Regulation of Nerve Growth Factor Receptor Gene Expression by Nerve Growth Factor in the Developing Peripheral Nervous System

Freda D. Miller, Thazhumpal C. Mathew, and Jean G. Toma

Department of Anatomy and Cell Biology, University of Alberta, Edmonton, Canada

Abstract. Nerve growth factor (NGF) is a target-derived neurotrophic protein that promotes the survival and growth of developing sympathetic and sensory neurons. We have examined NGF receptor gene expression in these neurons after NGF administration. Northern blot and in situ hybridization analyses demonstrated that NGF given systemically to neonatal rats increased levels of NGF receptor mRNA in sympathetic neurons within the superior cervical ganglion. This increase was accompanied by a differential regulation of genes associated with neurotransmitter phenotype; tyrosine hydroxylase mRNA was increased, but neuropeptide Y mRNA was not. NGF receptor mRNA levels were also increased in L4-L5 dorsal root ganglia, although this mRNA was not expressed uniformly in sensory neurons of control or NGF-treated animals. Levels of Tα1 α-tubulin mRNA, a marker of neuronal growth, also increased. In contrast to developing neurons, systemic NGF did not increase NGF receptor mRNA in nonneuronal cells of the sciatic nerve. To determine if NGF regulated NGF receptor gene expression at the transcriptional level, we examined PC12 cells. NGF treatment for 6 h increased NGF receptor mRNA fourfold; this increase was inhibited by cycloheximide. Nuclear run-off transcription assays demonstrated that the increase in steady-state NGF receptor mRNA levels was mediated at the transcriptional level. In contrast, although NGF treatment increased steady-state tyrosine hydroxylase mRNA levels, this effect was not blocked by cycloheximide, and was not due to increased transcription. These data raise the possibility that transcriptional regulation of NGF receptor gene expression by target-derived NGF could be a molecular mechanism for potentiating NGF's effects on neurons during developmental periods of neuronal competition and cell death.

INTERACTIONS between a developing peripheral neuron and its target organ are believed to partially determine the phenotypic fate of that neuron, and to play an important role in neuronal competition and cell death. Nerve growth factor (NGF)[1] is a target-derived neurotrophic factor involved in the survival and differentiation of developing sympathetic and neural crest–derived sensory neurons. NGF given systemically to neonatal rats promotes growth of sympathetic neurons (Levi-Montalcini and Booker, 1960*a*), and affects the neurotransmitter phenotype of both sensory and sympathetic neurons (Kessler and Black, 1980; Otten et al., 1980; Thoenen et al., 1971). Conversely, antibodies to NGF lead to the death of embryonic sensory neurons (Johnson et al., 1980; Aloe et al., 1981), and of neonatal or mature sympathetic neurons (Levi-Montalcini and Booker, 1960*b*; Angeletti et al., 1971; Gorin and Johnson, 1980). NGF synthesis in the target field of sympathetic neurons commences around the time of axonal contact (Davies et al., 1987). Together, these studies suggest that NGF plays an important role in regulating neuronal survival and differentiation.

NGF mediates its actions by binding to the high-affinity form of the membrane-bound NGF receptor (Green et al., 1986). The low-affinity form of the NGF receptor (Sutter et al., 1979), which has been cloned (Johnson et al., 1986; Radeke et al., 1987), is believed to provide an essential component of the high-affinity receptor (Hosang and Shooter, 1985; Green and Greene, 1986), and is capable, when expressed in mutant PC12 cells, of restoring functional responses to NGF (Hempstead et al., 1989). Thus, the same gene product is believed to encode components of both the high- and low-affinity binding sites, as well as a truncated form of the receptor (DiStefano and Johnson, 1988*b*).

NGF receptor mRNA is expressed in both neural and nonneural tissues during the development of rodents and chickens (Ernfors et al., 1988; Large et al., 1989). Wyatt et al. (1990) recently demonstrated that the amount of NGF receptor on developing trigeminal neurons increased at approximately the same time as initial target contact. One explanation for this observation is that NGF may directly increase expression of the NGF receptor gene, a hypothesis supported by studies demonstrating that NGF administered in the cerebrospinal fluid increased NGF receptor mRNA in basal forebrain cholinergic neurons (Higgins et al., 1989; Cavicchioli et al., 1989), and that NGF increased receptor mRNA in cultures of adult sensory neurons (Lindsay et al.,

1. *Abbreviations used in this paper*: DRG, dorsal root ganglia; NGF, nerve growth factor; SCG, superior cervical ganglia.

1990). Regulation of the number and density of NGF receptors on the surface of peripheral neurons by NGF in vivo could be a positive feedback mechanism that contributes to neuronal differentiation and survival.

In this study, we tested whether exogenous, systemic NGF regulates the levels of NGF receptor mRNA in developing peripheral neurons during the period of neuronal competition and cell death. Results demonstrate that systemic NGF increased levels of NGF receptor mRNA in neonatal sympathetic and sensory neurons, but not in developing nonneuronal cells of sciatic nerve that also express NGF receptor mRNA. In PC12 cells, the NGF-mediated increases in NGF receptor mRNA occur at the transcriptional level with characteristics that implicate an immediate early gene product. This increase in NGF receptor gene expression was accompanied by increased expression of tyrosine hydroxylase and Tα1 α-tubulin mRNAs in sympathetic and sensory neurons, respectively. These data indicate that NGF increases transcription of the NGF receptor gene in developing peripheral neurons, and that this increase is coincident with other NGF-mediated changes in neuronal gene expression. Subsequent increases in levels of the high-affinity NGF receptor would provide a cellular mechanism for potentiating the effects of NGF on NGF-responsive neurons, and may indicate a role for target-derived NGF in neuronal competition and cell death.

Materials and Methods

Animals and Surgical Procedures

Neonatal Sprague Dawley rats obtained from timed pregnant mothers were injected subcutaneously daily from postnatal days 2 to 11 with either 5 mg/kg (two experimental animals) or 10 mg/kg (one experimental animal) 2.5S NGF (generously provided by Dr. Richard Murphy, University of Alberta) dissolved in saline. Control littermates were injected daily with similar volumes of saline. Animals were subsequently killed at postnatal day 12 under deep anaesthesia (35 mg/kg sodium pentobarbital) and RNA was isolated from the sciatic nerve, the superior cervical ganglion, and L4-L5 dorsal root ganglia (DRG). Alternatively, animals were anaesthetized with sodium pentobarbital, transcardially perfused with 4% paraformaldehyde in phosphate buffer, and the sciatic nerve, the superior cervical ganglion, and dorsal root ganglia removed and processed for in situ hybridization or immunocytochemistry.

PC12 Cell Cultures

Stock cultures of PC12 pheochromocytoma cells (Tischler and Greene, 1975) were routinely maintained in complete medium consisting of 85% RPMI-1640 medium, 10% heat-inactivated horse serum, 5% FBS, 25 μg/ml streptomycin, and 50 U/ml penicillin (all from Sigma Chemical Co., St. Louis, MO). For each experiment, cells were plated onto 10-cm collagen-coated (rat tail collagen; Sigma Chemical Co.) tissue culture dishes (Corning Glass Works, Corning, NY) containing a total volume of 10 ml of complete medium. 24 h after plating, the cells were washed and maintained in PC-1 serum-free medium (Ventrex) containing the PC-1 supplement, 3 mM L-glutamine (Sigma Chemical Co.), 20 U/ml penicillin, and 20 μg/ml streptomycin until they reached 30–40% confluence. The medium was subsequently changed to PC-1 medium containing 200 ng/ml 2.5S NGF, and the cells were incubated for 2, 6, 24, 48, or 72 h before harvesting. Medium containing NGF was replaced every 24 h. For studies involving cycloheximide, the drug was added at a final concentration of 10 μg/ml for 6 or 12 h during the NGF treatment.

RNA Isolation and Analysis

Total cytoplasmic RNA was prepared from ganglia or nerve by a modification of the phenol/chloroform/isoamyl alcohol technique (Schibler et al., 1980). Total RNA (1–3 μg) was fractionated by electrophoresis on 1.2% agarose gels in the presence of 1 M formaldehyde (Rave et al., 1979) and transferred to nitrocellulose (Thomas, 1980). Antisense RNA probes were hybridized to the immobilized RNA as previously described for probes prepared by nick-translation (Lenoir et al., 1986) except that hybridizations were performed at 65°C, and blots were washed to a stringency of 0.05× SSC at 65°C. Nitrocellulose filters were subsequently exposed to XAR or XRP x-ray film (Eastman Kodak Co., Rochester, NY) for 2 h to 7 d. To confirm that equivalent amounts of RNA were loaded in each lane, ethidium bromide was added to the sample buffer before electrophoresis, and gels were photographed under ultraviolet illumination. In addition, the nitrocellulose was stained with methylene blue (Monroy, 1988) subsequent to hybridization.

