Changes of Nerve Growth Factor Synthesis in Nonneuronal Cells in Response to Sciatic Nerve Transection

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Abstract. The intact sciatic nerve contains levels of nerve growth factor (NGF) that are comparable to those of densely innervated peripheral target tissues of NGF-responsive (sympathetic and sensory) neurons. There, the high NGF levels are reflected by correspondingly high $mRNA^{NGF}$ levels. In the intact sciatic nerve, $mRNA^{NGF}$ levels were very low, thus indicating that the contribution of locally synthesized NGF by nonneuronal cells is small. However, after transection an increase of up to 15-fold in $mRNA^{NGF}$ was measured in 4-mm segments collected both proximally and distally to the transection site. Distally to the transection site, augmented $mRNA^{NGF}$ levels occurred in all three 4-mm segments from 6 h to 2 wk after transection, the longest time period investigated. The augmented local NGF synthesis after transection was accompanied by a reexpression of NGF receptors by Schwann cells (NGF receptors normally disappear shortly after birth). Proximal to the transection site, the augmented NGF synthesis was restricted to the very end of the nerve stump that acts as a "substitute target organ" for the regenerating NGF-responsive nerve fibers. While the $mRNA^{NGF}$ levels in the nerve stump correspond to those of a densely innervated peripheral organ, the volume is too small to fully replace the lacking supply from the periphery. This is reflected by the fact that in the more proximal part of the transected sciatic nerve, where $mRNA^{NGF}$ remained unchanged, the NGF levels reached only 40% of control values. In situ hybridization experiments demonstrated that after transection all nonneuronal cells express $mRNA^{NGF}$ and not only those ensheathing the nerve fibers of NGF-responsive neurons.

NERVE growth factor (NGF)[1], a well-characterized protein, is essential for the embryonic development and the maintenance of specialized properties of the sympathetic and neural crest–derived sensory neurons (9, 23, 38). The similarity of the effects resulting from the administration of anti-NGF antibodies and the interference with the retrograde axonal transport provided indirect evidence for NGF to act as a retrograde messenger, transferring information from the peripheral target tissues to the innervating neurons (12, 33). More recently this indirect evidence has been corroborated by the demonstration that while the density of sympathetic innervation of target tissues is correlated with the levels of NGF (18) and its mRNA (14, 34), the high levels of NGF in both sympathetic and sensory ganglia are not reflected by correspondingly high levels of $mRNA^{NGF}$ (5, 14), implying that NGF is accumulated in the ganglia by axonal transport from the periphery rather than by local synthesis. This interpretation was supported by the observation that the destruction of sympathetic nerve terminals by 6-hydroxydopamine or blockade of axonal transport by colchicine lead to a rapid decay of NGF in sympathetic ganglia ($t_{1/2}$ = 4–5 h) and to a concomitant increase in NGF levels in the corresponding peripheral target tissues (20). Similarly, ligation of the sciatic nerve which contains both sympathetic and sensory fibers resulted in a very rapid, up to 13-fold increase in NGF levels distal to the ligature and to a drop to <20% proximal to the ligature (19). However, these observations do not exclude the possibility that ligature or transection of the sciatic nerve might initiate an enhanced local synthesis of NGF. Such a possibility was suggested by the previous observation that sciatic nerve continuously releases NGF when brought into culture, indicating a local synthesis of NGF in the nerve in vitro (27).

We therefore aimed to investigate whether NGF synthesis might be up regulated in the transected sciatic nerve. Here, we have analyzed the time course of the changes in levels of NGF and its mRNA in defined small (4-mm) segments distal and proximal to the transection site over a period of 6 h to 2 wk after transection. The simultaneous determination of NGF and $mRNA^{NGF}$ together with the known time course of the degeneration of peripheral axons after transection (4, 32) allowed us to distinguish between NGF accumulation by retrograde transport and local synthesis. These experiments should provide insight into the mechanism(s), by which the synthesis of NGF in nonneuronal cells, particularly Schwann

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1. *Abbreviations used in this paper*: NGF, nerve growth factor.

cells, is regulated by the interaction with adjacent axons. Moreover, the detailed analysis of these parameters on the proximal side of the transection are of interest with respect to (*a*) the initiation and promotion of the regeneration of NGF-responsive nerve fibers after transection by local NGF synthesis and (*b*) the evaluation of whether and/or to what extent this locally enhanced synthesis could in part replace the interrupted supply from the peripheral target tissues.

Materials and Methods

Preparation of Sciatic Nerves

Wistar rats (male or female, 150–200 g) were ether anesthesized and the sciatic nerve was cut with scissors at the sciatic notch. The distal stump of the nerve was diverted into muscle tissue in order to prevent regrowth of new fibers into the peripheral nerve stump. After various time periods, animals were killed, the transected nerves were cut into 5 segments—A and B (proximal) and C, D, and E (distal)—of 4-mm in length each, and frozen immediately in Eppendorf caps which were kept on dry ice. Before further processing, the frozen nerves were weighed and homogenized as described previously for the determination of NGF (21) and mRNA^{NGF} (13). To locally inhibit new synthesis of NGF in a proportion of nerves a cuff (Velaspon^{R}, VEB, Jenapharm, GDR) was soaked in 0.5 mg/ml of actinomycin D, and 1 mg/ml of cycloheximide in PBS was applied around the distal (~8-mm) portion of the nerve.

Determination of NGF Levels

NGF levels were determined according to Korsching and Thoenen (21). The sensitivity of this two-site enzyme immunoassay was 0.01 fmol per assay corresponding to 0.1 ng NGF/g wet weight. All values were expressed as ng NGF/g wet weight and then normalized to contralateral control levels as indicated in legend to Fig. 1.

Determination of mRNA^{NGF}

mRNA^{NGF} levels were determined by the quantitative Northern blot procedure using a calibration standard of 920 bases. A shorter synthetic RNA^{NGF} fragment (510 bases) was added to the tissue samples to assess the recovery in total RNA preparations (see reference 13).

