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Neurobiology

Expression of nerve growth factor *in vivo* from a defective herpes simplex virus 1 vector prevents effects of axotomy on sympathetic ganglia

(neurotrophic factors/gene therapy/gene transfer)

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ABSTRACT **Sympathetic neurons in the superior cervical ganglion (SCG) of adult rats depend on target-derived nerve growth factor (NGF) for maintenance of tyrosine hydroxylase (TH) levels and the noradrenergic neurotransmitter system. Axotomy of a SCG results in NGF deprivation, causing a decline in TH activity; continuous local application of NGF can prevent this decline in TH activity. We now report that injection of a defective herpes simplex virus 1 vector that expresses NGF (pHSVngf) into a SCG can prevent the decline in TH activity that follows axotomy. SCG of adult rats were injected with either pHSVngf virus or pNFlac virus, which expresses *Escherichia coli* β-galactosidase. Analysis of RNA from pHSVngf-infected SCG indicated that the NGF gene was efficiently transcribed and processed. Furthermore, 4 days after pHSVngf injection animals underwent axotomy of the virus-injected SCG. After another 10 days, animals were sacrificed and both the injected–axotomized and contralateral control ganglia were assayed for TH activity. Axotomy of SCG injected with pNFlac virus produced a 50% decline in TH activity relative to control ganglia ($P = 0.02$). In contrast, SCG injected with pHSVngf virus did not show a decline in TH activity following axotomy; instead, these ganglia manifested an 18% increase in TH levels relative to control ganglia. These data demonstrate that herpes simplex virus 1 vectors can be used to modify neuronal physiology *in vivo*; specifically, expression of a critical gene product by neural cells that do not normally produce it has potential applications for gene therapy.**

The successful use of gene transfer methods to alter somatic cell physiology has created considerable interest in their application to ameliorating or correcting inherited and acquired human diseases. Human gene transfer was recently demonstrated in patients with a form of skin cancer (1). Gene therapy approaches may also be applicable to those neurological disorders in which limiting quantities of critical gene products are implicated in the disease process. In particular, neurotrophic factors play a pivotal role in the development and maintenance of neurons in both the peripheral and the central nervous system (CNS) (reviewed in refs. 2–5). Nerve growth factor (NGF) is the best-characterized neurotrophic factor. Depletion of NGF in developing animals results in the death of sympathetic neurons and many sensory neurons, indicating that NGF promotes neuronal survival (6–10). In the adult animal, however, NGF acts to maintain neuronal phenotype, such as the noradrenergic characteristics of sympathetic neurons (5, 9, 11) and the cholinergic phenotype of some CNS neurons (12–14). Disruption of NGF supply from targets of innervation to neuronal cell soma is observed after traumatic axon injury (11), after administration of pharmacological agents that interrupt axonal transport (15), or in metabolic disorders such as diabetes (16, 17). Whether NGF deprivation is the direct cause of toxic and/or metabolic neuropathy is uncertain; however, an important therapeutic role for NGF is suggested by the protective effect observed when it is coadministered with a neurotoxin (15). Disruption of CNS NGF supply may also produce disease. Cholinergic basal forebrain neurons are dependent on NGF produced by the hippocampus; disruption of this NGF supply may be involved in pathophysiology of Alzheimer disease (reviewed in ref. 18).

A potentially powerful gene therapy approach to neurological disorders due to deficiency in a critical gene product is to cause production of that gene product from neural cells that do not normally produce it. In adult sympathetic neurons, NGF-dependent maintenance of the noradrenergic neurotransmitter system depends on stimulation of synthesis of the catecholamine biosynthetic enzyme tyrosine hydroxylase (TH) (4, 9, 11, 19). Thus, sympathetic-neuron axotomy, which results in depletion of target-cell-derived NGF, causes a decline in neuronal TH levels (4, 11). This decline can be prevented by systemic (4) or local (11) application of NGF. Therefore, expression of NGF in sympathetic neurons and surrounding glia may be an effective therapeutic approach to peripheral nerve axotomy. NGF has been expressed from retrovirus vectors (20); however, retroviruses require at least one round of host cell DNA replication for integration and the resulting stable expression. Consequently, retrovirus vectors are not effective for gene transfer into postmitotic neurons and glia. Therefore, a defective herpes simplex virus 1 (HSV-1) vector system was developed to transfer genes into neurons in culture and *in vivo* (21–23, 42). In this report, we demonstrate that direct injection of a defective HSV-1 that expresses NGF, pHSVngf, into a superior cervical ganglion (SCG) directs the production of NGF RNA and prevents the decline in TH levels that follows axotomy.