Hybridization Probes

Probes to Tα1 and total α-tubulin mRNAs were prepared as previously described (Miller et al., 1987*a*, 1989*a*). For NGF receptor studies, a 310 nucleotide Eco RI/Bam HI fragment containing nucleotides 400–710 of the rat cDNA (Radeke et al., 1987) (kindly donated by Dr. Moses Chao, Cornell University Medical College) was subcloned into pGEM3, and radiolabeled antisense RNA probes were generated with SP6 RNA polymerase (Bethesda Research Laboratories, Gaithersburg, MD) and (32P)CTP (800 Ci/mmol; New England Nuclear, Boston, MA) under conditions described by Melton et al. (1984). Antisense RNA probes specific to mRNAs encoding tyrosine hydroxylase (plasmid K35) (Lewis et al., 1983) and neuropeptide Y (Allen et al., 1987) were generated from subclones provided by Dr. Gerry Higgins and Dr. Janet Allen, respectively. The clone for rat histone H3.3 mRNA (Devo 8) was previously isolated in a screen for mRNAs enriched in the embryonic rat brain (Miller et al., 1987*b*), and has since been fully sequenced and characterized (F. Miller, D. Feinstein, L. Mah, and R. Milner, manuscript in preparation).

Nuclear Run-Off Transcription Assays

Nuclear run-off transcriptions were performed as described by Greenberg and Ziff (1984; Groudine et al., 1981). Briefly, nuclei were isolated from PC12 cells that were 50% confluent after treatment for 6 or 12 h with or without 200 ng/ml NGF. After nuclear run-off transcription, the labeled, purified RNA was hybridized to linearized plasmid containing the inserts of interest immobilized on nitrocellulose. After washing, the filters were exposed to XAR x-ray film (Eastman Kodak Co.) for 1–7 d, and the hybridization signal was quantitated using an Ultrascan XL scanning laser densitometer (LKB Instruments, Inc., Gaithersburg, MD).

In Situ Hybridization

Ganglia or segments of sciatic nerve from perfused animals were cryoprotected in graded sucrose solutions and sectioned onto chromalum subbed slides. In situ hybridization was performed with antisense probes as previously described (Miller et al., 1989*b*). Hybridized slides were air-dried and apposed to Kodak XRP film for 12–24 h to obtain x-ray images. The slides were subsequently dipped in Kodak NTB-2 emulsion, and exposed for 2–7 d before development. Hybridization with a sense probe was performed to ensure specificity of hybridization. For viewing, slides were counterstained with hematoxylin and eosin, and alternate tissue sections stained with cresyl violet.

Analysis and Quantification

Northern blot and nuclear run-off results were quantitated using an Ultrascan XL scanning laser densitometer (LKB Instruments, Inc.). Representative Northern blots from different experiments were chosen for quantitation after ensuring that the amounts of total RNA in the pertinent lanes were identical. Several different film exposures of the same data were analyzed. Results are represented as an approximate value, or as a range of values. To ensure that the in situ hybridization and immunocytochemistry results were comparable and reproducible, we sectioned control and NGF-treated tissue onto the same slides (Miller et al., 1989*a*,*b*).

Results

Regulation of NGF Receptor and Tyrosine Hydroxylase mRNAs in the Developing Superior Cervical Ganglion by NGF

Systemic administration of NGF to neonatal rats dramati-

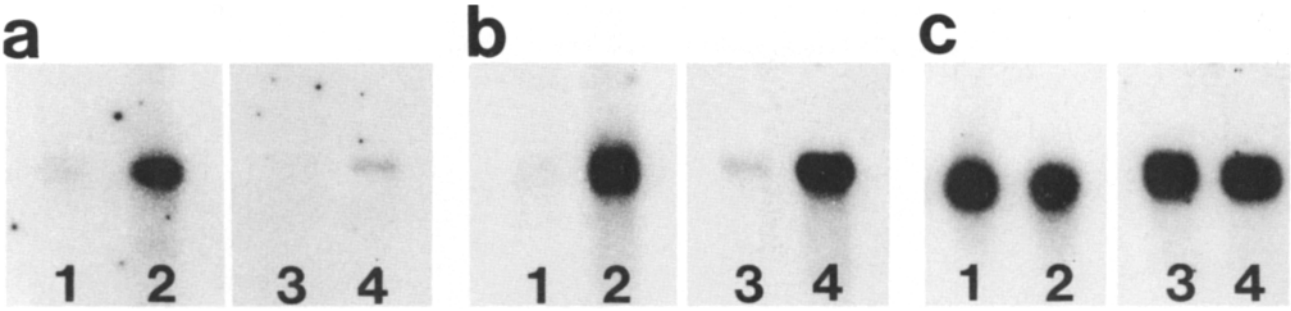


Figure 1. Expression of the NGF receptor, tyrosine hydroxylase, and neuropeptide Y mRNAs in the postnatal day 12 SCG with and without NGF treatment. Northern blot analysis of (*a*) NGF receptor, (*b*) tyrosine hydroxylase, and (*c*) neuropeptide Y mRNAs in equal amounts of total RNA from the SCG of an animal treated with 10 mg/kg NGF (lane *2*) and its control littermate (lane *1*), and equal amounts of total RNA from an animal treated with 5 mg/kg NGF (lane *4*) and its control littermate (lane *3*). Note that lanes *1* and *2* are not directly comparable to lanes *3* and *4* in the amount of RNA analyzed, the specific activity of the probe, or in the exposure time.

cally influences the differentiation of sympathetic neurons (Levi-Montalcini and Booker, 1960*a*; Snider, 1988; Thoenen et al., 1971). To assess any NGF-mediated changes in abundance of NGF receptor that might play a role in this response, we injected neonatal animals with 2.5S NGF from postnatal days 2–11, and isolated RNA from the superior cervical ganglia (SCG) at postnatal day 12. Northern blot analysis revealed that levels of NGF receptor mRNA increased 5–10-fold relative to total RNA synthesis in NGF-treated versus control SCG (Fig. 1 *a*). No significant differences were observed between one animal treated with 10 mg/kg (Fig. 1 *a*, lanes *1* and *2*) and those treated with 5 mg/kg 2.5S NGF (Fig. 1 *a*, lanes *3* and *4*).

To determine whether the increase in NGF receptor mRNA was specific, we examined the mRNAs encoding tyrosine hydroxylase and neuropeptide Y, two proteins associated with the transmitter phenotype of sympathetic neurons. Northern blot analysis demonstrated that, consistent with a previously reported increase in enzyme activity (Thoenen et al., 1971), tyrosine hydroxylase mRNA increased at least 10-fold in the SCG after administration of 10 mg/kg (Fig. 1 *b*, lanes *1* and *2*) or 5 mg/kg 2.5S NGF (Fig. 1 *b*, lanes *3* and *4*). In contrast to tyrosine hydroxylase, neuropeptide Y mRNA levels remained constant with NGF treatment (Fig. 1 *c*).

These data suggest that a specific program of gene expression is induced in developing sympathetic ganglia by systemic NGF. Alternatively, NGF may prolong the developmental process and maintain high neonatal levels of NGF receptor and tyrosine hydroxylase mRNAs. To differentiate between these two possibilities, we isolated total RNA from the SCG at postnatal day 1, 1 d before NGF treatment. Northern blot analysis demonstrated that systemic NGF increased NGF receptor mRNA levels at least 5–10-fold above those seen either at postnatal days 1 or 12 (Fig. 2 *a*). Longer exposures of similar blots revealed that, as demonstrated by Buck et al. (1987), NGF receptor mRNA increased 2–3-fold from postnatal day 1–12 in control animals, paralleling a similar increase in NGF content of the developing SCG (Korsching and Thoenen, 1988). In contrast, neither tyrosine hydroxylase nor neuropeptide Y mRNAs changed significantly in the SCG over the same developmental interval (Fig. 2, *b* and *c*). However, NGF treatment dramatically increased tyrosine hydroxylase mRNA over normal neonatal levels, as it does for NGF receptor (Fig. 2 *b*).