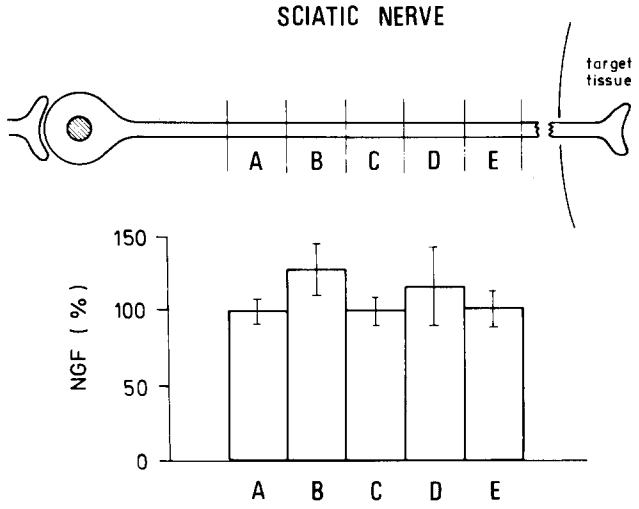

Figure 1. NGF levels in segments along the intact sciatic nerve. The nerve was cut into five consecutive proximo-distal 4-mm segments (segments A, B, C, D, and E) and NGF levels were determined as described in Materials and Methods. The values were normalized to those of segment A (1.2 ± 0.1 ng NGF per g wet weight). Values given are the mean ± SEM of six experiments. The values for the single experiments were determined in quadruplicate.

All NGF and mRNA^{NGF} values were expressed in terms of the unlesioned contralateral sciatic nerve (100%). The values given were expressed as the mean ± SEM.

18S ribosomal RNA was determined from 1/10 of the total RNA extractions using separate Northern blots as described previously (13).

In situ Hybridization of mRNA^{NGF}

The procedures for in situ hybridization of mRNA^{NGF}, of primary cultures, and of O_4 and Thy-1 immunohistochemistry and tissue sections are described in detail elsewhere (2). Briefly, ^{35}S-labeled cRNA probes were synthesized from recombinant pSP6 plasmids (160–180 bases) of both template orientations (RNA^{NGF+}, RNA^{NGF-}). The unspecific binding of ^{35}S-labeled probes to the tissue (which produced major difficulties) was eliminated by prehybridization with cold α-thio-UTP (30 umol/ml), replacement of dithiothreitol (DTT) by the more heat stable β-mercaptoethanol, and reducing the pH for hybridization from pH 7.5 to pH 5.5 (2).

^{125}I-NGF Autoradiography in Primary Cultures of Dissociated Rat Sciatic Cells

Preparation of ^{125}I-NGF and autoradiographic procedures were performed as described previously (28). Primary cultures were washed with a modified Krebs-Ringer solution supplemented with 1% BSA and incubated for 60 min at 37°C with 10 ng/ml ^{125}I-NGF in the same solution. The specificity of ^{125}I-NGF binding was established by adding 10 μg/ml of unlabeled NGF (see Fig. 8).

Results

Levels of NGF and mRNA^{NGF} in the Intact Sciatic Nerve

In the intact sciatic nerve, levels of NGF did not differ significantly between five consecutive 4-mm-long segments (Fig. 1). In particular, there was no evidence for a disto-proximal increase of NGF levels, to be expected in case of a substantial contribution of Schwann cells to the NGF transported from the periphery within sensory and sympathetic fibers of the sciatic nerve. The average NGF level of intact sciatic nerve amounts to 1.2 ± 0.1 ng of NGF/g wet weight and corresponds to levels of intermediately to densely innervated sympathetic organs such as heart atrium (1.0 ± 0.1 ng/g wet weight), iris (1.9 ± 0.3 ng/g wet weight), or vas deferens (2.1 ± 0.2 ng/g wet weight) (Table I). In contrast, the levels of mRNA^{NGF} were very low; i.e., 3.9 ± 1 pg/g wet weight as compared to 52 ± 1 pg/g in the heart atrium, 345 ± 5 pg/g in the iris, and 357 ± 5 pg/g in the vas deferens (Table I).

Time Course of Changes in NGF and mRNA^{NGF} Levels after Sciatic Nerve Transection

The time course for NGF and mRNA^{NGF} levels was followed from 6 h to 2 wk in two 4-mm-long segments proximal to and three corresponding segments distal to the site of transection. The time course of the NGF changes for all segments are presented in Fig. 3, and those for mRNA^{NGF} in Fig. 4, using identical scales for all segments. In view of the particular interest of the changes in NGF and mRNA^{NGF} in the proximal transection stump with respect to the initiation of the regeneration of NGF-responsive nerve fibers, the combined data of NGF and mRNA^{NGF} for the two proximal segments are presented in Fig. 2 using expanded scales.

Proximal Segment A. 6 h after transection the NGF levels dropped to 15% of control and then increased between days 2 and 6 to ~40% of control to remain at this level up to 14 d (Figs. 2 and 3). In contrast, the mRNA^{NGF} values re-

Table I. Levels of NGF and mRNA^{NGF}, and the Ratio of NGF to mRNA^{NGF} in Peripheral Target Tissues of NGF-responsive Neurons and in the Sciatic Nerve

Organ	NGF	mRNA^{NGF}	Ratio NGF/mRNA^{NGF}
	ng/g wet weight	*ng/g wet weight**	
Heart atrium‡	1.0 ± 0.1	0.052 ± 0.01	19
Iris‡	1.9 ± 0.3	0.345 ± 0.05	6
Vas deferens‡	2.1 ± 0.2	0.357 ± 0.05	6
Sciatic nerve (intact)	1.2 ± 0.1	0.0039 ± 0.001	308
Proximal stump§ segment B	0.94 ± 0.1	0.042 ± 0.01	23
Distal stump§ segment D	4.9 ± 0.6	0.030 ± 0.003	164

* The mRNA^{NGF} levels were determined as described in Materials and Methods.
‡ NGF levels were taken from references 17 and 18.
§ Levels were determined 3 d after transection.

mained in the range of the control level of 3.9 pg/g wet weight (Figs. 2 and 4). It has to be mentioned that in the first proximal segment the mRNA^{NGF} levels are very low and many segments had to be pooled to reach the detection limit of the adult rat sciatic nerve. The values determined up to days 3 and 7 are within the limits of the scattering of the method, using total RNA preparations (see Materials and Methods).

