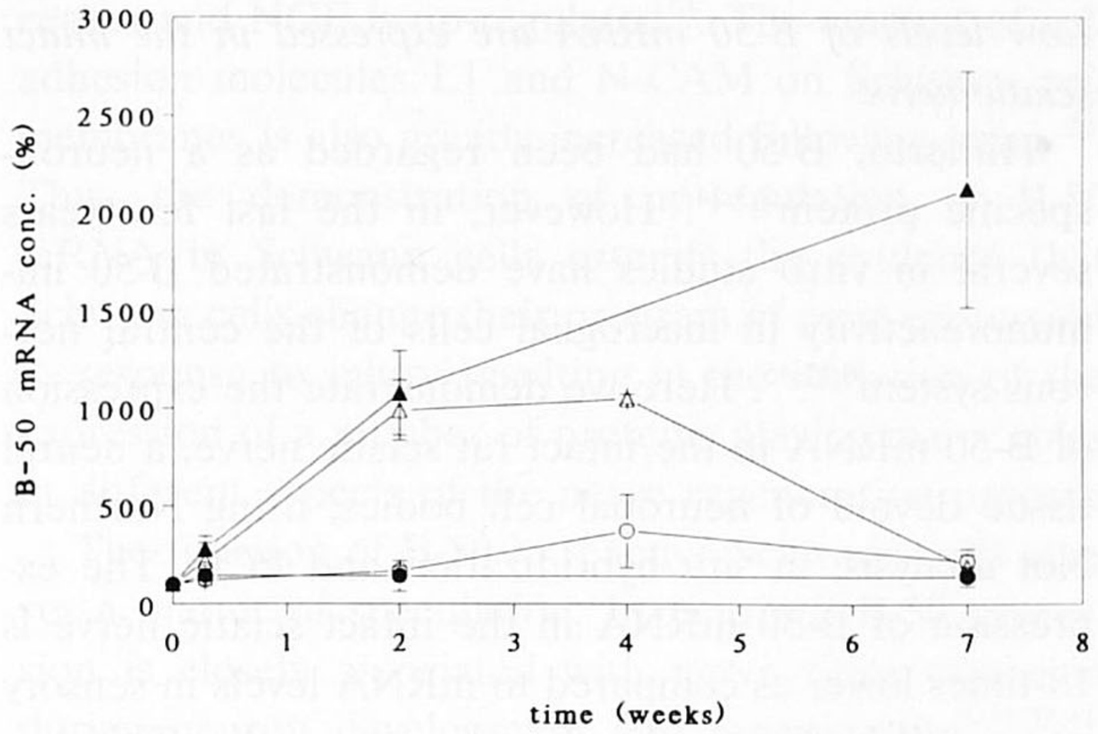

Fig. 5. Semi-quantitative analysis of the expression of B-50 mRNA in the proximal and distal nerve portions following crush or transection of the sciatic nerve. At 2 days, 2, 4, and 7 weeks, the amount of B-50 mRNA was determined by densitometric scanning of autoradiograms and normalized to the signal obtained with a GAPDH probe. B-50 mRNA levels in control non-injured nerves were set at 100% and the increased expression in the crushed samples was expressed as the percentage of the control samples. In the graph each point represents two independent observations and is shown as the mean and the range. (○) Crush, proximal nerve portion; (△) crush, distal nerve portion; (●) transection, proximal nerve portion; (▲) transection, distal nerve portion.

nerves (Fig. 1, regions A,C) and the study was extended to 7 weeks. Age-matched control rats were used to monitor B-50 mRNA expression in intact sciatic nerves. Both crush injury and transection caused a dramatic increase in the level of B-50 mRNA in the distal sciatic nerve stump (Fig. 4). The relative amounts of B-50 mRNA obtained by densitometric scanning of the autoradiogram are shown in Fig. 5. Following nerve crush, the expression of B-50 mRNA in the distal nerve portion was elevated 2 days post-injury and continued to increase up to 2 weeks following crush injury. The levels in the distal segment remained elevated at 4 weeks but returned to normal control levels 7 weeks after nerve crush. No change in B-50 gene expression was observed in the proximal stump (Figs. 4 and 5). In contrast, after transection the expression of B-50 mRNA in the distal portion was increased at 2 days post-lesion and continued to increase up to 7 weeks. No change in B-50 mRNA expression in the proximal portion of the transected nerve as compared with mRNA expression in age-matched controls was detected (Figs. 4 and 5).