Differential Regulation of NGF Receptor mRNA in Sympathetic Neurons and Nonneuronal Cells of the Sciatic Nerve

To determine whether NGF receptor mRNA was increased in neurons or nonneuronal cells of the SCG, we analyzed sections of control and NGF-treated superior cervical ganglia by in situ hybridization (Fig. 3). Adjacent sections were hybridized to probes specific for NGF receptor mRNA (Fig.

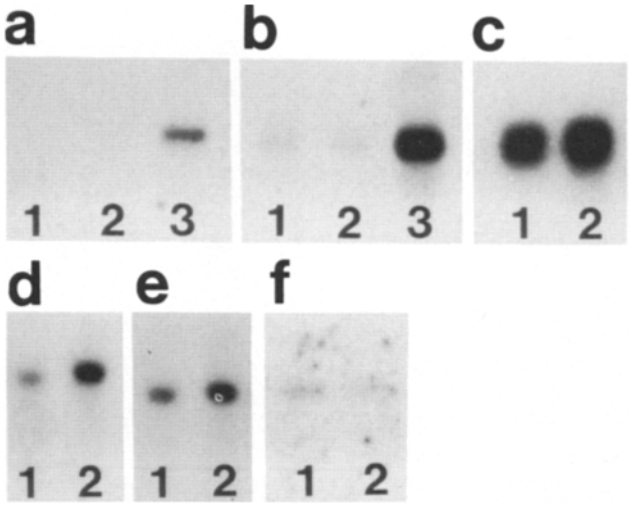
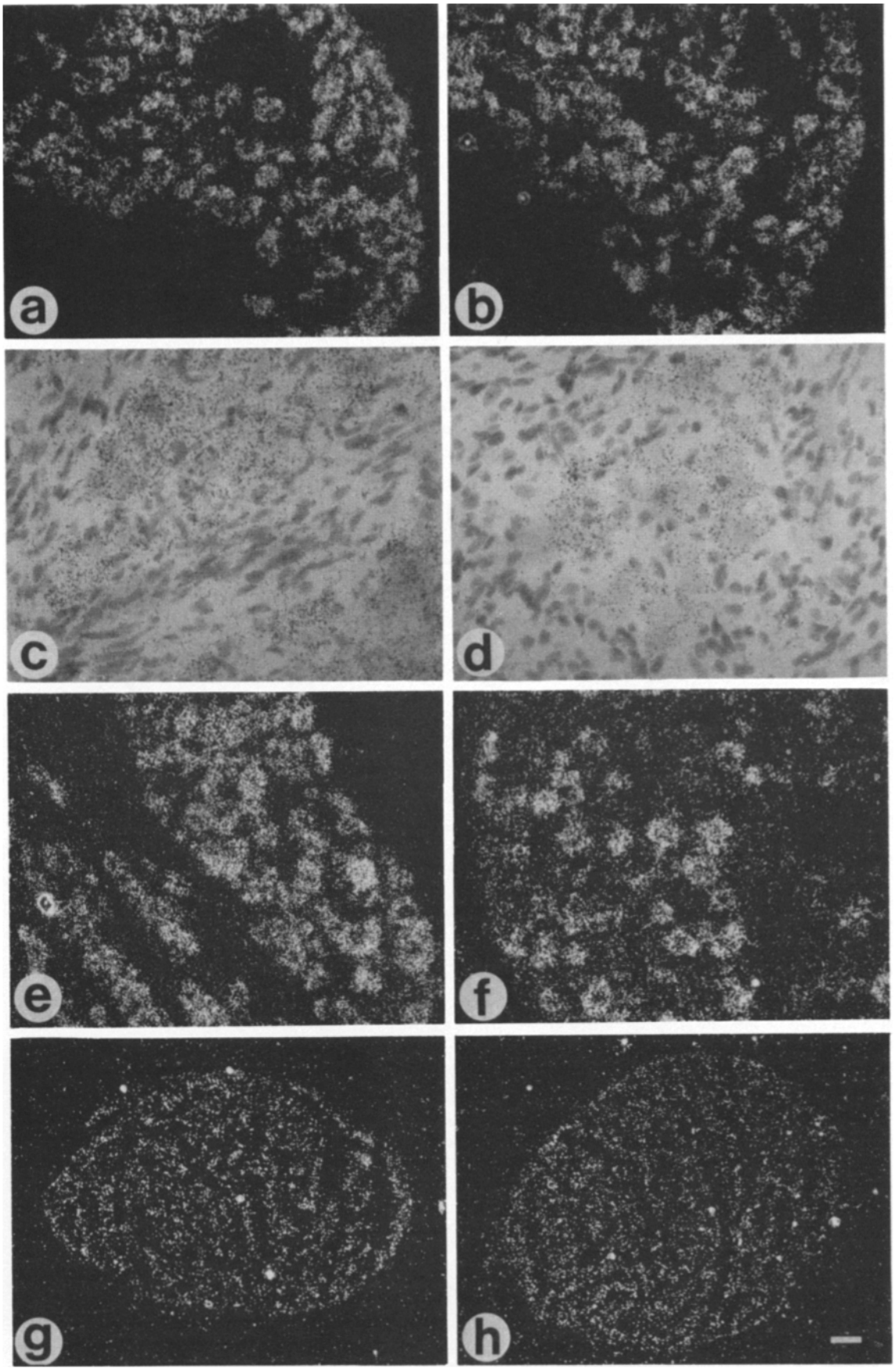


Figure 2. (*a–c*) Expression of NGF receptor, tyrosine hydroxylase, and neuropeptide Y mRNAs in the developing SCG. Northern blot analysis of (*a*) NGF receptor, (*b*) tyrosine hydroxylase, and (*c*) neuropeptide Y mRNAs in equal amounts of total RNA from the SCG of postnatal day 1 (lane *1*), postnatal day 12 (lane *2*, and NGF-treated postnatal day 12 (lane *3*) animals. (*d* and *e*) Expression of NGF receptor and Tα1 α-tubulin mRNAs in the postnatal day 12 L4-L5 DRG with and without NGF treatment. Northern blot analysis of (*d*) NGF receptor and (*e*) Tα1 α-tubulin mRNAs in equal amounts of total RNA from the L4-L5 DRG of an animal treated with 10 mg/kg NGF (lane *2*) and its control littermate (lane *1*). (*f*) Expression of NGF receptor mRNA in the sciatic nerve of NGF-treated animals. Northern blot analysis of equal amounts of total RNA isolated from the sciatic nerve of control (lane *1*) and NGF-treated (lane *2*) postnatal day 12 animals. Note that any differences in hybridization intensity in *c* can be attributed to differences in the amount of total RNA present in each lane.


a
b
c
d
e
f
g
h

3, *a* and *c*) or for tyrosine hydroxylase mRNA (Fig. 3, *b* and *d*), which is expressed in neurons, but not nonneuronal cells, of the ganglion. The SCG were enlarged in all of the NGF-treated animals (data not shown), as previously reported (Levi-Montalcini and Booker, 1960*a*; Thoenen et al., 1971). Increased hybridization to sections from NGF-treated versus control animals was observed for both NGF receptor and tyrosine hydroxylase mRNAs (data not shown), confirming the Northern blot results. The cellular localization of NGF receptor and tyrosine hydroxylase mRNAs was similar in control and NGF-treated animals, with silver grains being predominantly localized over neurons (Fig. 3, *a–d*). The NGF receptor probe did not hybridize significantly to the epineurium, or to any nonneuronal cells scattered throughout the ganglion.

Although these data indicate that NGF receptor mRNA is expressed primarily in neurons of control and NGF-treated ganglia, they do not rule out the possibility that NGF can increase low relative levels of NGF receptor mRNA in nonneuronal cells. To address this possibility, total RNA was isolated from the sciatic nerves of control and NGF-treated P12 animals. Northern blot analysis demonstrated that NGF receptor mRNA levels were similar in the sciatic nerve of control versus NGF-treated animals (Fig. 2*f*). Furthermore, the NGF receptor probe hybridized to a similar degree to cross-sections of control and NGF-treated sciatic nerve (Fig. 3, *g* and *h*).

