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Changes in Neurofilament Gene Expression Occur After Axotomy of Dorsal Root Ganglion Neurons: An *In Situ* Hybridization Study

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Neurofilaments (NFs) are predominant elements in large myelinated axons, where they are thought to serve the important function of maintaining axonal caliber. Previous studies have shown that changes in NF synthesis and axonal transport occur after axonal injury in rat dorsal root ganglion (DRG) cells. The resulting reduction in the NF supply to DRG axons is thought to be largely responsible for the observed decrease in axonal diameter in the proximal axonal stump after an injury. In the present study, we test the hypothesis that a change in NF gene expression precedes the changes in synthesis and transport of NF proteins. To address this hypothesis, the levels of mRNA encoding the 68-kilodalton (kd) neurofilament protein (NF68) in adult rat DRG neurons were assessed at different times after peripheral axotomy using *in situ* hybridization. For these studies we used a ^{35}S-labeled cDNA probe to NF68. The levels of NF68 mRNA in sensory neurons located in ipsilateral fourth and fifth lumbar DRG at 1, 7, and 14 days after sciatic nerve crush were compared to those in normal DRG neurons using quantitative autoradiography. In large DRG neurons ($> 1000\,\mu m^2$), the levels of NF68 mRNA were significantly reduced relative to normal at 1, 7, and 14 days after axotomy. Medium-sized cells ($601–1000\,\mu m^2$) exhibited a reduction only at 14 days postinjury, and small-sized cells were not significantly affected. These findings indicate that larger DRG neurons which give rise to large myelinated sensory axons exhibit a change in NF gene expression after axonal injury. The observed changes in NF68 mRNA levels temporally precede changes in NF synthesis and transport in injured DRG cells. Thus, a change in NF gene expression may be an important component of an effective regenerative response and a critical step at which axonal caliber is regulated in injured neurons.

KEY WORDS: neurofilaments; neurofilament messenger RNA; *in situ* hybridization; regeneration; nerve injury; axonal atrophy.

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INTRODUCTION

Neurofilaments (NFs) are major components of the axonal cytoskeleton. In rat, they are composed of three subunit proteins that are 68, 145, and 200 kilodaltons (kd) (Hoffman and Lasek, 1975; Liem *et al.*, 1978). Each NF protein is the product of a separate gene and these genes are transcribed only in neurons (Lewis and Cowan, 1985). The 68-kd subunit (NF68) is the most abundantly expressed of the three NF proteins (Liem and Hutchinson, 1982). Because axons are devoid of ribosomes, translation of NF mRNAs occurs only in the cell body and proximal dendrites of neurons. Within minutes of synthesis, the NF proteins are assembled into NFs (Black *et al.*, 1986). The majority of NFs are exported to the axon, where they are phosphorylated as they pass through the initial segment (Bennett and DiLullo, 1985; Oblinger, 1987). In the axon, NFs are continually moved along by slow axonal transport at a rate of 0.1–1 mm/day (Lasek and Hoffman, 1976; Black and Lasek, 1980; Brady and Lasek, 1982; Oblinger and Lasek, 1985; Oblinger *et al.*, 1987). Normally, little turnover of NFs occurs during axonal transport until the NFs reach the axon terminal, where they are degraded (Lasek and Hoffman, 1976; Roots, 1933). In normal neurons, this entire process of NF supply is apparently quite tightly regulated since NF number and density remain fairly constant in and along any given type of axon (Berthold, 1978; Price *et al.*, 1987).

NF content appears to be a primary determinant of axonal size (Friede and Samorajski, 1970; Berthold, 1978; Lasek *et al.*, 1983; Hoffman *et al.*, 1985). Thus, changes in any of the parameters of NF supply—transcription, translation, assembly, export from the cell body, posttranslational processing, transport, degradation—could conceivably affect axonal size by altering the axonal content of NFs. Axonal size is an important parameter in the nervous system since alterations in axon diameter can produce changes in impulse conduction (Hoffer *et al.*, 1979; Cragg and Thomas, 1961; Milner and Stein, 1981). A well-known consequence of nerve injury, particularly in chronic situations, is atrophy of axons in the proximal stump (Sunderland, 1978; Carlson *et al.*, 1979; Risling *et al.*, 1980; Dyck *et al.*, 1984). In light of the importance of axonal caliber to the functional properties of axons, it is important to gain a detailed understanding of the mechanisms by which axonal atrophy occurs after neuronal injury or in other types of pathological conditions.