Cellular distribution of B-50 mRNA in the sciatic nerve following nerve crush

In order to examine the cellular distribution of B-50 mRNA we used in situ hybridization. Tissue sections were prepared from the distal portions of transected sciatic nerves 2 days after injury and compared with sections obtained from age-matched control nerves. Sections of DRG were used to demonstrate both the difference in intensity of the signal between DRG and nerve samples and to demonstrate the specificity of the probe and the method used. A distinct pattern of B-50 mRNA expression was observed in the DRG of control animals (Fig. 6A). Differences in the amount of B-50 mRNA expression between individual sensory cell bodies, as reported previously, were clearly visible[40]. In sections of control nerves the autoradiographic signal

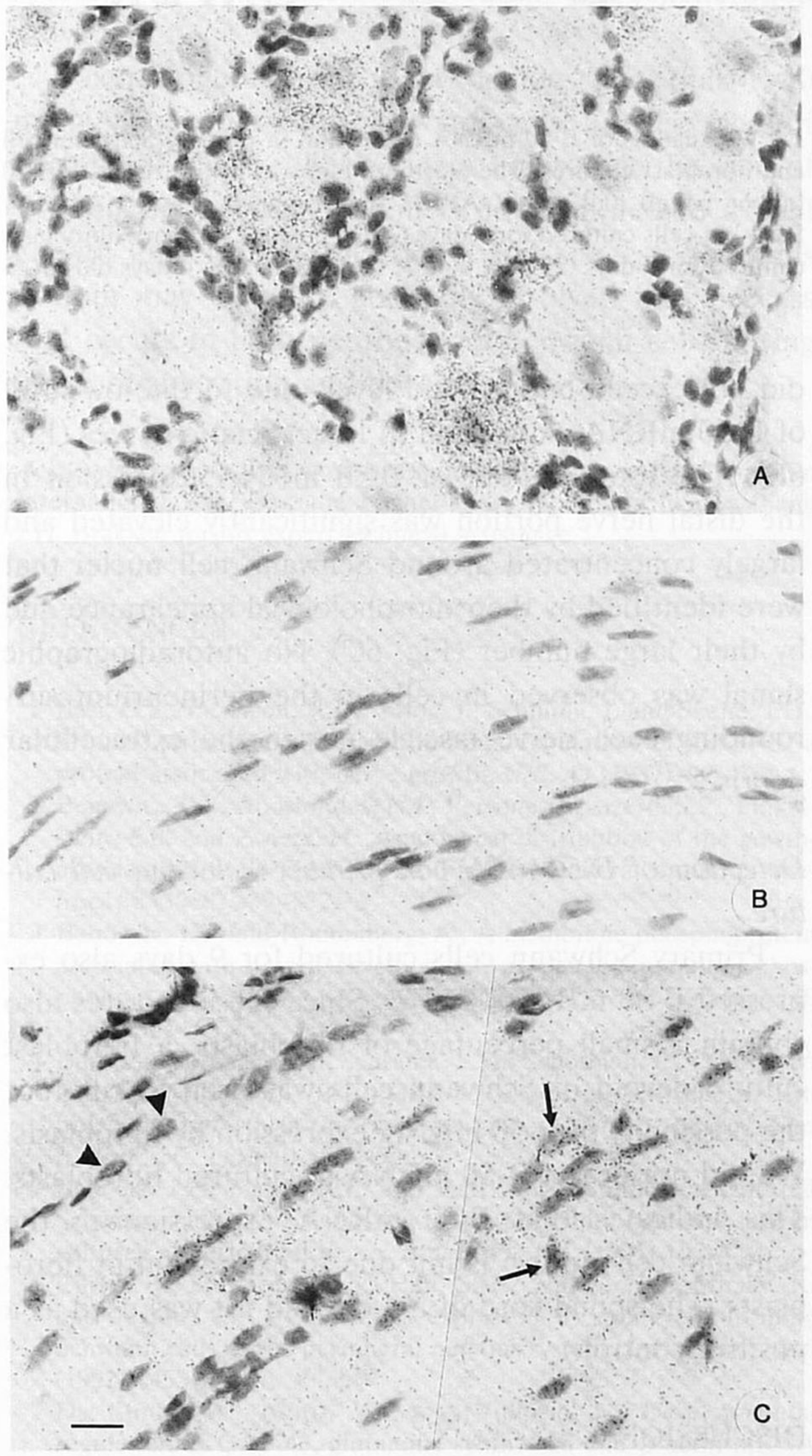

Fig. 6. Cellular localization of B-50 mRNA in DRG and sciatic nerve as assessed by in situ hybridization. A: control DRG; B: intact sciatic nerve; C: degenerating sciatic nerve 2 days following nerve transection. The photomicrograph of the control DRG clearly demonstrates the specific localization of silver grains and the differences in labelling between individual neurons. In situ hybridization of a non-injured control nerve demonstrates the low level of expression of B-50 mRNA as compared to sensory neurons, whereas after nerve transection the total amount of B-50 mRNA in the distal nerve portion is increased. B-50 mRNA is localized predominantly around Schwann cell nuclei in the degenerating nerve stump (arrows). Note that the cells in the perineurium are not labelled (arrowhead). Bar = 15 μm.