Regulation of NGF Receptor and Tα1 α-Tubulin mRNAs by Systemic NGF in Sensory Neurons of the DRG

To determine whether NGF increases NGF receptor mRNA in postnatal, neural crest–derived sensory neurons, as it does in sympathetic neurons, we isolated RNA from L4-L5 DRG of NGF-treated animals. Northern blot analysis demonstrated an increase of approximately fourfold in NGF receptor mRNA in the DRG of animals treated with 10 mg/kg (Fig. 2 *d*) or 5 mg/kg 2.5S NGF (data not shown). The magnitude of the increase was lower than that observed in the SCG of the same animals (Fig. 1 *a*).

To determine whether other changes in gene expression accompanied the observed increase in NGF receptor mRNA in the DRG, we examined Tα1 α-tubulin mRNA, which is expressed in all developing neurons (Miller et al., 1987*a*), and is regulated as a function of neuronal growth (Miller et al., 1989*a*). In contrast to NGF receptor mRNA, Tα1 mRNA was increased only approximately twofold in the DRG (Fig. 2 *e*), consistent with the fact that sensory neurons do not sprout significantly after systemic NGF administration (Levi-Montalcini and Booker, 1960*a*).

Previous studies have demonstrated that sensory neurons of the DRG are heterogeneous with regards to the presence of high-affinity NGF binding sites (Richardson et al., 1986). To determine the cellular localization of NGF receptor mRNA, sections of control and NGF-treated ganglia were analyzed by in situ hybridization (Fig. 3*f*). As a control, alternate sections were hybridized to a probe specific for Tα1 α-tubulin mRNA (Fig. 3 *e*). This analysis demonstrated that NGF receptor and Tα1 mRNAs were both predominantly localized to neurons in control (data not shown) and NGF-treated (Fig. 3, *e* and *f*) ganglia, with little or no detectable hybridization to nonneuronal cells. However, whereas the Tα1 α-tubulin probe hybridized uniformly to all DRG neurons, the NGF receptor probe did not, as previously observed in the embryonic chick (Ernfors et al., 1988).

NGF Regulation of NGF Receptor and Tyrosine Hydroxylase Gene Expression in PC12 Cells

To analyze the genetic mechanisms responsible for the NGF-induced increase in NGF receptor mRNA, we studied the PC12 pheochromocytoma cell line (Tischler and Greene, 1975), which responds to NGF with an increase in the number of NGF-binding sites (Bernd and Greene, 1984). To determine whether the NGF-induced increase in NGF binding sites was a consequence of elevated levels of NGF receptor mRNA, we isolated RNA from PC12 cells that had been exposed to NGF for timepoints ranging from 2 to 72 h. Northern blot analysis demonstrated that NGF receptor mRNA levels were similar to controls after 2 h, and were increased approximately fourfold at 6, 12, 24, and 48 h posttreatment (Fig. 4 *a*). The NGF-induced increase in NGF receptor mRNA observed at 6 and 12 h was completely inhibited by the addition of the protein synthesis inhibitor cycloheximide to the culture medium (Fig. 4 *c*).

Expression of tyrosine hydroxylase mRNA was also regulated by NGF in PC12 cells. Tyrosine hydroxylase mRNA did not change at 2 h, but was increased approximately twofold at 6 h, and threefold at 12, 24, and 48 h after NGF addition, as determined by Northern blots (Fig. 4 *b*). This increase was transient, and by 72 h, levels of tyrosine hydroxylase mRNA were similar in the control and NGF-treated PC12 cells (data not shown). In contrast to NGF receptor mRNA, the NGF-mediated increase in tyrosine hydroxylase mRNA was not affected by the concurrent addition of cycloheximide (Fig. 4 *d*).

To determine whether the changes in steady-state levels of NGF receptor and tyrosine hydroxylase mRNAs were a consequence of increased rates of transcription, we performed nuclear run-off transcription assays. Nuclei were isolated from PC12 cells cultured with and without 200 ng/ml NGF

Figure 3. (*a–d*) Expression of NGF receptor and tyrosine hydroxylase mRNAs in sympathetic neurons of NGF-treated postnatal day 12 animals. Sections of SCG from NGF-treated animals were hybridized with probes specific for (*a* and *c*) NGF receptor or (*b* and *d*) tyrosine hydroxylase mRNAs, coated with emulsion for autoradiography, developed, counterstained with hematoxylin and eosin, and visualized under darkfield (*a* and *b*) or brightfield (*c* and *d*) illumination. Note the clustering of grains over the large, pale-staining neurons in *c* and *d* and the relative lack of signal over the smaller, nonneuronal cells. (*e* and *f*) Expression of NGF receptor and Tα1 α-tubulin mRNAs in sensory neurons of NGF-treated postnatal day 12 animals. Sections of L4-L5 DRG from NGF-treated animals were hybridized with probes specific for (*e*) Tα1 α-tubulin or (*f*) NGF receptor mRNAs and, following autoradiography, visualized under darkfield illumination. (*g* and *h*) Expression of NGF receptor mRNA in the sciatic nerve of NGF-treated postnatal day 12 animals. Sections of sciatic nerve from control (*g*) and NGF-treated (*h*) animals were hybridized with probes specific for NGF receptor mRNA and, after autoradiography, visualized under darkfield illumination. Bars: (*a*, *b*, *e*, and *f*) 10 μm; (*c* and *d*) 5 μm; (*g* and *h*) 20 μm.

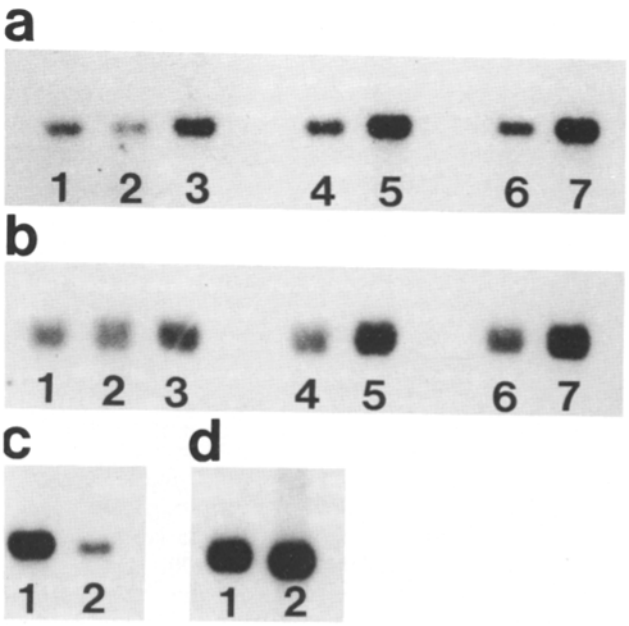


Figure 4. (*a* and *b*) Expression of NGF receptor and tyrosine hydroxylase mRNAs in NGF-treated PC12 cells. Northern blot analysis of (*a*) NGF receptor and (*b*) tyrosine hydroxylase mRNAs in equal amounts of total RNA from control PC12 cells (lanes *1*, *4*, and *6*) or from PC12 cells treated with 200 ng/ml 2.5S NGF for 2 h (lane *2*), 6 h (lane *3*), 24 h (lane *5*), and 48 h (lane *7*). (*c* and *d*) Expression of NGF receptor and tyrosine hydroxylase mRNAs in NGF-treated PC12 cells with and without cycloheximide treatment. Northern blot analysis of (*c*) NGF receptor, and (*d*) tyrosine hydroxylase mRNAs in equal amounts of total RNA from PC12 cells treated with 200 ng/ml 2.5S NGF for 12 h with (lane *2*), and without (lane *1*) 10 μg/ml cycloheximide.

for 6 or 12 h, and the relative levels of transcription of the NGF receptor and tyrosine hydroxylase genes determined (Fig. 5). For comparison, we also examined transcription rates for the replication-independent histone H3.3 mRNA, which does not increase with NGF treatment of PC12 cells, and for total α-tubulin mRNA, which does (J. Toma and F. Miller, unpublished observations). These experiments demonstrated that NGF treatment increased the transcription rate of the NGF receptor gene approximately three- to fourfold. This increase is equivalent to the observed increase in steady-state NGF receptor mRNA levels (Fig. 4), suggesting

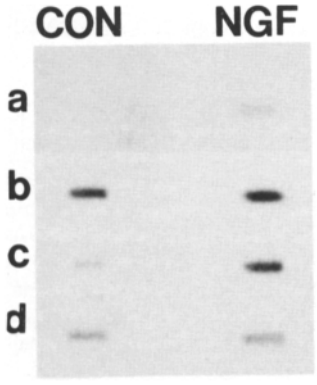


Figure 5. Nuclear run-off transcription of (*a*) NGF receptor, (*b*) histone H3.3, (*c*) total α-tubulin, and (*d*) tyrosine hydroxylase mRNAs in PC12 cells cultured with (*NGF*) or without (*CON*) 200 ng/ml 2.5S NGF for 12 h. Note that *a* comes from a darker exposure of the same experiment shown in *b–d*.

that NGF mediates its effects upon this gene mainly at the transcriptional level. A similar fourfold elevation of transcription rate was observed for total α-tubulin mRNA. In contrast, NGF did not affect the transcription rate of tyrosine hydroxylase mRNA, and only slightly increased that for histone H3.3 mRNA (Fig. 5).