MATERIALS AND METHODS

Construction of pHSVngf. The rat NGF gene was kindly provided by G. Heinrich (University Hospital, Boston); exon IV encodes all of prepro-NGF (24). An NGF minigene was constructed that lacked the sequence from 90 base pairs (bp) into the first intron to 277 bp before the start of exon IV (within intron III). The 3′ untranslated region of the NGF

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Abbreviations: CNS, central nervous system; HSV-1, herpes simplex virus 1; NGF, nerve growth factor; RT–PCR, reverse transcription–polymerase chain reaction; SCG, superior cervical ganglion (ganglia); TH, tyrosine hydroxylase.

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gene (8 bp after the NGF stop codon), which is highly A+T-rich (25) and perhaps susceptible to rapid turnover, was replaced with the human growth hormone 3′ untranslated region (nucleotides 1858–2157; ref. 26). The resulting 1.8-kilobase (kb) NGF minigene was inserted into a defective HSV-1 vector (pHSVngf; Fig. 1).

Virus Production and Cell Culture Studies. pHSVngf was packaged into HSV-1 particles by using a deletion-mutant packaging system (27). The titer of the virus stock was 5×10^5 infectious particles of pHSVngf and 1×10^6 plaque-forming units of D30EBA (helper virus) per ml. For *in vivo* experiments, the virus was concentrated ≈80-fold by the following procedure (all manipulations were performed at 4°C). Virus stock (10 ml) was centrifuged at 10,000 × *g* for 10 min. The supernatant was layered onto 2 ml of 25% sucrose in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline and centrifuged at 77,000 × *g* for 12–16 hr. The pellet was resuspended in 100 μl of Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline.

NIH 3T3 mouse fibroblasts were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 5% horse serum, at 37°C in an atmosphere of 5% CO_2. Cells were plated (5×10^4 cells per cm^2) and allowed to grow for 48 hr before infection with 20 μl of unconcentrated virus. Eight hours later the virus containing medium was removed, and fresh medium was added. Medium was harvested 24 hr later for NGF ELISA and 48 hr later for NGF bioassay.

ELISA and Bioassay of NGF. Two-site ELISAs were performed with polyclonal antibodies (gift of D. Sinicropi, Genentech) directed against human NGF. Murine NGF was used as a standard.

A neonatal sympathetic neuron survival assay (28) was used to measure the amount of bioactive NGF produced by pHSVngf-infected cells. Approximately 4×10^4 neonatal SCG neurons were plated on collagen and cultured with medium consisting of 50% Ham's F12 containing 10% fetal bovine serum and 50% conditioned medium from the infected cells. One day after plating, rapidly dividing cells were killed by treatment with 5 μM 1-β-D-arabinofuranosylcytosine, and neuron survival was scored 72 hr later by counting the cells in at least 10 high-power fields. Control cultures contained no conditioned medium and had exogenous NGF (100 ng/ml) added to them.