Studies have shown that decreased amounts of pulse-labeled NF proteins are transported in proximal regions of both motor axons (Hoffman and Lasek, 1980) and sensory axons of dorsal root ganglion (DRG) cells (Oblinger and Lasek, 1985, 1987) after sciatic nerve injury. In addition, it is known that the incorporation of radiolabeled amino acids into NF proteins is reduced in DRG neurons 2 weeks after axotomy (Oblinger and Lasek, 1985, 1987). These observations suggest that decreased synthesis of NF proteins is a major reason for the reduced levels of axonally transported NFs in injured neurons. At this point, it is important to determine whether or not this change in synthesis is the result of a change in the level of mRNA for NF proteins in axotomized neurons. A cDNA probe to the NF68 gene of mouse has recently been cloned (Lewis and Cowan, 1985). We have used this probe for *in situ* hybridization studies in DRG cells. *In situ* hybridization is an extremely sensitive method for detecting changes in the level of specific mRNAs in individual cells. In the present study,

we examined the time course of the change in NF68 mRNA levels in axotomized DRG neurons and report that a reduction is present as early as 1 day after peripheral nerve crush. We also report that the axotomy response of larger DRG neurons is more pronounced than that of smaller neurons.

MATERIALS AND METHODS

Nerve Injury

Adult male Sprague Dawley rats (250–300 g) were anesthetized by intraperitoneal injection of Chloropent (Fort Dodge Laboratories, Fort Dodge, Iowa) at a dose of 3 ml/kg. Peripheral axons of DRG neurons were injured by crushing the sciatic nerve in the midthigh. The crush axotomy involved exposing the sciatic nerve at a site about 50–55 mm from the L5 ganglion and crushing it three consecutive times for 30 sec with No. 5 Dumont forceps. At postinjury times of 1, 7, and 14 days, the animals were killed by ether overdose and the L4 and L5 DRGs ipsilateral to the injury were removed. Two rats at each postcrush time point were prepared; control L4 and L5 ganglia were obtained from a normal, untreated rat.

Tissue Preparation

Ganglia were placed into 4% paraformaldehyde for 1 hr. The DRGs were then dehydrated with an increasing series of ethanol solutions and embedded in paraffin. The blocks were sectioned at 10 μm and the histological sections were mounted on gelatin chrome-alum subbed slides. Slides were stored in covered slide boxes at room temperature until used.

Preparation and Labeling of the cDNA Probe

The neurofilament probe consisted of a 1.2-kb cDNA encoding a region of the 68-kd mouse NF protein (Lewis and Cowan, 1985). It was provided to us in the plasmid pUC8 by Dr. Nick Cowan (NYU School of Medicine, New York). We used this plasmid to transform JM105 bacteria and subsequently isolated plasmid DNA from 1-liter bacterial cultures using cesium chloride centrifugation. For *in situ* hybridization, only the purified insert cDNA was used. To isolate the insert, plasmid DNA was digested with *Eco*R1 (Promega Biotec). The digested DNA was precipitated with 1/20th vol of saturated ammonium acetate and 2 vol of ethanol at -70°C for 30 min, pelleted, and resuspended in TE buffer (10 m*M* Tris, 1 m*M* EDTA, pH 8). Next, the DNA was boiled for 5 min and then run on a large-sized 1% agarose gel; the gel run was stopped after 30 min and a strip of DEAE paper (Schleicher and Schuell) was inserted into a razor slit in the gel just in front of the insert band. Another strip of DEAE paper was placed behind the 1.2-kb insert band to collect plasmid DNA. The gel run was continued for another 30 min to allow DNA to run onto the paper. Subsequently, the paper strip containing the insert DNA was removed, and the

DNA was eluted from the paper by incubation in 1.5 *M* NaCl at 37°C overnight and concentrated using a Centricon 30 concentrator unit (Amicon Corp.).