Discussion

These results demonstrate that NGF increases levels of its own receptor mRNA in neonatal peripheral neurons as part of a specific program of NGF-induced gene expression. This program includes coordinate upregulation of tyrosine hydroxylase mRNA in sympathetic neurons, and Tα1 α-tubulin mRNA in both sympathetic (Mathew and Miller, 1990) and sensory neurons. The NGF-mediated increase in NGF receptor mRNA is specific to neurons, which are known to display high-affinity NGF receptor binding sites (Richardson et al., 1986), but is not observed in nonneuronal cells of the sciatic nerve that also express NGF receptor mRNA. In PC12 cells the NGF-induced increase in NGF receptor mRNA is mediated at the transcriptional level, with characteristics that implicate an immediate early gene product in the observed transcriptional activation. Together, these data predict that one direct result of NGF binding to its high-affinity receptor on developing and mature neurons in vivo is increased transcription of the NGF receptor gene. This would provide a cellular mechanism for potentiating the effects of NGF on NGF-responsive neurons during development, collateral sprouting, and physiological situations where NGF is increased either locally or systemically.

NGF mediates its biological effects by binding to the high-affinity form of the membrane-bound NGF receptor (Green et al., 1986). Since we have determined levels of NGF receptor mRNA, which is believed to encode components of both the high- and low-affinity binding sites (Hosang and Shooter, 1985; Green and Greene, 1986; Hempstead et al., 1989), as well as a truncated form of the receptor (DiStefano and Johnson, 1988*b*), it is not possible to make definitive statements about the protein produced as a function of the observed increases. However, the in situ hybridization studies presented here correlate well with the reported localization of high-affinity NGF binding sites on sympathetic and sensory neurons (Richardson et al., 1986). Furthermore, Bernd and Greene (1984) have previously demonstrated that NGF increases the number and density of high- and low-affinity receptors on PC12 cells, and Verge et al. (1989) have shown that administration of NGF prevented an axotomy-induced decrease in high-affinity binding sites on lesioned sensory neurons. It therefore seems likely that the NGF-mediated increases in neuronal NGF receptor mRNA levels lead to a corresponding increase in high-affinity NGF receptors.

NGF Selectively Induces NGF Receptor and Tyrosine Hydroxylase mRNAs in Developing Sympathetic Neurons

Administration of NGF to neonates has dramatic effects on sympathetic neurons, causing increased terminal sprouting (Levi-Montalcini and Angeletti, 1968), increased dendritic aborization (Snider, 1988), and increased activity of enzymes involved in catecholamine biosynthesis (Thoenen et

al., 1971). NGF treatment in doses that caused these changes increased NGF receptor mRNA levels 5–10-fold in P12 sympathetic neurons. This increase can be only partially explained by NGF-mediated rescue of neonatal sympathetic neurons, since NGF treatment permits only 30% more SCG neurons to survive (Hendry and Campbell, 1976; Hendry, 1977). The actual relative increase in NGF receptor mRNA on a per neuron basis is difficult to estimate, since the ratio of nonneuronal cells to neurons is increased by NGF treatment (Hendry and Campbell, 1976).

Elevated NGF receptor mRNA levels in sympathetic neurons are coincident with, and may play a role in, the induction of tyrosine hydroxylase mRNA. Thoenen et al. (1971) have previously demonstrated that the specific activity of tyrosine hydroxylase increased approximately fivefold with systemic NGF treatment, an increase that can be explained by the 10-fold increase in mRNA reported here. Previous studies demonstrated that NGF regulation of tyrosine hydroxylase is time and dose dependent in the SCG (Max et al., 1978; Kornblum and Johnson, 1982), in cultured sympathetic neurons (Hefti et al., 1982; Raynaud et al., 1988), and in adrenal chromaffin cells (Acheson et al., 1984). For example, studies using adrenal chromaffin cells demonstrated that tyrosine hydroxylase is first induced by NGF following a lag time of 36 h (Acheson et al., 1984). It may be that NGF must first "prime" these cells by increasing NGF receptor levels, in a manner analogous to the "priming" of PC12 cells (Bernd and Greene, 1984), to produce a maximal increase in tyrosine hydroxylase.

In addition to increasing NGF receptor and tyrosine hydroxylase mRNAs above early neonatal levels, systemic NGF prevents a developmentally programmed decrease in Tα1 α-tubulin mRNA in sympathetic neurons (Mathew and Miller, 1990). In contrast, neuropeptide Y mRNA, which is associated with the neurotransmitter phenotype of a subset of sympathetic neurons in the SCG (Ekblad et al., 1984), does not change. Together, these data indicate that NGF directly or indirectly regulates a specific program of gene expression in neonatal sympathetic neurons.

Although these studies were carried out with systemic NGF, the results may have implications for the role target-derived NGF plays in neuronal competition and cell death, which are ongoing in the superior cervical ganglion during the period we chose for NGF administration (Hendry, 1977). Based upon our data, we hypothesize that initial exposure of a developing sympathetic neuron to target organ-derived NGF would increase NGF receptor and tyrosine hydroxylase mRNAs, and maintain elevated levels of Tα1 α-tubulin mRNA. The increased mRNA levels could provide protein essential for expansion of the terminal arbor, and/or for neuronal maturation. In addition, increased NGF receptor mRNA could produce an increase in the number and density of high- and low-affinity neuronal receptors, as it does in PC12 cells (Bernd and Greene, 1984), thus, increasing net binding capacity and providing a "sink" for NGF. One prediction of such a feedback mechanism is that early-arriving neurons, which have elevated receptor levels and binding capacity, would compete more effectively than later-arriving neurons for limiting concentrations of target-derived NGF. One recent study supports this hypothesis; target contact is correlated with a significant increase in NGF receptor mRNA in developing trigeminal neurons (Wyatt et al., 1990).

Systemic NGF Increases NGF Receptor mRNA Levels in Neonatal Sensory Neurons

Our data demonstrate that NGF treatment increased NGF receptor mRNA approximately fourfold in the postnatal L4-L5 DRG. However, these sensory neurons did not express NGF receptor mRNA uniformly, as previously observed in the embryonic chick (Ernfors et al., 1988) and consistent with the observation that only 50% of L4-L5 neurons bind NGF with high affinity (Richardson et al., 1986; Verge et al., 1989). Since we did not quantitate the relative levels of NGF receptor mRNA on a per neuron basis, it is possible that NGF treatment increased this mRNA only within a defined population of DRG neurons.

Previous studies of postnatal sensory neurons after NGF administration failed to demonstrate significant increased sprouting (Levi-Montalcini and Booker, 1960*a*), although a subset of DRG neurons hypertrophied (Kornblum and Johnson, 1982), and levels of substance P, a marker for sensory neurons, increased (Kessler and Black, 1980; Otten et al., 1980). The relative lack of neuronal sprouting after NGF administration is consistent with the small, twofold increase in Tα1 α-tubulin mRNA reported here. In sympathetic neurons, which sprout extensively with NGF treatment (Levi-Montalcini and Booker, 1960*a*), levels of Tα1 α-tubulin mRNA increase 5–10-fold (Mathew and Miller, 1990).

Similar NGF-induced genetic changes may have relevance not only in developing peripheral neurons, but also during the sprouting and growth of mature neurons. Increased available target-derived NGF has been implicated in the collateral sprouting of mature sensory neurons (Diamond et al., 1987), and levels of Tα1 α-tubulin mRNA increased during the collateral sprouting of mature sympathetic neurons (Mathew and Miller, 1990). In the central nervous system, administration of NGF in the cerebrospinal fluid leads to increased NGF receptor mRNA in basal forebrain cholinergic neurons (Higgins et al., 1989; Cavicchioli et al., 1989). Furthermore, NGF increased NGF receptor mRNA in cultures of mature sensory neurons (Lindsay et al., 1990). These studies all suggest that NGF-induced changes in genes like NGF receptor and Tα1 α-tubulin could play a physiologically relevant role in the mature animal.