Proximal Segment B. Also in this segment immediately adjacent to the transection site, NGF levels dropped rapidly within 6 h. However, in comparison to segment A the initial decrease was somewhat smaller, i.e., 32% as compared with 15% in segment B. Within the following days there was, as in segment A, a gradual increase in the NGF levels equilibrating somewhat below control levels from day 3 to 14 (Fig. 2 *B*). In contrast to the proximal segment A, the mRNA^{NGF} levels changed markedly during the whole observation period. An initial increase 6 h after transection was followed by a transient reduction between 12 and 24 h, which then was followed by a very marked increase to 11-fold levels at day 3 which then gradually decreased to fivefold levels after 14 d. It should be noted that the high mRNA^{NGF} levels in this segment did not result in correspondingly high NGF levels throughout the 14-d observation period (Fig. 2 *B*).

Distal Segment C. After nerve transection, NGF was rapidly accumulated in this distal segment immediately adjacent to the transection site, thus confirming previous experiments (19, 25). The NGF levels rapidly reached a maximum at 24 h to decrease to still sevenfold elevated levels at day 3. The NGF levels remained at this increased level throughout the 14-d observation period (Fig. 3). The mRNA^{NGF} changes can be divided into two phases. There was an early rapid 13-fold increase reached already 6 h after nerve transection. At 12 and 24 h, mRNA^{NGF} dropped to fivefold levels of control. A second maximum (14-fold of control) was reached 3 d after transection and then the values remained constant at about 11-fold levels up to 14 d.

Distal Segments D and E. In contrast to segment C there was no initial rapid increase of NGF levels. This is in agree-

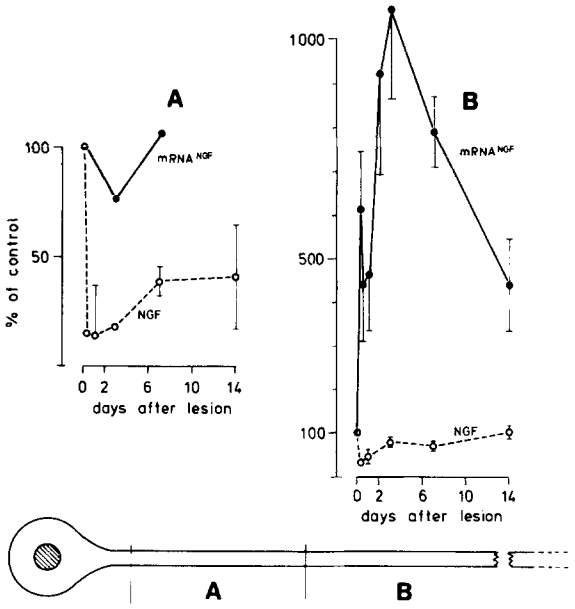

Figure 2. Comparison of NGF and mRNA^{NGF} levels in the proximal segments A and B of the transected sciatic nerve. The NGF values given represent the mean ± SEM of two experiments. The values for the single experiments were determined in quadruplicate. The mRNA^{NGF} values are derived from triplicate determinations obtained in four independent series of experiments. As an exception, mRNA^{NGF} values of segment A are single determinations because a high number of segments (18) had to be pooled for the quantification (see also Results). For time points of measurements, see Figs. 3 and 4. (*Solid circles*) mRNA^{NGF}; (*open circles*) NGF.

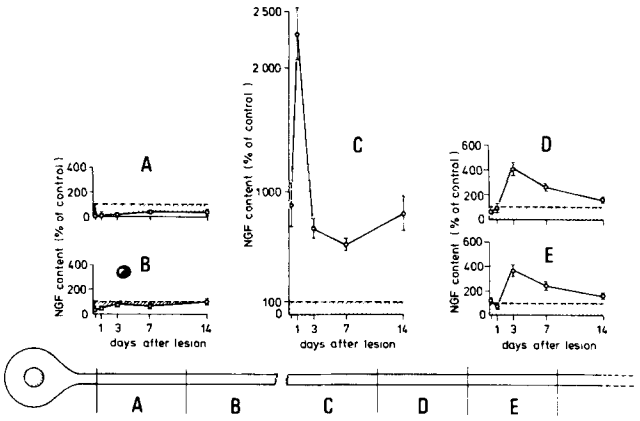

Figure 3. Time course of NGF levels in the sciatic nerve after transection in segments A, B, C, D, and E. Contralateral levels are indicated by the dashed line. The site of transection was between B and C. Values are the means ± SEM from quadruplicate determinations. Time points of measurements were taken at 6 h, 24 h, 3 d, 7 d, and 14 d.