The purified insert was labeled with ^{35}S-dCTP (Amersham Corp.) using a nick-translation kit (Bethesda Research Labs) at 15°C for 1.5 hr. An additional 0.75 U of DNAse I (Promega Biotec) was added to each 50 μl reaction mixture in order to produce probes that averaged 50–300 bp in size. Following nick translation, the labeled cDNA was separated from unbound nucleotides and concentrated using a Centricon 30 concentrator unit. The size range of the final labeled cDNA was confirmed on polyacrylamide gels using *Hae*III-digested pBR322 DNA (Sigma) as a marker. The labeled probe was aliquoted into microfuge tubes and stored at −20°C until used (generally within 1–2 weeks).

Hybridization Conditions

Slides of DRG sections were deparaffinized in xylene for 10 min and then run through a decreasing series of alcohols. In all subsequent steps, solutions contained 0.1% added DEPC (diethyl pyrocarbonate); Tris solutions were made using DEPC-treated sterile water. The sections were rehydrated in 0.1 *M* Tris, pH 7.4 and then permeabilized in 0.3% Triton X-100 for 15 min at room temperature. After rinsing in 0.01 *M* Tris (pH 7.4), the sections were digested with proteinase *K* (1 μg/ml in 0.01 *M* Tris, pH 7.4) at 37°C for 15 min. The sections were then rinsed with 2X SSC (15 m*M* NaCl, 1.5 m*M* sodium citrate) for 15 min at room temperature and prehybridized at 37°C for 2 hr in a chamber humidified with paper towels soaked with 2X SSC and 50% formamide. The prehybridization buffer contained 1.2 *M* NaCl, 0.1 *M* Tris (pH 7.6), 40 μg/ml sodium pyrophosphate, 80 μg/ml Ficoll, 80 μg/ml polyvinylpyrrolidine, 100 μg/ml yeast total RNA (Sigma type III), 20 μg/ml salmon sperm DNA, and 20 μg/ml DTT (dithiothreitol). Just before placing on the tissue, the prehybridization buffer was boiled for 5 min, placed on ice, and diluted with an equal volume of deionized formamide.

For hybridization, 5 ng of labeled insert (1.2×10^5 cpm) was used for each DRG section. An appropriate amount of labeled DNA for a series of slides was vacuum dried and then suspended in hybridization buffer. This buffer was the same as the prehybridization buffer, with the addition of 20% dextran sulfate and 15 mg/ml polyoxyadenylic acid. The hybridization buffer containing probe was boiled for 5 min and quickly cooled to denature the DNA. Next an equal volume of deionized formamide was added to the hybridization mixture and the mixture was brought to 500 nmol/ml unlabeled α-thio-CTP (New England Nuclear). The addition of α-thio nucleotide was used to reduce nonspecific binding of the ^{35}S-labeled probe to the tissue (Bandlow *et al.*, 1987). Ten microliters of the complete hybridization mixture was pipetted onto each DRG section and the sections were incubated for 16 h at 37°C in the humidified chambers.

After hybridization, the slides were dipped in 2X SSC and then rinsed twice in 2X SSC for a total of 20 min. They were then rinsed in 1.5 liters of 0.5X SSC containing 5 m*M* DTT for 10 hr at room temperature. Finally, the sections were dehydrated with an increasing series of ethanol/300 m*M* ammonium acetate (pH 5.5) and allowed to dry overnight.

Detection of Hybridization and Quantitative Analysis

Thoroughly dried slides were dipped into 37°C Kodak NTB2 emulsion diluted 1 : 1 with 600 m*M* ammonium acetate in the dark. Coated slides were allowed to dry for 2 hr, placed into black slide boxes containing desiccant, and stored at 4°C. After 4 days, the slides were developed with Kodak D19 developer for 3 min at 15°C, rinsed with water, fixed for 3 min in Kodak fixer, and rinsed with water for 40 min. The tissue was then lightly stained with cresyl violet, dehydrated in graded ethanols, cleared in xylene, and coverslipped with Permount. Sections were examined under the light microscope for the presence of reduced silver grains.