NGF Does Not Regulate NGF Receptor Gene Expression in Developing Nonneuronal Cells

The data presented here suggest that expression of NGF receptor mRNA is correlated with the presence of a functional, high-affinity receptor on peripheral neurons. A similar correlation does not seem to exist for nonneuronal cells of the ganglia or the sciatic nerve. Developing sciatic nerve contains NGF receptor mRNA and protein, as previously demonstrated (Heumann et al., 1987*b*; Yan and Johnson, 1988) and confirmed here. It is likely that these represent low-affinity NGF binding sites, since Schwann cells cultured from neonatal sciatic nerve express only low-affinity receptor (DiStefano and Johnson, 1988*a*). After transection of the adult sciatic nerve, both NGF receptor mRNA and protein are reexpressed (Taniuchi et al., 1986; Heumann et al., 1987*a*), coincident with localized production of NGF itself (Heumann et al., 1987*a*,*b*). Our studies, which demonstrate that systemic NGF does not increase NGF receptor mRNA in nonneuronal cells in vivo, suggest that localized produc-

tion of NGF after nerve injury does not itself cause increased NGF receptors. Although NGF administered systemically may not have complete access to the nerve as the blood/nerve barrier develops postnatally, the same is not true for nonneuronal cells of the peripheral ganglia. Thus, NGF differentially regulates NGF receptor mRNA in neurons versus nonneuronal cells of the developing peripheral nervous system. This conclusion is supported by in vitro studies demonstrating that NGF does not regulate expression of NGF receptor mRNA in cultured Schwann cells (Lemke and Chao, 1988), or in cultured nonneuronal cells of adult sensory ganglia (Lindsay et al., 1990). One potential explanation for these observations is cell type–specific gene regulation. However, the more likely, alternative explanation is lack of high-affinity NGF receptors on developing, NGF receptor mRNA-producing nonneuronal cells.

NGF Increases Transcription of the NGF Receptor Gene in PC12 Cells

NGF increased NGF receptor gene expression in PC12 cells within 6 h of treatment. The increase in steady-state mRNA levels was approximately equal to the increase in transcription rate, indicating that NGF mediates its effects primarily at the transcriptional level. Inhibition of protein synthesis by cycloheximide blocked the increase, suggesting that the NGF receptor gene may be the "target" of one or more of the NGF-inducible immediate early gene products (Sheng and Greenberg, 1990). Although the promoter of this gene has been suggested to resemble that of a constitutively-expressed gene (Sehgal et al., 1988), it contains a recently described binding site (Christy and Nathans, 1989) for the zinc finger protein zif-268 (or, alternatively, NGFIA, Egr-1, or Krox 24) (Sikhatme et al., 1988; Lemaire et al., 1988; Milbrandt, 1987; Christy et al., 1988) from nucleotides -161 to -152. The zif-268 gene product is rapidly induced in PC12 cells by NGF (Milbrandt, 1987), as well as by a variety of other extracellular stimuli (Bartel et al., 1989). Together, these data raise the possibility that binding of NGF to PC12 cells or neurons at the high-affinity receptor results in the rapid production of the zif-268 protein product, which subsequently plays a role in increasing transcription of the NGF receptor gene. Interestingly, zif-268 is also induced by certain patterns of neuronal activity: it is, for example, dramatically increased in postsynaptic, hippocampal neurons by a stimulus sufficient to induce long-term potentiation (Cole et al., 1989). It is thus tempting to speculate that NGF receptor gene expression may be modulated by both NGF and neuronal activity, potentially via the same immediate early gene product. This would provide one mechanism for coordinating trophic input and neuronal activity at the cellular level.

Our results also indicate that NGF increased tyrosine hydroxylase mRNA levels within 6 h of treatment, that levels remained elevated for up to 48 h, and that by 72 h they returned to control levels. The increased steady-state mRNA levels were not coincident with increased transcription and were not sensitive to cycloheximide, suggesting that the underlying mechanisms are posttranscriptional in nature. Previous studies have reached similar conclusions regarding NGF induction of tyrosine hydroxylase in the superior cervical ganglion (Rohrer et al., 1987), sympathetic neurons (Hefti et al., 1982; Raynaud et al., 1988), and adrenal chromaffin cells (Acheson et al., 1984).

There is, however, some discrepancy in the literature regarding effects of NGF on tyrosine hydroxylase in the PC12 pheochromocytoma cell line. Several laboratories have reported that NGF does not increase tyrosine hydroxylase activity in PC12 cells (Edgar and Thoenen, 1978; Goodman and Herschman, 1978; Hatanaka, 1981; Greene and Tischler, 1982), but does in cell lines derived from the same tumor (Goodman and Herschman, 1978) in subcloned derivatives, (Hatanaka, 1981), and in PC12 cells themselves in the presence of glucocorticoids (Otten and Towbin, 1980). More recent reports indicate that NGF treatment of PC12 cells increased transcription of the tyrosine hydroxylase gene for 1-2 h after treatment, leading to a transient twofold increase in steady-state mRNA levels (Leonard et al., 1987; Gizang-Ginsberg and Ziff, 1990). In the present studies, we did not detect a significant increase in tyrosine hydroxylase mRNA levels until 6 h post-NGF, and this increase did not coincide with increased transcription. These data may indicate that NGF has two effects on the synthesis of tyrosine hydroxylase: it induces a rapid, transient increase in transcription of the gene, followed by a more long-term posttranscriptionally mediated increase in steady-state mRNA levels. Previous studies focusing primarily on long-term increases (1–5 d) in tyrosine hydroxylase mRNA or protein would therefore have concluded that the increase was mediated independent of transcriptional activation.

In summary, NGF induces a specific program of gene expression in developing sympathetic and sensory neurons that includes increases in transcription of the NGF receptor gene. This type of feedback loop provides a molecular mechanism for potentiating the effects of NGF on NGF-responsive neurons, and perhaps for enhancing the "fitness" of one neuron over another during the period of neuronal competition and cell death.

We thank Yanling Ma, Li-Juan Duan, Sangeeta Pareek, and Cheryl Richards for excellent technical assistance, and Richard Murphy for the gift of 2.5S NGF. We also thank Ann Acheson, Bob Campenot, Richard Murphy, and Phil Barker for frequent discussions and advice.

This work was supported by grants from the Medical Research Council of Canada, the Alberta Heritage Foundation for Medical Research (AHFMR), and the Canadian Networks of Centers of Excellence. Freda Miller is an AHFMR Scholar, T. Chako Mathew is supported by a studentship from the Alberta Paraplegic Association, and Jean Toma by a fellowship from the Rick Hansen Foundation for Spinal Cord Research.

Received for publication 11 June 1990 and in revised form 2 October 1990.

References

Acheson, A. L., K. Naujoks, and H. Thoenen. 1984. Nerve growth factor-mediated enzyme induction in primary cultures of bovine adrenal chromaffin cells: specificity and level of regulation. *J. Neurosci.* 7:1771–1780.

Allen, J., J. Novotny, J. Martin, and G. Heinrich. 1987. Molecular structure of mammalian neuropeptide Y: analysis by molecular cloning and computer-aided comparison with crystal structure of avian homologue. *Proc. Natl. Acad. Sci. USA.* 84:2532–2536.

Aloe, L., E. Mugnaini, and R. Levi-Montalcini. 1975. Light and electron microscopic studies on the excessive growth of sympathetic ganglia in rats injected daily from birth with 6-OHDA and NGF. *Arch. Ital. Biol.* 113:326–353.

Aloe, L., C. Cozzari, P. Calissano, and R. Levi-Montalcini. 1981. Somatic and behavioural postnatal effects of fetal injections of nerve growth factor antibodies in the rat. *Nature (Lond.).* 291:413–415.

Angeletti, P. U., R. Levi-Montalcini, and F. Caramia. 1971. Analysis of the effects of the antiserum to the nerve growth factor in adult mice. *Brain Res.* 92:343–355.