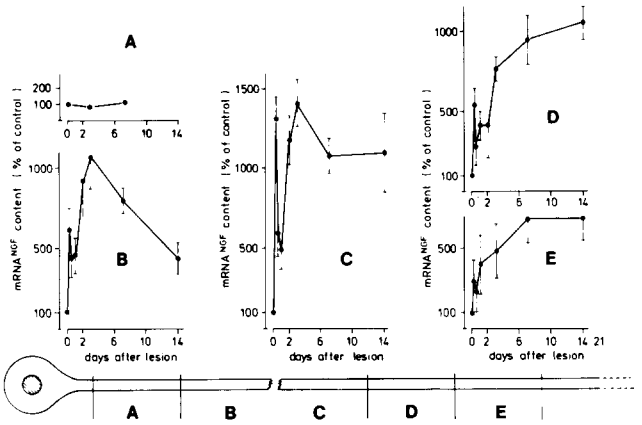

Figure 4. Time course of mRNA^{NGF} levels in the sciatic nerve after transection in segments A to E. The values given are means ± SEM of between three and four determinations pooled from four independent series of experiments with the exception of the values given for segment A (see Results). Time points of measurements were taken at 6 h, 12 h, 24 h, 48 h, 3 d, 7 d, and 14 d.

Table II. Influence of Actinomycin D and Cycloheximide on the Levels of NGF, mRNA^{NGF}, and 18S Ribosomal RNA in Segment C

Cuff* containing	NGF	mRNA^{NGF}	18S
	ng/g wet weight	*pg/g wet weight*	*au*
Saline	6.7	164	100
Drugs	7.4	<8	95

* The drugs were applied for 6 h in a cuff placed around the distal nerve stump. The concentrations of cycloheximide and actinomycin D used were 1 mg/ml and 0.5 mg/ml, respectively.

ment with previous results demonstrating that the rapid NGF increase after nerve crush was confined to a 2-mm segment immediately adjacent to the distal side of the ligature (19). Accordingly, after nerve transection there was only a slow increase in NGF levels reaching a maximum of 400% after 3 d to slowly decline to 250% in the subsequent days to reach in both segments a level of 160% at 14 d. The changes in mRNA^{NGF} in the distal segments D and E were less marked than in segment C; particularly the initial very rapid increase

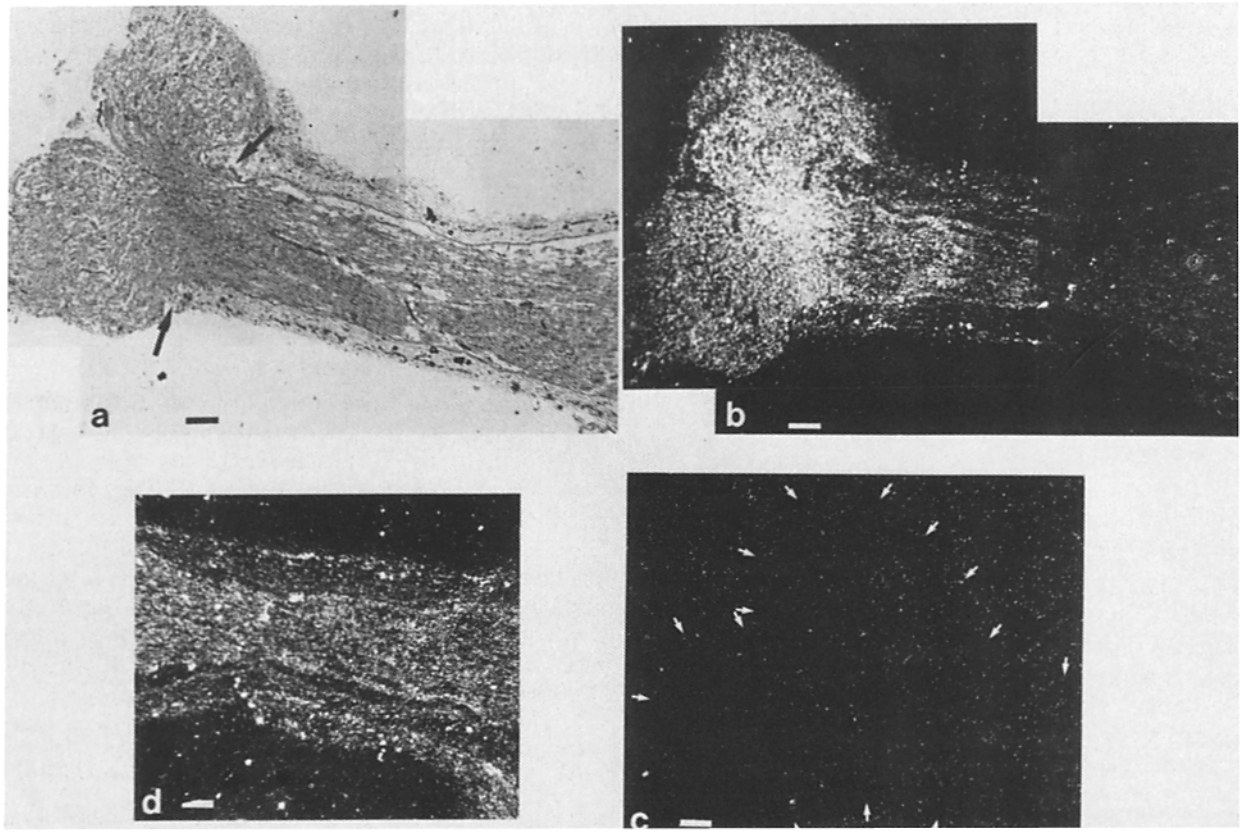

Figure 5. Localization of mRNA^{NGF} in cryostat longitudinal sections of adult rat sciatic nerve 4 d after transection by in situ hybridization. Phase-contrast (*a*) and dark-field photographs (*b*) of a section through the proximal stump were hybridized with a ^{35}S-labeled cRNA^{NGF+} probe, showing dense labeling over the neuroma structure (left from arrows at the proximal tip of the epineurium) and over the adjacent 2.4 mm of the proximal stump. No specific signal above background is seen over the residual proximal part of the stump. The dark-field photograph of longitudinal sections through the distal stump hybridized with the ^{35}S-cRNA^{NGF+} probe (*d*) demonstrates that the grain density is above background and evenly distributed throughout the section whereas sections hybridized with a ^{35}S-cRNA^{NGF-} probe of the opposite polarity (*c*) show no detectable signal above background. Levels of mRNA^{NGF} in intact nerve are below sensitivity limits (not shown). Exposure time, 22 d. Bar, 0.04 mm.