Total cell areas and numbers of silver grains overlying DRG neurons were quantified using an image analysis system that consisted of a Nikon microscope, a video camera, a Panasonic TV monitor, a digitizing tablet, a Leading Edge computer, and Microcomp software (Southern Micro Instruments Corp.). All of the data presented in this report were obtained from a single hybridization run. That is, all experimental and control slides were reacted under identical conditions using the same batch of buffers and the same stock of labeled probe and exposed for the same period of time. To minimize experimenter bias, the analysis was conducted under blinded conditions. That is, identifying labels on the slides were covered with opaque tape and coded with numbers so that the experimental condition remained unknown until the end of the quantitative analysis. For quantitation, only neurons in which a clearly visible nucleus and nucleolus were present were used since it has been shown that in mature DRG cells, the nucleus is approximately in the center of these spherical cells (Pannese *et al.*, 1972). Using these criteria, 36–57 neurons per experimental condition were randomly selected from the experimental material for analysis. In conducting the analysis, we found that DRG neurons were readily clustered into three size categories. These size groupings were small cells ($< 600\ \mu m^2$), medium-sized cells ($600–1000\ \mu m^2$), and large cells ($> 1000\ \mu m^2$). Most of the cells that were analyzed in the present experiment fell into the large or medium category (very few small cells having a clearly defined nucleus and nucleolus were present in the material). To establish the statistical significance of differences between experimental and control material, a two-tailed Student's *t* test at the 95% confidence level was used.

Two controls were included in the experiment. In the no-probe condition, slides were treated exactly as described above except that the "hybridization" was done without any labeled cDNA. In the RNAse condition, the tissue was incubated in 100 μg/ml RNase I (Sigma) in 1X *Eco*R1 reaction buffer for 1 hr at 37°C prior to the prehybridization step. These slides were reacted separately from the experimental material to prevent RNAse contamination.

RESULTS

In situ hybridization of normal DRG neurons using the ^{35}S-labeled cDNA probe revealed abundant levels of mRNA for the NF68 protein. Figure 1 illustrates a representative autoradiogram of a normal L5 ganglion. The reduced silver grains which appeared as small black dots over the tissue are evidence of successful

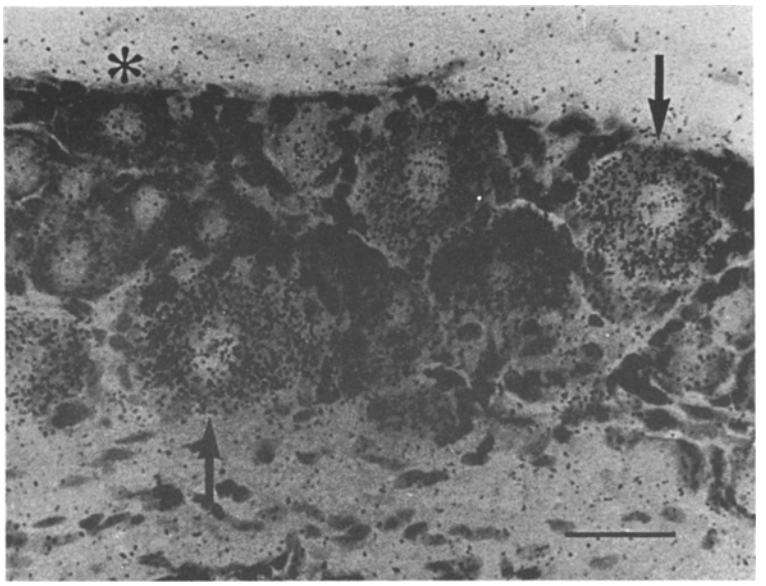

Fig. 1. Autoradiogram of a normal DRG after *in situ* hybridization with ^{35}S-labeled NF68 cDNA. In this control ganglion, silver grains are found over large neurons and smaller-sized neurons but the density of grains is apparently greater over the larger neurons. For quantitative analysis of grain density, only cells with a clearly identifiable nucleolus were used. Two suitable large cells (arrows) and a small neuron (asterisk) are indicated. The autoradiogram was exposed for 4 days; the bar equals 50 μm.

hybridization of the labeled cDNA probe with specific mRNA in the rat tissue. Several controls for the specificity of hybridization were utilized. One of these was built into each section. The DRG contains nonneuronal satellite and Schwann cells as well as regions of axons (those of sensory neurons as well as those of ventral motor neurons that course through the ganglion). Since NF proteins are expressed only in neurons and axons are devoid of ribosomes, regions of the ganglion that do not contain neuronal cell bodies should show the absence of significant grains. This can easily be seen in Fig. 1, where the bottom of the photomicrograph is a region of the ganglion that contains axons and their associated glia. Such regions contained very low numbers of grains and were subtracted as background from grain counts in subsequent quantitative analysis. Additional controls were provided by the analysis of sections that were reacted without labeled probe and of sections which were digested with RNase prior to hybridization. Such material contained very low levels of grains (data not shown).