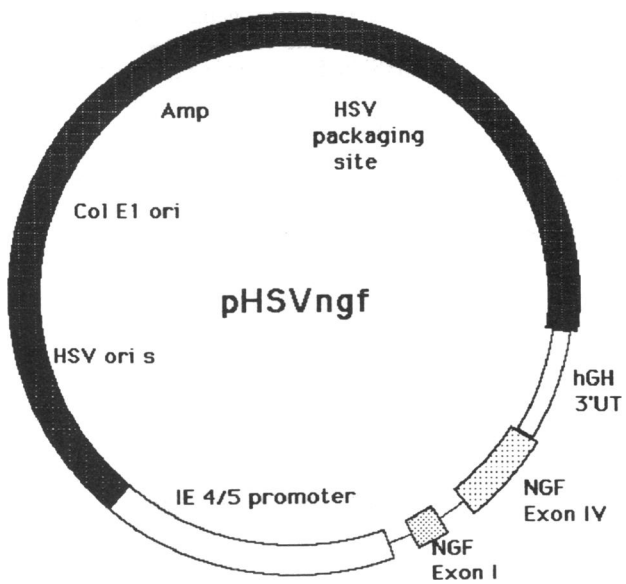

FIG. 1. Structure of pHSVngf. The vector contains two genetic elements from HSV-1, the ori_s and the a sequence (HSV packaging site), that are sufficient for packaging into viral particles. It also contains a transcription unit composed of the HSV-1 IE (immediate early) 4/5 promoter (unfilled segment) and the NGF minigene (stippled) with the human growth hormone (hGH) 3′ untranslated region (3′UT). For replication and selection in *Escherichia coli*, pHSVngf contains the Col E1 origin (ori) of replication and the β-lactamase gene (Amp).

Direct Injection of pHSVngf Virus into SCG and Subsequent Axotomy of the SCG. Adult female Sprague–Dawley rats weighing 175–225 g were anesthetized with halothane [3.5% (vol/vol) with 5 liters of O_2 per minute], and the SCG was exposed unilaterally. Two microliters of pHSVngf virus, pNFlac virus (expresses *E. coli* β-galactosidase; ref. 23), or isotonic saline was injected directly into the ganglion by using a Hamilton syringe equipped with a 27-gauge beveled needle. Surgical wounds were closed. Four days after injection, axotomies were performed on the injected ganglia as described (11). In brief, the ganglia were exposed and both major efferent branches of the SCG (internal and external carotid branches) were cut close to the ganglia, without severing visible vasculature. The wounds were again closed. In each animal, the contralateral, unperturbed, SCG served as an internal control. Ten days later, both the ipsilateral (experimental) ganglion and the contralateral (control) ganglion were removed and assayed for TH activity.

Measurement of TH Activity. Each ganglion was homogenized in 75 μl of distilled water in a glass/Teflon homogenizer. Ten microliters of each homogenate was assayed for TH activity (11) using tetrahydrobiopterin as cofactor.

Analysis of RNA from pHSVngf-Infected NIH 3T3 Cells and SCG. Infections of cultured NIH 3T3 cells and SCG ($n = 7$ for each group) were performed as described above. Total RNA was isolated by the acid/guanidinium method (29) from NIH 3T3 cells 48 hr after infection and from SCG 1 week after infection. Reverse transcription (RT) was performed with random hexamer primers (5 μM final concentration) in PCR buffer (50 mM KCl/10 mM Tris·HCl, pH 9.0/1.5 mM $MgCl_2$/0.01% gelatin/0.1% Triton X-100) containing 20 units of RNasin (Promega), 1 mM each dATP, dTTP, dGTP, and dCTP, and 50 units of avian myeloblastosis virus reverse transcriptase for 10 min at 23°C, 45 min at 42°C, and 10 min at 95°C. PCR amplification was performed in a volume of 100 μl with 100 ng of each oligonucleotide and 2 units of *Taq* polymerase (Promega) for 30 cycles (95°C, 1 min; 60°C, 1 min; and 72°C, 1.5 min). The oligonucleotides used for amplification were an antisense oligonucleotide from the 3′ untranslated region of the human growth hormone gene (5′-GTTTGTGTCGAACTTGCTGTAGGTCTG-3′) and a sense oligonucleotide from the NGF exon I (nucleotides 11–33, exon Ib; ref. 24). PCR amplification of pHSVngf DNA isolated from *E. coli* or HSV-1 particles was performed with 40 ng of template as described above, except the RT step was omitted.

Northern analysis of SCG RNA was performed as described (30); 15 μg of total RNA was fractionated in a 0.7% agarose/formaldehyde gel, transferred to Nytran membrane, and heat-immobilized. The probes were NGF exon IV and full-length GAP-43 (43-kDa growth-associated protein) cDNA (30). ^{32}P-radiolabeled probes were prepared by random hexamer-primed nick-translation (31).