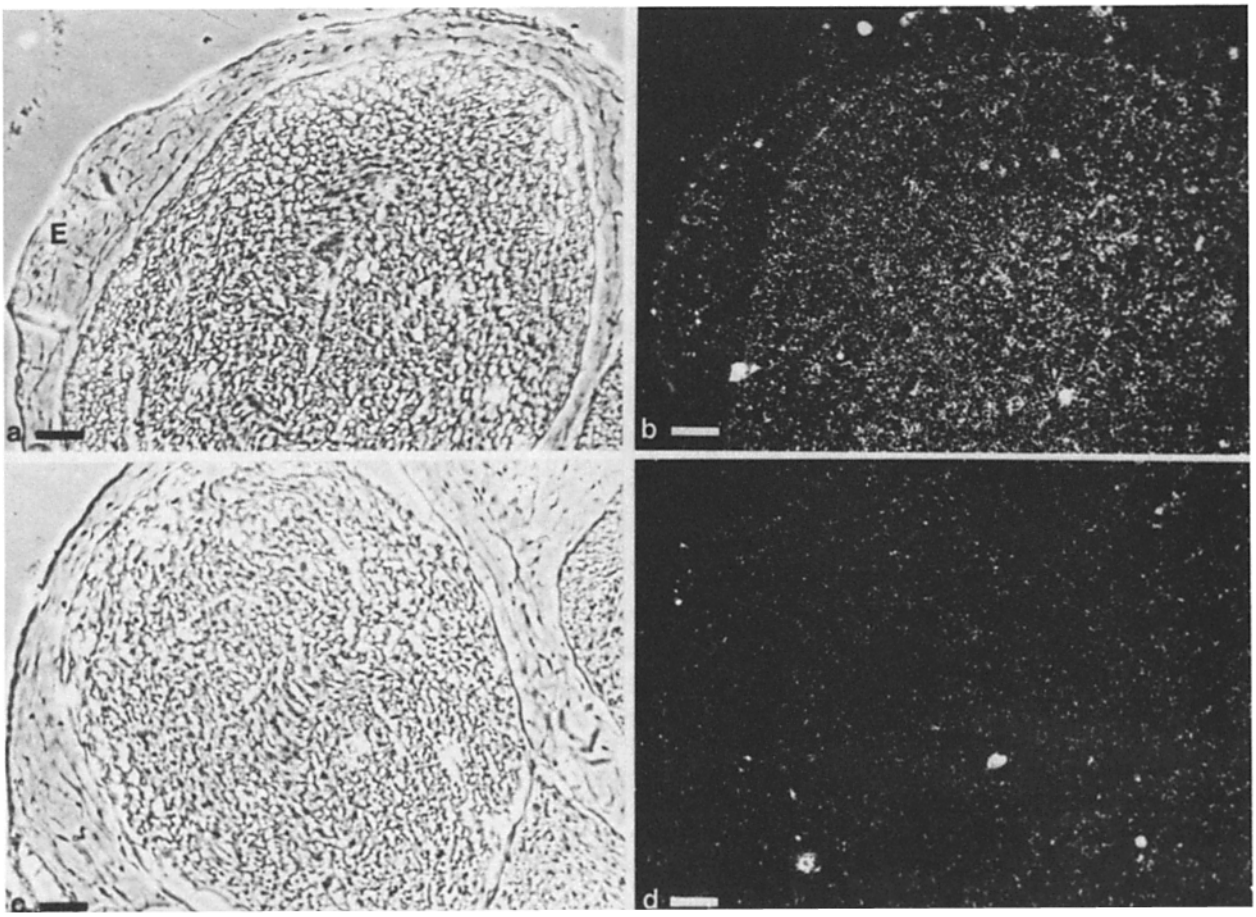

Figure 6. Localization of mRNA^{NGF} in cryostat transverse sections of the distal sciatic nerve stump 6 h after transection. Phase-contrast (*a*) and dark-field photographs (*b*) of sections hybridized with a ^{35}S-cRNA^{NGF+} probe showing homogeneous and dense labeling of the nerve with the exception of the epineurium (*E*) which is only sparsely labeled. There is no specific signal over sections hybridized with the ^{35}S-cRNA^{NGF-} probe (*c* and *d*). Exposure time, 22 d. Bar, 0.04 mm.

within 6 h was much smaller, in segment E barely detectable. The subsequent increase to levels between four- and fivefold in segment D and 4–10-fold in segment E was much slower than in segment C.

Effect of Inhibitors of Protein and mRNA Synthesis on the Initial Increases in NGF and mRNA^{NGF}

To decide whether the initial rapid accumulation of NGF resulted exclusively from accumulation by the still intact axonal transport from the periphery or additionally by local synthesis actinomycin D and cycloheximide were applied by a cuff to the distal nerve region (see Materials and Methods). This approach was chosen since previous experiments with cultured irides had shown that actinomycin D and cycloheximide inhibited the initial increase of both mRNA^{NGF} (13) and NGF (3). In the present experiments the NGF increase within the first 6 h was not affected by these drugs, whereas the rapid increase in mRNA^{NGF} was abolished (Table II). Since the total levels of 18S ribosomal RNA remained unchanged during this initial period after transection (see below), it can be concluded that the increase in mRNA^{NGF} does not reflect a general increase in RNA synthesis but is a specific effect.