Qualitative examination of sections of normal DRG revealed that large neurons apparently contained more grains that did smaller-sized cells. We classified cells by the following sizes—small cells ($< 600\,\mu m^2$), medium-sized cells ($601–1000\,\mu m^2$), and large cells ($> 1000\,\mu m^2$)—and always assessed only those neurons in which a clearly defined nucleolus was present. Using these criteria, quantitative analysis using a computer-based image analysis system for grain counting revealed that the grain

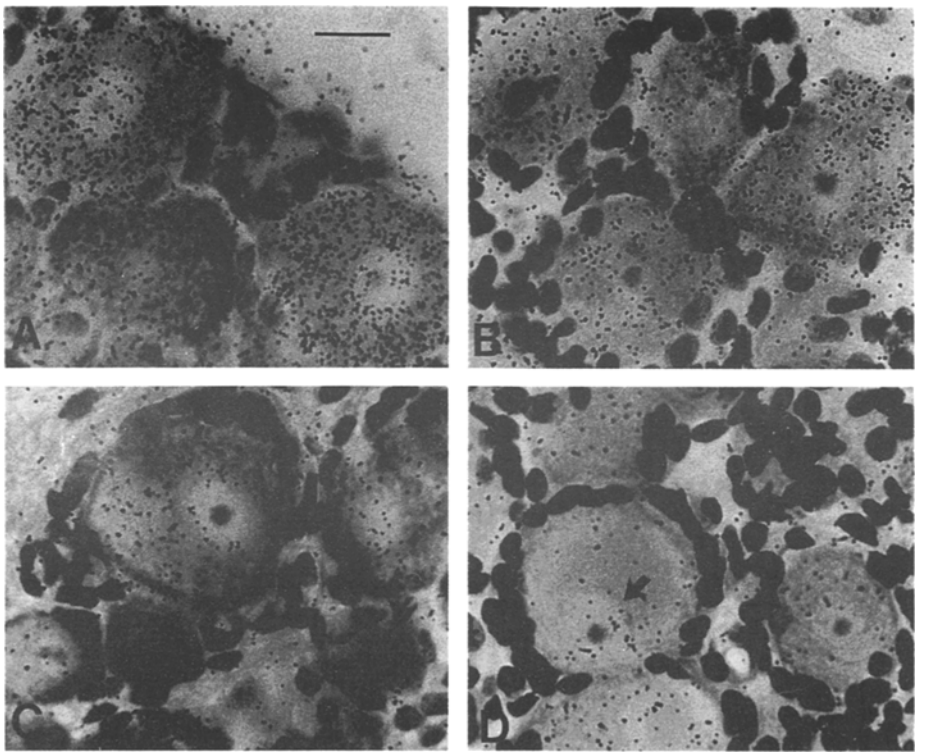

Fig. 2. Autoradiograms of normal DRG neurons and neurons at different times after peripheral axotomy that were hybridized with ^{35}S-labeled NF68 cDNA. (A) Normal neurons; (B) 1 day postaxotomy; (C) 7 days postaxotomy; (D) 14 days postaxotomy. Most of the neurons in these panels were classified as large cells ($> 1000\,\mu m^2$). All of the sections were hybridized under identical conditions, and the autoradiograms were exposed for the same length of time (4 days). Note the strong hybridization signal (silver grains) over the normal neurons. With increasing time after injury, the apparent density of silver grains declined progressively. Visible signs of chromatolysis were found at 7 and 14 days. An example of a large chromatolytic neuron with an eccentric nucleus is indicated by the small arrow in D. The bar indicates 25 μm.

density over large cells was significantly greater ($p < 0.05$) than that over medium or small cells in normal ganglia (Fig. 3). When the data were examined from the standpoint of total numbers of grains per cell rather than grain density, larger cells were found to have a higher absolute number of grains than did smaller cells (not shown). These findings indicate that, in normal DRG, the large neuronal cell bodies had a higher level of expression of NF68 mRNA than did smaller neurons.