RESULTS

pHSVngf Virus Can Direct the Efficient Synthesis of Biologically Active NGF in Cultured Fibroblasts. We tested the ability of pHSVngf virus to infect 3T3 fibroblasts and direct the synthesis of NGF. First, we measured the amount of immunoreactive NGF that was synthesized in 3T3 fibroblasts. The cells were infected with pHSVngf virus or mock infected, 8 hr later the medium was replaced with fresh medium, and 24 hr later the medium was harvested. The amount of NGF secreted into the medium was quantitated by a two-site ELISA. The medium contained 12 ng of NGF per

Table 1. pHSVngf-infected 3T3 cells secrete NGF into the culture medium as measured by ELISA

Dilution of medium	NGF, ng/ml
1:4	10.16
1:8	11.24
1:16	12.16
1:32	12.8
1:64	11.84
(Average)	11.64

NIH 3T3 cells (2×10^5) were infected with 20 μl of pHSVngf virus. Eight hours later the medium was removed and replaced with 1 ml of fresh medium. Twenty-four hours later the medium was harvested and NGF levels were determined by a two-site ELISA. Samples were diluted with assay diluent and compared with a murine NGF standard.

ml (Table 1). In three repetitions of the experiment, the amount of NGF secreted varied from 10 to 34 ng/ml.

Next, we tested whether the NGF produced by 3T3 cells infected with pHSVngf was biologically active, as determined by the ability to support the survival of neonatal sympathetic neurons. 3T3 cells were infected with pHSVngf virus or mock infected, the inoculum was replaced with fresh medium, and 48 hr later the medium was harvested and assayed for NGF bioactivity. In two representative experiments (Table 2), the media from pHSVngf-infected 3T3 cells dramatically increased survival of sympathetic neurons compared with medium from mock-infected cultures.

NGF RNA Is Expressed and Correctly Spliced in pHSVngf-Infected SCG. We investigated whether direct injection of pHSVngf virus into SCG of adult rats resulted in efficient synthesis and processing of NGF RNA. Concentrated pHSVngf virus (2 μl) or pNFlac virus (2 μl) was directly injected into a SCG, unilaterally. One week later, injected and uninjected (control) ganglia were harvested and total RNA was prepared (29). RT-PCR was used to determine whether the NGF transcription unit in pHSVngf was efficiently transcribed and correctly processed; the two oligonucleotides used as primers allowed us to distinguish between an unprocessed NGF minigene primary transcript and spliced RNA. RNA extracted from pHSVngf-infected SCG (Fig. 2, lanes 2 and 3) yielded a PCR product (801 bp) consistent with correctly spliced NGF RNA. This PCR product was obtained only from SCG infected with pHSVngf virus: RNA from control (lane 6) or pNFlac-infected (lane 7) SCG did not yield any PCR product. Furthermore, the template for the PCR amplification was the product of transcription of pHSVngf and subsequent splicing, since (*i*)

Table 2. Medium from pHSVngf-infected NIH 3T3 fibroblasts can support the survival of cultured sympathetic neurons

Trial	Added NGF, ng/ml	pHSVngf, μl	No. of neurons surviving
1	0	0	100
		10	8,400
		20	18,200
	100	0	29,600
2	0	0	0
		10	17,100
		20	30,000
	100	0	32,650

NIH 3T3 cells were plated at 5×10^4 per well and grown for 48 hr before infection. Eight hours later the medium was removed and replaced with 1 ml of fresh medium. Forty-eight hours later the medium was harvested and its ability to support sympathetic neuron survival was assayed. The neuron counts represent the mean of duplicate plates; the experiment was performed twice.