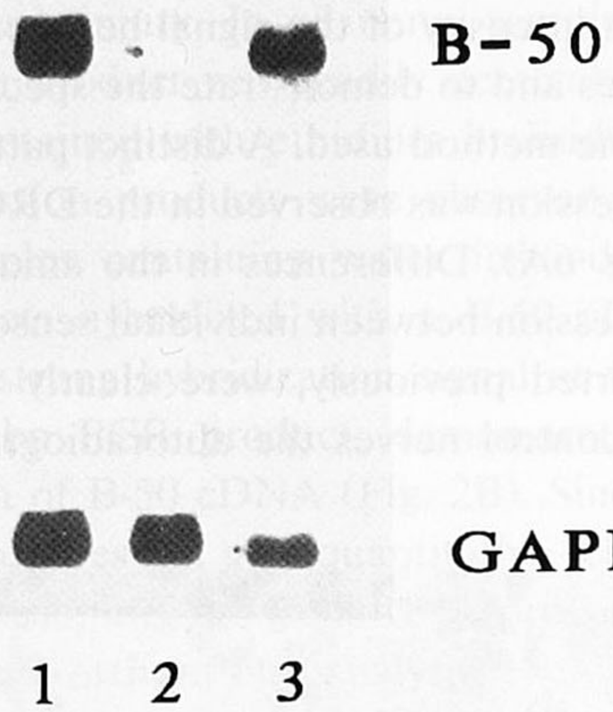

Fig. 7. Analysis of B-50 mRNA expression in primary Schwann cell and fibroblast cultures. The Northern blot was hybridized with B-50 (upper panel) and with GAPDH (lower panel). Lane 1, primary Schwann cells cultured for 9 days (20 μg); lane 2, primary fibroblasts cultured for 9 days (20 μg); lane 3, spinal cord of a 4 days old rat (5 μg).

did not exceed background levels due to the low level of B-50 mRNA expression in intact sciatic nerves (Fig. 6B). The total amount of B-50 mRNA expression in the distal nerve portion was significantly elevated and largely concentrated around Schwann cell nuclei that were identified by their morphological appearance and by their large number (Fig. 6C). No autoradiographic signal was observed in cells in the perineurium surrounding each nerve fascicle nor in the extracellular debris.

Detection of B-50 mRNA in primary Schwann cell culture

Primary Schwann cells cultured for 9 days also expressed B-50 mRNA (Fig. 7). Since these cultures also contain a small percentage of fibroblasts, a fibroblast culture devoid of Schwann cells was used to rule out the possibility of B-50 mRNA expression by fibroblasts. We did not detect B-50 mRNA in cultured fibroblasts. This indicates that B-50 mRNA expression in the Schwann cell culture is not due to expression in fibroblasts. The spinal cord of a 4-day-old rat was used as a positive control.

DISCUSSION

In this paper we have demonstrated: (1) the presence of low levels of B-50 mRNA in the intact sciatic nerve, (2) the induction of B-50 mRNA expression in the degenerating portion of a lesioned peripheral nerve, (3) downregulation of B-50 mRNA expression upon peripheral nerve regeneration, (4) the localization of B-50 mRNA in reactive Schwann cells in the transected sciatic nerve.

Low levels of B-50 mRNA are expressed in the intact sciatic nerve

Hitherto, B-50 had been regarded as a neuron-specific protein[2,3,24]. However, in the last few years several in vitro studies have demonstrated B-50 immunoreactivity in macroglial cells of the central nervous system[8,12,45]. Here we demonstrate the expression of B-50 mRNA in the intact rat sciatic nerve, a neural tissue devoid of neuronal cell bodies, using Northern blot analysis, in situ hybridization and PCR. The expression of B-50 mRNA in the intact sciatic nerve is 18-times lower as compared to mRNA levels in sensory nerve cell bodies in the dorsal root ganglia. This low level of expression may account for previous failures to detect B-50 mRNA in non-neuronal cells in the rat sciatic nerve in vivo[1]. In addition, the low levels of B-50 mRNA may explain our inability to detect B-50 mRNA in the intact nerve with a sensitive in situ hybridization protocol with which we could clearly demonstrate B-50 mRNA in reactive Schwann cells in the distal portion of transected sciatic nerves. Formally we cannot exclude the possibility that B-50 mRNA in the intact sciatic nerve is transported down axons and is thus of neuronal origin. Recently it has been demonstrated that B-50 immunoreactivity in intact peripheral nerves is confined to non-myelin-forming Schwann cells, a relatively small subpopulation of Schwann cells in the peripheral nervous system[6]. In view of this and our finding that B-50 mRNA is expressed in Schwann cells located in the distal nerve stump following nerve transection, we are confident that the presence of B-50 mRNA in the intact sciatic nerve is due to local expression and does not derive from an axonal source.