To examine the effect of axotomy on the levels of mRNA to NF68 in individual DRG cells, ganglia were harvested at intervals of 1, 7, and 14 days after crush of the ipsilateral distal sciatic nerve and analyzed by *in situ* hybridization. Figure 2 illustrates examples of autoradiograms obtained from DRG neurons at these postaxotomy times. In these experiments, sections of normal DRG and axotomized DRG were hybridized at the same time, using the same batch of buffers and probe, and exposed for identical periods of time to make the experimental conditions as identical as

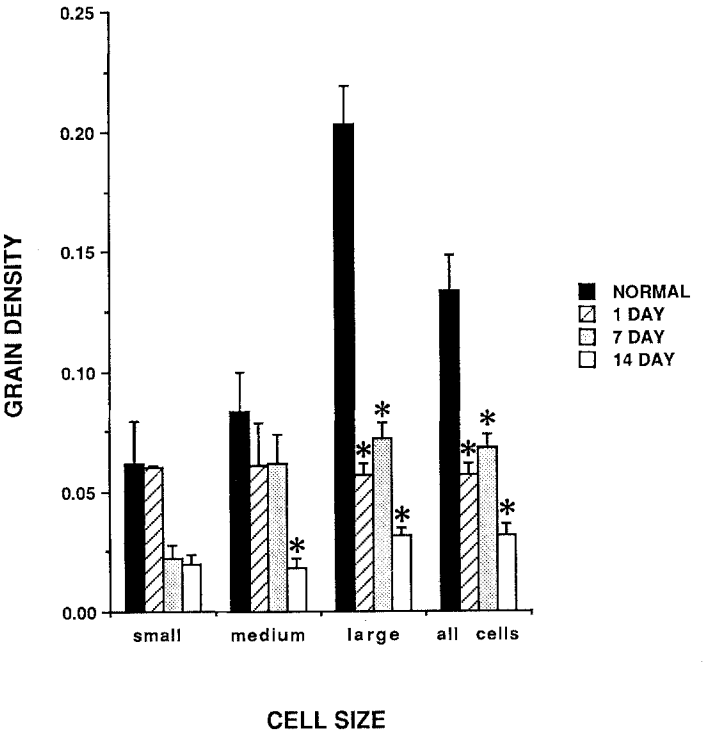

Fig. 3. Quantitation of grain densities over normal and axotomized DRG neurons after *in situ* hybridization with NF68 cDNA. The mean numbers of autoradiographic silver grains per square micrometer neuron area are plotted for neurons of different sizes. Horizontal bars represent the standard error of the means. Small cells, $< 600\,\mu m^2$; medium cells, $601–1000\,\mu m^2$; large cells, $> 1000\,\mu m^2$. Compared to large-sized normal cells, the levels of NF68 mRNA were significantly reduced in large neurons at 1, 7, and 14 days after axotomy. NF68 mRNA levels were significantly reduced in medium-sized cells at 14 days but not at 1 or 7 days postaxotomy and the levels in small-sized cells did not show a significant reduction at any postaxotomy time. Neuronal areas and grain densities were measured using a computer-assisted image analysis system. Asterisks indicate significance at $p < 0.05$ using Student's t test.

possible. Inspection of these autoradiograms revealed a pattern of progressively reduced labeling with increasing time after axotomy. In addition, material from 7 and 14 days postaxotomy revealed the presence of chromatolytic neurons that had light staining cytoplasm and eccentric nuclei (Fig. 2D)

To quantity the change in NF68 mRNA levels in axotomized neurons, an image analysis computer system was used to measure cell areas, count grains, and determine grain densities over individual cells. Background labeling (grains over regions of the DRG that contained axons and glia but no neurons) were measured separately for each section and subtracted from the counts. Figures 3 and 4 illustrate the results of this analysis. Compared to the normal control DRG, the grain densities over large neurons were significantly reduced ($p < 0.05$) at 1, 7, and 14 days postaxotomy. For