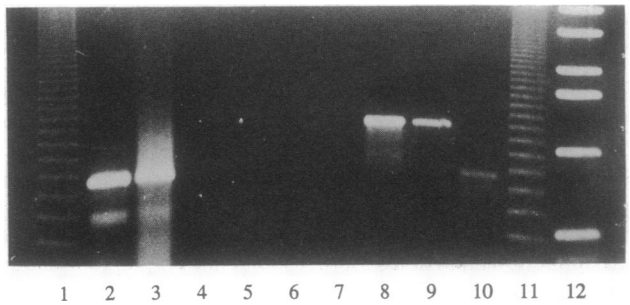

FIG. 2. RT-PCR analysis of RNA isolated from pHSVngf-infected NIH 3T3 cells or SCG. Total RNA was isolated (29) from NIH 3T3 cells and from SCG. RT-PCR was performed using two oligonucleotides to distinguish between unprocessed and spliced NGF minigene RNA transcripts. Lanes 1 and 11, 123-bp "ladder" (size markers); lanes 2 and 3, RT-PCR product from two different preparations of RNA from pHSVngf-infected SCG; lanes 4 and 5, reactions as in lanes 2 and 3 but performed without reverse transcriptase; lane 6, RT-PCR on RNA from uninfected SCG; lane 7, RT-PCR on RNA from pNFlac-infected SCG; lane 8, PCR on pHSVngf DNA isolated from *E. coli*; lane 9, PCR on pHSVngf DNA isolated from HSV-1 particles; lanc 10, RT-PCR on RNA isolated from pHSVngf-infected NIH 3T3 cells; lane 12, 1-kilobase-pair ladder.

no PCR product was seen when reverse transcriptase was omitted (lanes 4 and 5) and (*ii*) the PCR product amplified from pHSVngf DNA purified from *E. coli* (lane 8) or from HSV-1 particles (lane 9) was larger (1170 bp) because it contained the intron sequence. In addition, the RT-PCR product obtained using RNA isolated from pHSVngf-infected NIH 3T3 fibroblasts (lane 10) was the same size as that observed from pHSVngf-infected SCG, indicating that processing of the NGF minigene primary transcript was similar in cultured cells and *in vivo*.

To obtain a quantitative estimate of NGF minigene transcript levels in infected SCG, we performed Northern analysis on total RNA extracted from pHSVngf virus-infected SCG (Fig. 3, lanes 1 and 2), control ganglia (lanes 3 and 5), and ganglia injected with pNFlac virus (lane 4). As shown in Fig. 3*A*, only the pHSVngf-infected ganglia contained a transcript that hybridized with a NGF coding-sequence probe. The observed band was the expected size (1326 nucleotides) for a processed NGF minigene transcript initiated at the HSV IE 4/5 promoter and utilizing the human growth hormone polyadenylylation site. Fig. 3*B* shows hybridization of the same blot to the neuron-specific gene

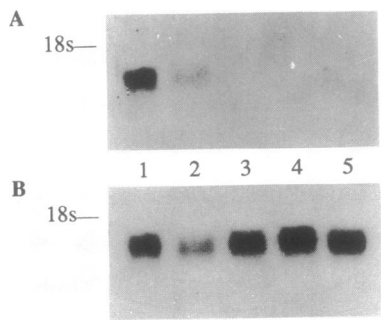

FIG. 3. Northern analysis of RNA isolated from pHSVngf-infected and control SCG. The SCG RNA samples used in Fig. 2 were subjected to Northern analysis (30) with a probe from the NGF coding sequence (*A*, 6-hr exposure) or from the GAP-43 (43-kDa growth-associated protein) coding sequence (*B*, 3-hr exposure). Each lane contained 15 μg of total RNA except lane 2, which contained 10 μg. Lanes 1 and 2, RNA from two groups of pHSVngf-infected SCG; lanes 3 and 5, RNA from two groups of uninfected SCG; and lane 4: RNA from pNFlac-infected SCG. Position of 18S rRNA is indicated.

GAP-43, indicating that each sample contained a considerable amount of intact neuronal RNA. These data demonstrate that direct infection of SCG with pHSVngf virus results in efficient transcription of the NGF minigene and correct processing of the primary transcript.