Bartel, D. P., M. Sheng, L. F. Lau, and M. E. Greenberg. 1989. Growth factors and membrane depolarization activate distinct programs of early response gene expression: dissociation of *fos* and *jun* induction. *Genes & Dev.*

3:304–313.
Bernd, P., and L. A. Greene. 1984. Association of ^{125}I-nerve growth factor with PC12 pheochromocytoma cells. *J. Biol. Chem.* 259:15509–15516.
Buck, C. R., H. J. Martinex, I. B. Black, and M. V. Chao. 1987. Developmentally regulated expression of the nerve growth factor receptor gene in the periphery and brain. *Proc. Natl. Acad. Sci. USA.* 84:3060–3063.
Cavicchioli, L., T. P. Flanigan, G. Vantini, M. Fusco, P. Polato, G. Toffano, F. S. Walsh, and A. Leon. 1989. NGF amplifies expression of NGF receptor messenger RNA in forebrain cholinergic neurons of rats. *Eur. J. Neurosci.* 1:258–262.
Chandler, C. E., L. M. Parsons, M. Hosang, and E. M. Shooter. 1984. A monoclonal antibody modulates the interaction of nerve growth factor with PC12 cells. *J. Biol. Chem.* 259:6882–6889.
Christy, B. A., L. F. Lau, and D. Nathans. 1988. A gene activated in mouse 3T3 cells by serum growth factors encodes a protein with "zinc finger" sequences. *Proc. Natl. Acad. Sci. USA.* 85:7857–7861.
Christy, B., and D. Nathans. 1989. DNA binding site of the growth factor-inducible protein Zif268. *Proc. Natl. Acad. Sci. USA.* 86:8737–8741.
Cole, A. J., D. W. Saffen, J. M. Baraban, and P. F. Worley. 1989. Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature (Lond.).* 340:474–476.
Davies, A. M., C. Bandtlow, R. Heumann, S. Korsching, H. Rohrer, and H. Thoenen. 1987. Timing and site of nerve growth factor synthesis in developing skin in relation to innervation and expression of the receptor. *Nature (Lond.).* 326:353–358.
Diamond, J., M. Couglin, L. MacIntyre, M. Holmes, and B. Visheau. 1987. Evidence that endogenous β nerve growth factor is responsible for the collateral sprouting, but not the regeneration, of nociceptive axons in adult rats. *Proc. Natl. Acad. Sci. USA.* 84:6596–6600.
DiStefano, P. S., and E. M. Johnson, Jr. 1988*a*. Nerve growth factor receptors on cultured rat Schwann cells. *J. Neurosci.* 8(1):231–241.
DiStefano, P. S., and E. M. Johnson, Jr. 1988*b*. Identification of a truncated form of the nerve growth factor receptor. *Proc. Natl. Acad. USA.* 85: 270–274.
Edgar, D. H., and H. Thoenen. 1978. Selective enzyme induction in a nerve growth factor-responsive pheochromocytoma cell line (PC 12). *Brain Res.* 154:186–190.
Ekblad, E., L. Edvinsson, C. Wahlestedt, R. Uddman, R. Hakanson, and F. Sundler. 1984. Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. *Regul. Pept.* 8:225–235.
Ernfors, P., F. Hallbook, T. Ebendal, E. M. Shooter, M. J. Radeke, T. P. Misko, and H. Persson. 1988. Developmental and regional expression of β-nerve growth factor receptor mRNA in the chick and rat. *Neuron.* 1: 983–996.
Gizang-Ginsberg, E., and E. B. Ziff. 1990. Nerve growth factor regulates tyrosine hydroxylase gene transcription through a nucleoprotein complex that contains c-Fos. *Genes & Dev.* 4:477–491.
Goodman, R., and H. R. Herschman. 1978. Nerve growth factor-mediated induction of tyrosine hydroxylase in a clonal pheochromocytoma cell line. *Proc. Natl. Acad. Sci. USA.* 75:4587–4590.
Gorin, P. D., and E. M. Johnson, Jr. 1980. Effects of long-term nerve growth factor deprivation on the nervous system of the adult rat: an experimental autoimmune approach. *Brain Res.* 198:27–42.
Green, S. M., and L. A. Greene. 1986. A single M_r ~103,000 ^{125}I-β-nerve-growth factor-affinity-labeled species represents both the low and high affinity forms of the nerve growth factor receptor. *J. Biol. Chem.* 261: 15316–15326.
Green, S. M., R. M. Rydel, J. L. Connolly, and L. A. Greene. 1986. PC12 mutants that possess low-but not high-affinity nerve growth factor receptors neither respond to nor internalize nerve growth factor. *J. Cell Biol.* 102:830–843.
Greenberg, M. E., and E. B. Ziff. 1984. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. *Nature (Lond.).* 311:433–438.
Greene, L. A., and A. S. Tischler. 1982. PC12 pheochromocytoma cultures in neurobiological research. *Adv. Cell. Neurobiol.* 3:373–414.
Groudine, M., M. Peretz, and H. Weintraub. 1981. Transcriptional regulation of hemoglobin switching on chicken embryos. *Mol. Cell Biol.* 1:281–288.
Hatanaka, H. 1981. Nerve growth factor-mediated stimulation of tyrosine hydroxylase activity in a clonal rat pheochromocytoma cell line. *Brain Res.* 222:225–233.
Hefti, F., H. Gnahn, M. E. Schwab, and H. Thoenen. 1982. Induction of tyrosine hydroxylase by nerve growth factor and by elevated K+ concentrations in cultures of dissociated sympathetic neurons. *J. Neurosci.* 2:1554–1566.
Hempstead, B. L., L. S. Schleifer, and M. V. Chao. 1989. Expression of functional nerve growth factor receptors after gene transfer. *Science (Wash. DC).* 243:373–375.
Hendry, I. A. 1977. Cell division in the developing sympathetic nervous system. *J. Neurocytol.* 6:299–309.
Hendry, I. A., and J. Campbell. 1976. Morphometric analysis of rat superior cervical ganglion after axotomy and nerve growth factor treatment. *J. Neurocytol.* 5:351–360.
Heumann, R., S. Korsching, C. Bandtlow, and H. Thoenen. 1987*a*. Changes of nerve growth factor synthesis in nonneuronal cells in response to sciatic nerve transection. *J. Cell Biol.* 104:1623–1631.
Heumann, R., D. Lindholm, C. Bandtlow, M. Meyer, M. J. Radeke, T. P. Miso, E. Shooter, and H. Thoenen. 1987*b*. Differential regulation of mRNA encoding nerve growth factor and its receptor in rat sciatic nerve during development, degeneration, and regeneration: role of macrophages. *Proc. Natl. Acad. Sci. USA.* 84:8735–8739.
Higgins, G. A., S. Koh, K. S. Chen, and F. H. Gage. 1989. NGF induction of NGF receptor gene expression and cholinergic neuronal hypertrophy within the basal forebrain of the adult rat. *Neuron.* 3:247–256.
Hosang, M., and E. M. Shooter. 1985. Molecular characteristics of nerve growth factor receptors on PC12 cells. *J. Biol. Chem.* 260:655–662.
Johnson, D., A. Lanahan, C. R. Buck, A. Sehgal, C. Morgan, E. Mercer, M. Bothwell, and M. Chao. 1986. Expression and structure of the human NGF receptor. *Cell.* 47:545–554.
Johnson, Jr., E. M., P. D. Gorin, L. D. Brandeis, and J. Pearson. 1980. Dorsal rat ganglion neurons are destroyed by exposure in utero to maternal antibody to nerve growth factor. *Science (Wash. DC).* 210:916–918.
Kessler, J. A., and I. B. Black. 1980. Nerve growth factor stimulates the development of substance P in sensory ganglia. *Proc. Natl. Acad. Sci. USA.* 77:649–652.
Kornblum, H. I., and E. M. Johnson, Jr. 1982. Time and dose dependencies of effects of nerve growth factor on sympathetic and sensory neurons in neonatal rats. *Brain Res.* 234:41–51.
Korsching, S., and H. Thoenen. 1988. Developmental changes of nerve growth factor levels in sympathetic ganglia and their target organs. *Dev. Biology.* 126:40–46.
Large, T. H., G. Weskamp, J. C. Helder, M. J. Radeke, T. P. Misko, E. M. Shooter, and L. F. Reichardt. 1989. Structure and developmental expression of the nerve growth factor receptor in the chicken central nervous system. *Neuron.* 2:1123–1134.
Lemaire, P., O. Revelant, P. Bravo, and P. Charnay. 1988. Two mouse genes encoding potential transcription factors with identical DNA-binding domains are activated by growth factors in cultured cells. *Proc. Natl. Acad. Sci. USA.* 85:4691–4695.
Lemke, G., and M. Chao. 1988. Axons regulate Schwann cell expression of the major myelin and NGF receptor genes. *Development (Camb.).* 102:499–504.
Lenoir, D., E. Battenberg, M. Kiel, F. E. Bloom, and R. J. Milner. 1986. The brain-specific gene 1B236 is expressed postnatally in the developing rat brain. *J. Neurosci.* 6:522–530.
Leonard, D. G. B., E. B. Ziff, and L. A. Greene. 1987. Identification and characterization of mRNAs regulated by nerve growth factor in PC12 cells. *Mol. Cell. Biol.* 7:3156–3167.
Levi-Montalcini, R., and P. U. Angeletti. 1968. Nerve growth factor. *Physiol. Rev.* 48:534–569.
Levi-Montalcini, R., and B. Booker. 1960*a*. Excessive growth of the sympathetic ganglia evoked by a protein isolated from mouse salivary glands. *Proc. Natl. Acad. Sci. USA.* 42:373–384.
Levi-Montalcini, R., and B. Booker. 1960*b*. Destruction of the sympathetic ganglia in mammals by an antiserum to the nerve-growth promoting factor. *Proc. Natl. Acad. Sci. USA.* 42:384–391.
Lewis, E. J., A. W. Tank, N. Weiner, and D. M. Chikaraishi. 1983. Regulation of tyrosine hydroxylase mRNA by glucocorticoid and cyclic AMP in a rat pheochromocytoma cell line. *J. Biol. Chem.* 258:14632–14637.
Lindsay, R. M., E. M. Shooter, M. J. Radeke, T. P. Misko, G. Dechant, H. Thoenen, and D. Lindholm. 1990. Nerve growth factor regulates expression of the nerve growth factor receptor gene in adult sensory neurons. *Eur. J. Neurosci.* 2:389–396.
Mathew, T. C., and F. D. Miller. 1990. Increased expression of Tα1 α-tubulin mRNA during collateral and NGF-induced sprouting of sympathetic neurons. *Dev. Biol.* 141:84–92.
Max, S. R., H. Rohrer, U. Otten, and H. Thoenen. 1978. Nerve growth factor-mediated induction of tyrosine hydroxylase in rat superior cervical ganglia *in vitro*. *J. Biol. Chem.* 253:8013–8015.
Melton, D. A., D. A. Krieg, M. R. Rabaphiati, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* 12:7035–7056.
Milbrandt, J. 1987. A nerve growth factor-induced gene encodes a possible transcription regulatory factor. *Science (Wash. DC).* 238:797–799.
Miller, F. D., C. C. G. Naus, M. Durand, F. E. Bloom, and R. J. Milner. 1987*a*. Isotypes of α-tubulin are differentially regulated during neuronal maturation. *J. Cell Biol.* 105:3065–3073.
Miller, F. D., C. C. G. Naus, G. A. Higgins, F. E. Bloom, and R. J. Milner. 1987*b*. Developmentally regulated rat brain mRNAs: molecular and anatomical characterization. *J. Neurosci.* 7:2433–2444.
Miller, F. D., W. Tetzlaff, M. A. Bisby, J. W. Fawcett, and R. J. Milner. 1989*a*. Rapid induction of the major embryonic α-tubulin mRNA, Tα1, during nerve regeneration in adult rats. *J. Neurosci.* 9:1452–1463.
Miller, F. D., G. Ozimek, R. J. Milner, and F. E. Bloom. 1989*b*. Regulation of neuronal oxytocin mRNA by ovarian steroids in the mature and developing hypothalamus. *Proc. Natl. Acad. Sci. USA.* 86:2468–2472.
Monroy, A. F. 1988. Staining immobilized RNA ladder. *Focus.* 10:1.
Nja, A., and D. Purves. 1978. The effects of nerve growth factor and its antiserum on synapses in the superior cervical ganglion of the guinea-pig. *J. Physiol.* 277:53–75.
Osborn, M., and K. Weber. 1982. Immunofluorescence and immunocytochemical procedures with affinity purified antibodies: tubulin-containing structures. *Methods Cell Biol.* 24:97–132.