B-50 mRNA expression is up-regulated in Schwann cells in the distal nerve stump following nerve injury

Following sciatic nerve injury, the amount of B-50 mRNA expressed by Schwann cells increased substantially. The initial up-regulation of B-50 mRNA was observed between 1 and 2 days post-injury coinciding with characteristic morphological and biochemical events that occur in a nerve after axotomy. During this post-lesion period the fragmentation of the myelin sheath becomes apparent and the axolemma disintegrates[33]. As a result of these changes the intimate contact between the Schwann cells and axons is lost in the distal portion of the nerve. The Schwann cells switch from a myelin-producing state to an active proliferating non-myelin-forming state[19,29]. During this transition period the pattern of gene expression in Schwann cells changes dramatically[16]. The expression of the major myelin proteins, including P_0 and MBP, is sharply reduced[35] and the expression of the NGF re-

ceptor and NGF is up-regulated[14]. The amount of cell adhesion molecules L1 and N-CAM on Schwann cell membranes is also greatly increased following injury[20]. Thus the demonstration of up-regulation of B-50 mRNA in Schwann cells extends the evidence that Schwann cells change their program of gene expression in response to injury resulting in the initiation of the expression of a number of proteins playing major roles in different aspects of the nerve regeneration process.

The function of B-50 in reactive Schwann cells is as yet a matter of speculation. In neurons, B-50 expression is closely associated with nerve fibre extension during neuron development and regeneration[4,31,32,41]. The protein is localized at the cytoplasmic face of the neuronal membrane in growth cones and in the growing axon shaft[39]. Zuber et al.[48] were able to demonstrate that stable transfection of non-neuronal cells with a B-50 expression vector resulted in the formation of membrane extensions in these non-neuronal cells. This suggests that B-50 affects the ability of a cell to modify its shape and may thus be required in vivo in situations were membraneous growth is necessary. Recently it has been demonstrated that Schwann cells at the neuromuscular junction extend numerous processes with lengths upto 300 μm following nerve injury[28]. These changes in Schwann cell shape, although perhaps less dramatic than in regenerating neurons may require B-50. This would be consistent with the notion that B-50 is a protein involved in the regulation of neural cell shape.

In contrast to Schwann cells in the distal portion of the nerve, Schwann cells located in the proximal portion of the injured sciatic nerve maintain intimate contact with axons and do not up-regulate their B-50 mRNA expression. When axon–Schwann cell contact is disrupted by primary culture of Schwann cells prepared from post-natal sciatic nerves these Schwann cells acquire an immature phenotype and cease their production of myelin[23]. Such cultured Schwann cells express abundant levels of B-50 mRNA for up to 9 days in culture. We do not currently understand which processes in the nerve may govern the down-regulation of B-50 as a nerve is permitted to regenerate. The loss of axon–Schwann cell contact occurring in vitro as a result of Schwann cell culturing and in vivo in the distal degenerating nerve portion appears to be a prerequisite for the induction of B-50 gene expression. When axon–Schwann cell contact is not restored in vivo, as is the case following nerve transection and ligation, B-50 mRNA levels remain high up to at least 7 weeks following axotomy. The re-establishment of axon–Schwann cell contact, however, is not sufficient to ensure an immediate down-regulation of B-50 mRNA expression. Within 4 weeks following a crush lesion most neurons have grown a new axon down the existing endoneural tubes and have attained contact with the ensheathing Schwann cells. B-50 mRNA expression, however, is still high at this point in time. Myelinization of peripheral axons by Schwann cells is initiated as early as 8 days post-lesion and is fully underway at 4 weeks after lesioning. Therefore the down-regulation of B-50 mRNA expression following target cell re-innervation does not appear to correspond to a clearly defined event in the regeneration nerve.

Taken together our data demonstrate that B-50 mRNA is expressed in Schwann cells, a major class of peripheral glial cells. Our data imply that the role of B-50 in the nervous system is not confined to axonal growth and synaptic plasticity, and suggests that this protein may be more generally involved in a process that occurs in both neurons and Schwann cells during nerve regeneration and development.

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