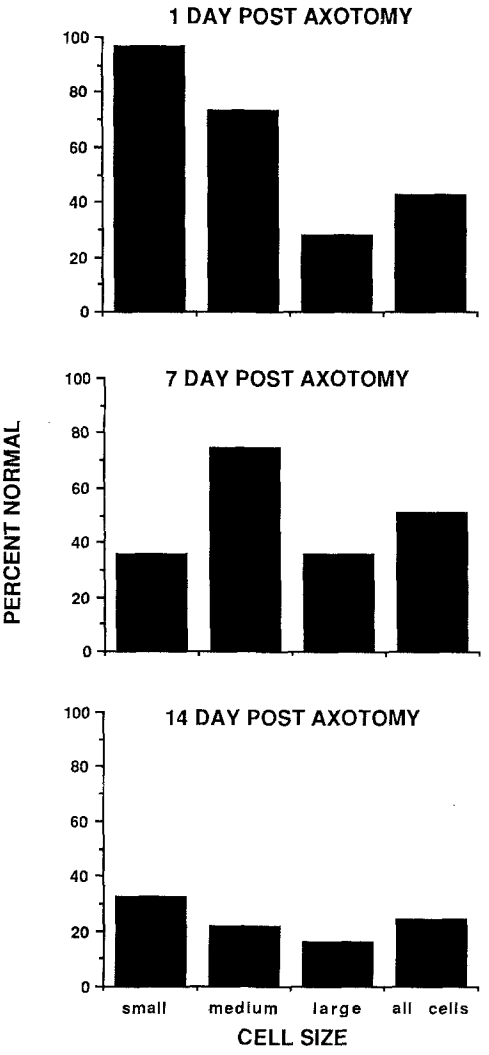

Fig. 4. Levels of NF68 mRNA in axotomized DRG neurons expressed as a percentage of those in normal DRG neurons. The mean grain densities for neurons of different sizes were determined from autoradiograms of sections hybridized with the NF68 cDNA probe using a computer-assisted image analysis system. The values are plotted as a percentage of the mean grain densities over normal DRG neurons. Size categories were identical to those described in the legend to Fig. 3. Note that with increasing time after peripheral axotomy, the levels of mRNA for NF68 in axotomized neurons relative to those in normal cells were progressively reduced.

the medium-sized neurons, the grain densities were significantly reduced at day 14 ($P < 0.05$) but not at 1 or 7 days postaxotomy. In contrast, although a trend toward decreased labeling was noticed, the grain densities over small-sized neurons were not statistically different from controls at any of the postaxotomy times. This may be due partly to the fact that only a few small-sized neurons having nucleoli were available in the experimental material (typically five or six) since the majority of the cells suitable for counting fell into the large or medium-sized category.

When the data were expressed as a percentage of the control labeling, the pattern of signal reduction as a function of time after injury was also evident (Fig. 4). For example, the average grain densities (combined sizes) fell to 43% of control values by 1 day, to 51% of controls by 7 days, and to 24% of controls by 14 days after axotomy (Fig. 4). In addition, it was apparent that the large cells showed a reduction earlier

than the medium-sized cells. Thus, the quantitative analysis confirmed that mRNA levels for the NF68 protein were significantly reduced after peripheral axotomy of DRG cells.

DISCUSSION

The results of this study show that, in rat lumbar DRG neurons, a reduction in the levels of mRNA for the NF68 protein occurs after crush axotomy of the sciatic nerve. This decrease begins as early as 1 day after axotomy and continues through day 14, the longest postaxotomy interval examined in the present study. Previous pulse-labeling studies had shown that peripheral axotomy of DRG cells was followed by a reduction in the synthesis and axonal transport of NF proteins 2 weeks after injury (Oblinger and Lasek, 1985, 1987). Our present findings indicate that these changes are preceded by a reduction in NF mRNA levels. Our observations confirm and extend the findings in a recent study by Hoffman *et al.* (1987) that examined mRNA levels for NF68 at 2 weeks after injury of DRG cells using *in situ* hybridization.

The clear directionality of expression and regulation of axonal size is reinforced by the present results. Axon caliber in large myelinated fibers in mammals is determined largely by NF number and density (Friede and Samorajski, 1970; Lasek *et al.*, 1983; Hoffman *et al.*, 1985). Thus, the pathway of expression of axonal caliber can be defined by the following three main steps in NF supply: transcription of NF genes, translation of NF mRNA, and translocation of NFs into and along axons (Lasek *et al.*, 1983). Reductions in the size of DRG axons after axotomy have been well characterized (Sunderland, 1978; Carlson *et al.*, 1979; Dyck *et al.*, 1984; Hoffmann *et al.*, 1987). Thus, changes in axonal caliber which ensue after injury may be the culmination of the cascade of events which begins with a change at an important regulatory step for axonal size: mRNA levels for NF proteins.