Otten, U., and M. Towbin. 1980. Permissive action of glucocorticoids in induction of tyrosine hydroxylase by nerve growth factor in a pheochromocytoma cell line. *Brain Res.* 193:304–308.

Otten, U., M. Goedert, N. Mayer, and F. Lembeck. 1980. Requirement of nerve growth factor for development of substance P-containing sensory neurones. *Nature (Lond.).* 287:158–159.

Radeke, M. J., T. P. Misko, C. Hsu, L. A. Herzenberg, and E. M. Shooter. 1987. Gene transfer and molecular cloning of the rat nerve growth factor receptor. *Nature (Lond.).* 325:593–597.

Rave, N., R. Crkvenjakov, and H. Boedtker. 1979. Identification of procollagen mRNAs transferred to diazobenzyloxymethyl paper from formaldehyde agarose gels. *Nucleic Acids Res.* 6:3559–3567.

Raynaud, B., N. Faucon-Biguet, S. Vidal, J. Mallet, and M. J. Weber. 1988. Regulation of neurotransmitter metabolic enzymes and tyrosine hydroxylase mRNA level by nerve growth factor in cultured sympathetic neurones. *Development (Camb.).* 102:361–368.

Richardson, P. M., V. M. K. Verge Issa, and R. J. Riopelle. 1986. Distribution of neuronal receptors for nerve growth factor in the rat. *J. Neurosci.* 6(8):2312–2321.

Rohrer, H., U. Otten, and H. Thoenen. 1978. On the role of RNA synthesis in the selective induction of tyrosine hydroxylase by nerve growth factor. *Brain Res.* 159:436–439.

Schibler, K., M. Tosei, A.-C. Pittet, L. Fabiani, and P. Wellames. 1980. Tissue-specific expression of mouse α-amylase genes. *J. Mol. Biol.* 142: 93–116.

Sehgal, A., N. Patil, and M. V. Chao. 1988. A constitutive promoter directs expression of the nerve growth factor receptor gene. *Mol. Cell. Biol.* 8: 3160–3167.

Sheng, M., and M. E. Greenberg. 1990. The regulation and function of c-*fos* and other immediate early genes in the nervous system. *Neuron.* In press.

Snider, W. D. 1988. Nerve growth factor enhances dendritic arborization of sympathetic ganglion cells in developing mammals. *J. Neurosci.* 8(7): 2628–2634.

Sukhatme, V. K., X. Cao, L. C. Chang, C-H. Tasi-Morris, D. Stamenkovich, P. C. P. Ferreira, D. R. Cohen, S. A. Edwards, T. B. Shows, T. Curran, M. M. Le Beau, and E. D. Adamson. 1988. A zinc finger-encoding gene co-regulated with c-*fos* during growth and differentiation, and after cellular depolarization. *Cell.* 53:37–43.

Sutter, A., R. J. Riopelle, R. M. Harris-Warrick, and E. M. Shooter. 1979. Nerve growth factor receptors: characterization of two distinct classes of binding sites on chick embryo sensory ganglia cells. *J. Biol. Chem.* 254: 5972–5982.

Taniuchi, M., H. B. Clark, and E. M. Johnson, Jr. 1986. Induction of nerve growth factor receptor in Schwann cells after axotomy. *Proc. Natl. Acad. Sci. USA.* 83:4094–4098.

Thoenen, H., P. V. Angeletti, R. Levi-Montalcini, and R. Kettler. 1971. Selective induction by nerve growth factor of tyrosine hydroxylase and dopamine-β-hydroxylase in the rat superior cervical ganglia. *Proc. Natl. Acad. Sci. USA.* 68:1598–1602.

Thomas, P. S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA.* 77:5201–5215.

Tischler, A. S., and L. A. Greene. 1975. Nerve growth factor-induced process formation by cultured rat pheochromocytoma cells. *Nature (Lond.).* 258: 341–342.

Verge, V. M. K., R. J. Riopelle, and P. M. Richardson. 1989. Nerve growth factor receptors on normal and injured sensory neurons. *J. Neurosci.* 9(3):914–922.

Wyatt, S., E. M. Shooter, and A. M. Davies. 1990. Expression of the NGF receptor gene in sensory neurons and their cutaneous targets prior to and during innervation. *Neuron.* 2:421–427.

Yan, Q., and E. M. Johnson, Jr. 1988. An immunohistochemical study of the nerve growth factor receptor in developing rats. *J. Neurosci.* 8(9):3481–3498.