Changes in Ribosomal RNA Levels

In the distal segment C, 18S ribosomal RNA levels were unchanged during the first 12 h after transection and then rose sharply to threefold levels between 12 and 24 h. They remained at this elevated level throughout the observation period. Similar changes of ribosomal RNA were also observed in segments B, D, and E. In contrast, there were no significant changes in 18S ribosomal RNA levels in the most proximal segment A. This increase in ribosomal RNA corresponds to a maximal increase in general protein synthesis preceding initiation of DNA synthesis (30) which starts 24 h after transection of the sciatic nerve (8, 26).

Cellular Localization of NGF Synthesis by In Situ Hybridization and Receptor Binding of ^{125}I-NGF

According to the low levels of mRNA^{NGF} in intact sciatic nerves there was no specific labeling in both longitudinal and transverse sections of intact sciatics. However, in longitudinal (Fig. 5) and transverse (Fig. 6) sections prepared 6 h and 4 d after transection there was a homogeneous labeling of the whole distal segment, whereas in the proximal segment only the part of the nerve stump immediately adjacent to the transection site was labeled. Moreover, the portion of the

proximal stump was intensely labeled, which in a neuroma-like structure (24) contained outgrowing neurites and co-migrating cells (left to the arrows indicating the border of the transected epineurium in Fig. 5 *a*). In contrast, a neuroma-like structure was not found at the border of the distal stump (not shown). However, the homogeneous distribution of the label (the epineurium was generally weakly labeled) in longitudinal and transverse sections indicates that not only the nonneuronal cells surrounding the axons of sympathetic and sensory neurons contain $mRNA^{NGF}$, but also those around the motoneuron axons. This interpretation is supported by the observation that in the transected facialis nerve, which contains exclusively motoneuron axons, similar changes in $mRNA^{NGF}$ were observed (data not shown). Furthermore, in cells dissociated from sciatic nerves that had been stripped of the epineurium, all (O_4-positive) Schwann cells and all (Thy-1-positive) fibroblast-like cells were labeled (Fig. 7). In contrast, the expression of new NGF receptors was restricted to O_4-positive Schwann cells as shown by specific ^{125}I-NGF binding (Fig. 8).

Discussion

The simultaneous determination of NGF and $mRNA^{NGF}$ in the sciatic nerve permits the estimation of the relative contributions of locally synthesized NGF by nonneuronal cells and of NGF transported from the periphery by sympathetic and sensory fibers present in the sciatic nerve.

Previous findings have shown that the high levels of NGF in the target fields of peripheral sympathetic and sensory neurons and also of NGF-responsive central cholinergic neurons are reflected by correspondingly high $mRNA^{NGF}$ levels (5, 14, 22). In contrast, in the intact sciatic nerve, the high levels of NGF (1.2 ± 0.1 ng/g wet weight) are not correlated with correspondingly high $mRNA^{NGF}$ levels (3.9 pg/g wet weight) and therefore the ratio between NGF and $mRNA^{NGF}$ is extremely high (308) as compared to intermediately or densely innervated target tissues such as the heart atrium (19), iris (6), and vas deferens (6) (Table I). These observations indicate that the major part of the NGF present in the intact sciatic originates from retrograde transport and that the contribution by local synthesis is very small. This is also supported by the observation that there is no proximo-distal NGF gradient in the sciatic nerve. Such a gradient had to be expected should NGF synthesized by the nonneuronal cells in the sciatic nerve contribute substantially to the NGF transported from the periphery as suggested by Rush and collaborators (1, 6, 31). This small contribution of locally synthesized NGF is further illustrated by the fact that the destruction of sympathetic nerve terminals by 6-hydroxydopamine leads to a drop of NGF levels to ~5% of control in sympathetic ganglia (20).