The method of *in situ* hybridization is a powerful tool for examining changes in gene expression in the nervous system. The resolution is good because the method can detect specific mRNAs in individual cells without destroying cell morphology. This has advantages over other methods such as northern blotting which rely on loading equal amounts of RNA on gels (not all of which is the specific mRNA of interest) and on defining net changes in RNA in large groups of cells. In spite of the advantages, however, the *in situ* method does not allow us to conclude that the reduced levels of mRNA are the result of changes in transcription rather than changes in the stabilization of the mRNA in the cytoplasm. At present, it is not technically possible to directly measure the synthesis of specific mRNAs in single identifiable cells.

Our *in situ* hybridization studies have revealed that small DRG neurons normally have much lower levels of NF68 mRNA than do large cells. This is consistent with the fact that smaller cells give rise to smaller-diameter fibers that contain fewer NFs than the large myelinated sensory axons that arise from large DRG perikarya. Unlike the report of Hoffman *et al.* (1987), we found that large and small neurons differed in their response to axotomy since the large cells exhibited a significant reduction in NF68 mRNA while the small-sized neurons did not. A number of factors may be

important in this result. First, it is possible that large neurons are simply more responsive to axotomy than small cells. It is also possible that larger neurons generate a faster response than small cells. For example, small cells may down regulate their levels of NF mRNA but on a slower time scale than the 14-day period we examined. We are currently investigating this possibility by examining a longer time course after axotomy. These studies should also define the time course of recovery of mRNA levels in axotomized DRG neurons. A third possibility for the lack of response of small cells is that the detectability of a reduction in mRNA levels in small neurons is below the resolution of our present experimental methods. The latter possibility is likely since the normal signal in most small cells was low.

One question that arises in considering the results of this study is, Are all mRNA species reduced in DRG cells after axotomy or is the reduction in NF68 mRNA specific? While a definitive answer to this question must await further *in situ* hybridization studies using specific probes for other mRNA species such as tubulins and actin in DRG cells, several lines of evidence suggest that the NF changes are specific. In other cell types, such as goldfish retinal ganglion cells, the levels of mRNA and the level of synthesis of tubulin increase after axotomy (Burrell *et al.*, 1980; Neumann *et al.*, 1983). In addition, in rat DRG cells, the synthesis of tubulin has been shown to increase (Hall, 1982) or not to change (Oblinger and Lasek, 1987), depending on axotomy conditions; and tubulin mRNA levels have been shown to increase on northern blots of whole DRG after axotomy (Hoffman *et al.*, 1987). Thus, it is likely that tubulin genes follow a different pattern of change in expression than the NF genes after axotomy in DRG neurons.

What possible function could down regulation of NF levels serve for the injured neuron? Presumably, the accompanying change in axonal diameter does not have an adverse effect on the function of the neuron since the cell is disconnected from its target and, thus, does not need to maintain maximal conduction properties. It is conceivable that the change in axonal caliber of proximal axons after axotomy is simply a side effect of a response that is aimed at regenerating the distal portion of the axon in an efficient manner. It is thought that the essential components of an effective injury response include an increase in the synthesis of materials important to specific stages of the regenerative response and a decrease in the synthesis of materials that are nonessential or possibly detrimental (McQuarrie, 1983). For example, in the early stages of regeneration, when axonal sprouting and daughter axon elongation occur, the production and efficient delivery of actin (for growth cone motility) and microtubule proteins (to support elongation of the new axon and provide the basis for vesicle transport in the sprouts) are of a high priority. It is possible that reducing NF density in the axon may enable a more efficient delivery of microtubules, actin, and many other materials simply by decreasing the viscosity of the axoplasm. Because NFs and microtubules appear to be linked by extensive cross-bridges in the axon (Hirokawa, 1982), reducing the number of NFs may enable microtubules to move at faster rates by axonal transport. In fact, it has been shown that microtubule protein transport is accelerated in proximal regions of injured DRG axons during the same period of time in which the levels of NF transport are reduced (Oblinger, 1985). At later stages of regeneration, when reconnection of the new axons with targets has occurred and radial growth of the new axon must ensue, the insertion of stabilizing

NFs to increase the diameter of the sprouts becomes an essential component of the growth response. It is likely that an increase in NF production would occur during the later stages of a regenerative response, but this has not yet been investigated. Thus, the down regulation of NFs in the early stages of regeneration may serve to facilitate the growth response. In this case, a change in NF gene expression may be an important component of an effective regenerative response.

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