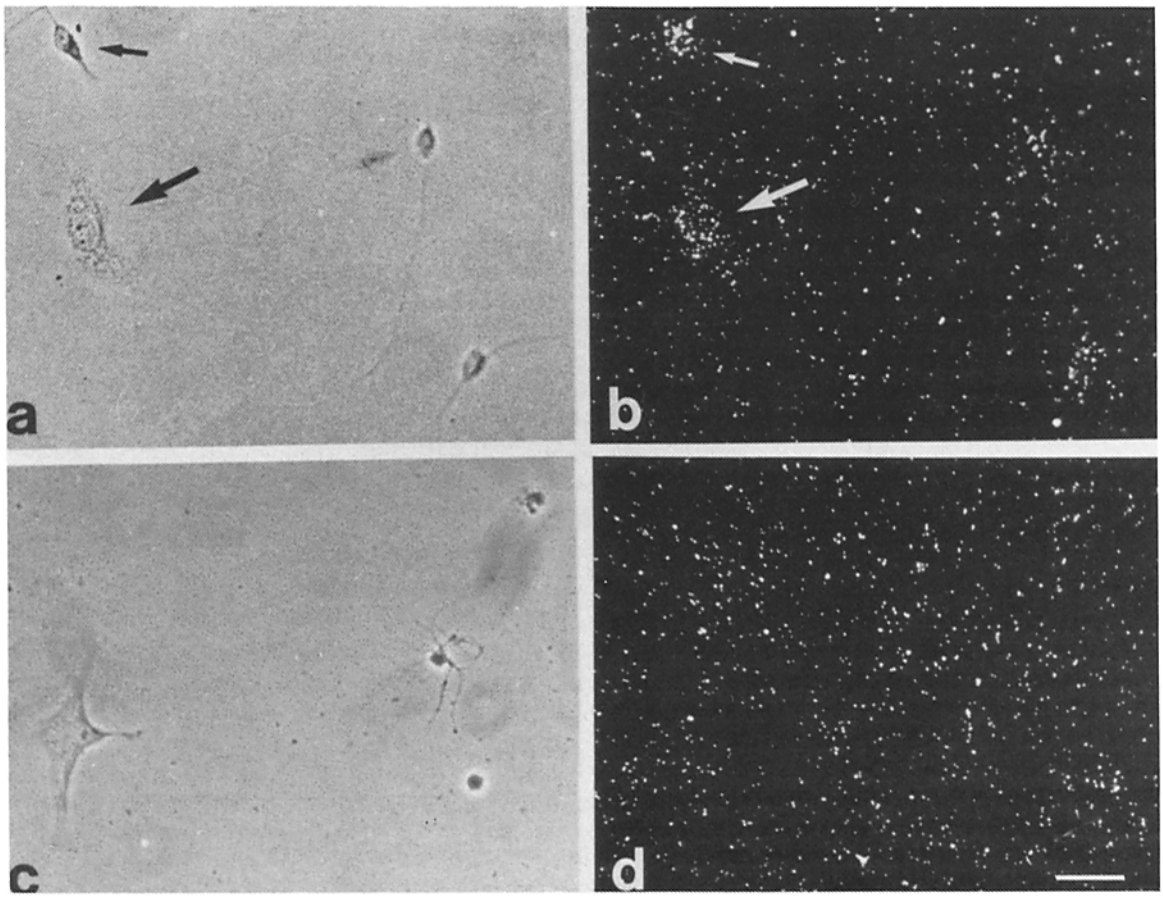

Figure 7. Dissociated cells of sciatic nerves after 48 h in culture. Phase-contrast (*a*) and dark-field photographs (*b*) of cultured cells hybridized with the ^{35}S-$cRNA^{NGF+}$ showing that both cell types, the fibroblasts (*large arrow*) and the Schwann cells (*small arrow*) are labeled. No specific signal is seen over cells hybridized with the ^{35}S-$cRNA^{NGF-}$ probe (*c* and *d*). Exposure time, 18 d. Bar, 0.04 mm.

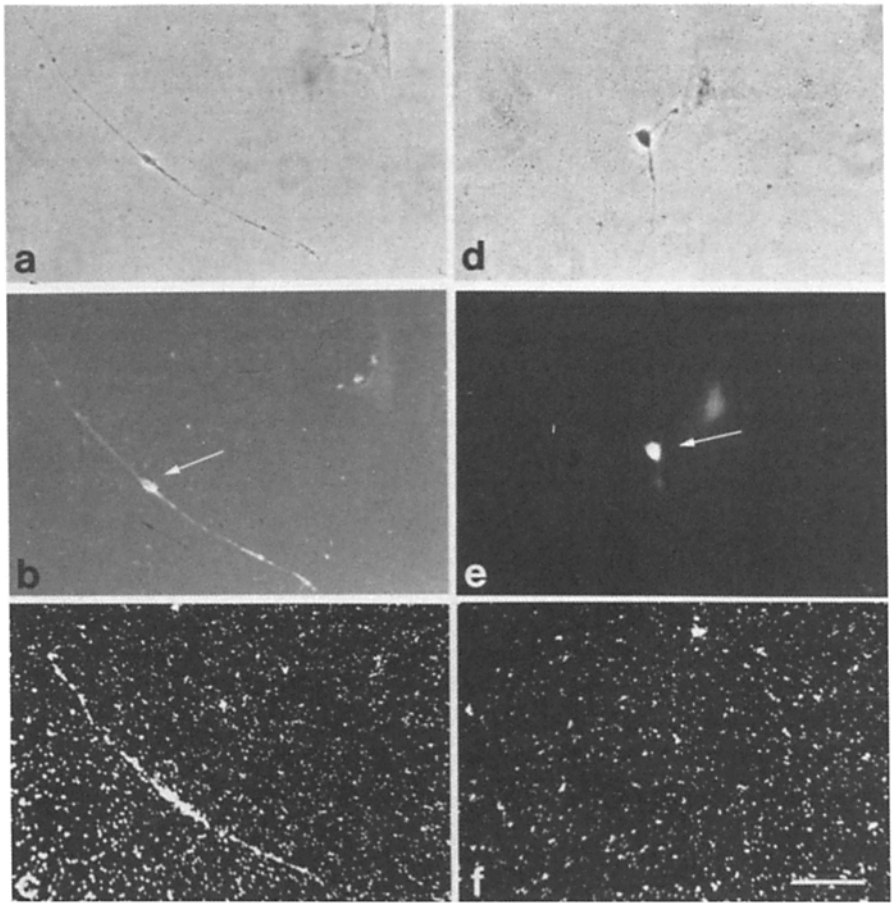

Figure 8. Localization of NGF receptors on dissociated sciatic nerve cells after 48 h in culture by ^{125}I-NGF binding and autoradiography. Phase-contrast (*a* and *d*), fluorescence (*b* and *e*), and dark-field (*c* and *f*) photographs demonstrate that exclusively Schwann cells identified by O_4 monoclonal antibody staining (*arrows* in *b* and *e*) were specifically labeled with ^{125}I-NGF (*c*). Control cultures (*d–f*) were incubated with a 1,000-fold excess of cold NGF over ^{125}I-NGF and were not labeled (*f*). Exposure time, 6 wk. Bar, 0.04 mm.

The transection of the sciatic nerve leads to marked changes in the levels of NGF and mRNA^{NGF} both distal and proximal to the transection site. The changes observed in the distal part, accompanied by a degeneration of the axons disconnected from their cell bodies (4) are of special interest with respect to the regulatory functions of the axons (32) on the synthesis of NGF by the nonneuronal cells. The events taking place proximally to the transection site are of particular interest with respect to the initiation of the regeneration of NGF-responsive nerve fibers. Thus, the events taking place proximally and distally to the transection site will be discussed separately.

Time Course of NGF and mRNA^{NGF} Changes Distal to the Transection Site

The changes in the NGF levels in the first segment (C) distal to the transection site are the result of the initial NGF accumulation by axonal transport from the periphery that slowly declines, the proteolytic degradation (11) of the accumulated NGF as Wallerian degeneration progresses (4), and the augmented local NGF synthesis reflected by the time course of increases of mRNA^{NGF} levels.

The fact that cycloheximide and actinomycin D abolish the initial (first 6 h after transection) rapid increase in mRNA^{NGF} but not that of NGF supports the earlier interpretation of Korsching and Thoenen (19) that the initial rapid increase in NGF results from axonal transport from the periphery rather than from local synthesis. This interpretation is also supported by the observation that in the more distal segments D and E, in spite of an initial rapid increase in mRNA^{NGF} (although smaller than in segment C), there is no initial increase in NGF. By the time peripheral axons have completely degenerated (4), NGF results exclusively from local synthesis by nonneuronal cells. Possible mechanisms that determine the levels of NGF in the absence of removal by axonal transport from the periphery include accumulation in the extracellular space (19), extracellular proteolytic degradation (11), and removal by the local circulation. Additionally the storage and/or degradation by Schwann cells themselves has to be considered: Taniuchi et al. (37) have demonstrated the reappearance of NGF receptors (NGF receptors have been shown to be present consistently in earlier developmental stages in the chick) (27a, 40) distal to the transection site using affinity labeling of the NGF receptors and subsequent immunoprecipitation of the ligand–receptor complex. Furthermore, in preliminary experiments we have demonstrated that ~24 h after transection a marked increase in the expression of the mRNA of the NGF receptor starts in all segments distal to the transection site. Autoradiographic studies with ^{125}I-labeled NGF demonstrated that only Schwann cells express NGF receptors (Fig. 8). Experiments are in progress to determine the possible NGF storage capacity of Schwann cells for exogeneous NGF, and ultimately to decide which proportion of the NGF in the distal stump represents NGF in the extracellular space no more removed by axonal transport.

In situ hybridization experiments have demonstrated that mRNA^{NGF} distal to the transection site is homogeneously

distributed both in longitudinal and transverse sections. Moreover, in cell preparations dissociated from the sciatic nerve, all Schwann cells and fibroblasts were shown to contain mRNA^{NGF} (Fig. 7). Thus reactive NGF production after transection is not confined to the nonneuronal cells adjacent to the nerve fibers of NGF-responsive neurons, but also includes the nonneuronal cells ensheathing motoneuron axons. Consistently, mRNA^{NGF} increases were also found after transection of the facialis nerve at a level containing exclusively motoneuron axons (data not shown).

The rapid increase in mRNA^{NGF} in the sciatic nerve after transection that occurs without change in 18S ribosomal RNA is reminiscent of the situation in the rat iris brought into culture (13). Shelton and Reichardt (35) demonstrated that selective sympathetic and sensory denervation of the iris did not result in an increase in mRNA^{NGF} levels. They suggested that the rapid initial increase in cultured irides results from "tissue damage" of the dissection procedure. Such a traumatic effect might be responsible for the very rapid increase in mRNA^{NGF} that occurred both in the distal and proximal segment immediately adjacent to the transection site (segment B and C). However, the fact that the marked increase in mRNA^{NGF} occurring in all distal segments (whereas proximally it remained confined to the segment immediately adjacent to the transection site; i.e., segment B) suggests that under physiological conditions, the production of mRNA^{NGF} in Schwann cells and fibroblast-like cells is repressed by the presence of the intact axons. Experiments are in progress to determine if the enhanced production of mRNA^{NGF} and the expression of the mRNA of NGF receptors return to normal upon the arrival of regenerating axons after nerve crush.

Time Course of NGF and mRNA^{NGF} Changes Proximal to the Transection Site

In contrast to the extensive mRNA^{NGF} changes in the distal nerve stump, the changes in mRNA^{NGF} proximal to the transection are strictly confined to the immediately adjacent segment B. Moreover, in situ hybridization experiments have shown that only part of this segment is involved in the NGF synthesis. An additional difference to the reaction in the distal segment was revealed by preliminary experiments that demonstrate that in the proximal segment there was no comparably augmented expression of mRNA of NGF receptors. Therefore, the NGF produced by the nonneuronal cells (including motoneuron-associated Schwann cells) is available to support the regeneration of NGF-responsive fibers, which remove the newly synthesized NGF by (retrograde) axonal transport (36). The NGF levels are lower in the proximal (0.94 ng/g wet weight) than in the distal segments (4.9 ng/g wet weight) because it is only proximally that the augmented quantity of NGF produced by local synthesis can be removed by axonal transport. The situation in the proximal segment (ratio between NGF and mRNA^{NGF} levels = 23) therefore reflects the situation of peripheral innervated target tissues (mean ratio = 10; Table I). The very end of the proximal sciatic stump could be considered to function as a "substitute target." However, although the NGF concentrations correspond to those of a relatively densely innervated peripheral target organ (17, 18) the volume is by far too small to compensate for the interrupted supply from the periphery. This is reflected by the fact that the NGF levels in the next proximal segment A (Fig. 2), which does not show any significant changes in mRNA^{NGF} after transection, reach only 40% of the control values of the intact sciatic nerve. This is a reasonable explanation for the observation that the local administration of NGF to the site of transection reduces the morphological and biochemical changes in dorsal root ganglia (7, 16), indicating not only that the quantity of NGF produced locally is limiting but that also the receptors of the outgrowing nerve fibers are not saturated and are able to take up locally applied NGF, necessary for the maintenance of dorsal root ganglion neurons.

In situ hybridization experiments demonstrated that 4 d after transection the mRNA^{NGF}-specific signal was most intense in the region of the proximal stump which, in a neuroma structure (24), contained the outgrowing axons and co-migrating cells (Fig. 5). Recently, it has been shown that neurites do not grow into an environment that lacks Schwann cells when reactive gliosis was prevented at the proximal stump (10). Thus the local production of neurotrophic molecules seems to be essential for the regeneration of neurons after injury of the sciatic nerve. It seems to be very likely that, in addition to NGF, other neurotrophic molecules are produced which may act on neurons nonresponsive to NGF such as motoneurons.

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