pHSVngf Virus Can Prevent Some of the Effects of Axotomy of SCG *in Vivo*. Next, an experiment was performed to determine whether pHSVngf virus could alter the physiology of cells *in vivo*. The SCG of the adult rat contains the cell bodies of sympathetic neurons whose axons project to target tissues that produce NGF. Unilateral axotomy of a SCG interrupts its NGF supply and results in an ipsilateral decline in TH activity over a 10-day period. Because local or systemic administration of NGF can prevent the decline in TH activity observed in the axotomized ganglion (4, 11), we tested whether direct infection of a SCG with pHSVngf virus could have a similar protective effect. We unilaterally injected a SCG with concentrated pHSVngf virus (2 μl) or pNFlac virus (2 μl). Four days later, SCG axotomy was performed ipsilateral to the injection. Ten days later, TH activity was assayed in both the injected/axotomized ganglion and the contralateral, control ganglion. Axotomy of ganglia injected with pNFlac virus reduced TH activity by 50% (P = 0.02) compared with the contralateral control ganglion (Fig. 4). In contrast, injection of ganglia with pHSVngf virus prevented a decline in TH activity after axotomy; instead, pHSVngf directed an 18% increase in TH activity. Similar results were obtained in another experiment, in which pHSVngf virus injection, but not saline injection, was able to prevent the decline in TH activity produced by axotomy (data not shown). These data show that pHSVngf virus stimulates TH activity in neurons in the axotomized SCG, thereby preventing some of the deleterious effects produced by axon injury.

DISCUSSION

Injury to the nervous system frequently disrupts the normal interactions between neurons and their target cells. In cases where the target cell provides the innervating neuron with a neurotrophic factor, such disruptions lead to a deficiency in neurotrophic factor supply. NGF is synthesized and secreted by the target tissues of sympathetic neurons. NGF is taken up by sympathetic neurons and retrogradely transported to the cell soma, where it functions, in the adult, to maintain the noradrenergic neurotransmitter system, in part by stimulating TH synthesis (4, 11). Prior studies have demonstrated that after axotomy, the amount of TH activity within sympathetic neurons of the SCG declines as a consequence of diminished NGF levels (11). Treatment with NGF at the time of axotomy prevents this decline in TH levels (11).

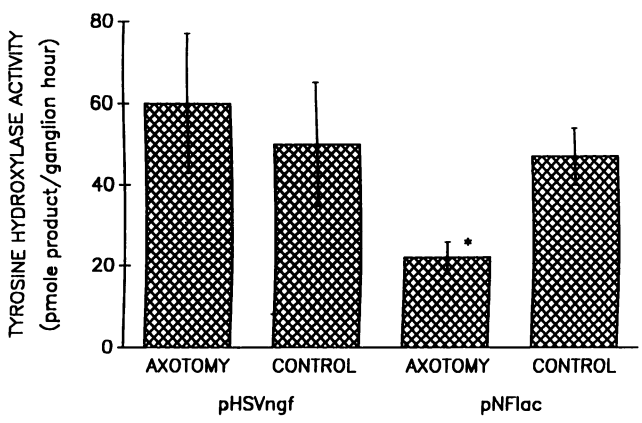

FIG. 4. Direct injection of pHSVngf virus, but not pNFlac virus, into a SCG prevents the decline in TH levels that follows axotomy. Adult rats were anesthetized, and in each rat the left SCG was exposed and injected with 2 μl of concentrated pHSVngf (n = 10) or pNFlac (n = 9) virus. Four days later, the animals underwent axotomy of the left SCG. In all animals the right SCG was not injected or axotomized; it served as an internal control. Ten days after axotomy, animals were sacrificed and both the injected/axotomized and contralateral, control SCG were harvested and TH assays were performed. Data are shown as mean ± SEM for each group of ganglia: pHSVngf + axotomy; control SCG (right SCG from animals receiving pHSVngf virus/axotomy on the left); pNFlac + axotomy; control SCG (right SCG from animals receiving pNFlac virus/axotomy on the left). The difference between the pNFlac virus/axotomy group and all other groups is statistically significant (P = 0.02 by analysis of variance).

The present study shows that direct infection of cells in the SCG with pHSVngf virus results in the transcription and correct processing of NGF RNA that, upon translation, yields sufficient quantities of NGF to prevent the decline in TH levels caused by axotomy. First, pHSVngf virus was injected into a SCG and allowed to express NGF for 7 days before total RNA was isolated. RT–PCR analysis of RNA from control, pNFlac-infected, and pHSVngf-infected SCG demonstrated that correctly spliced NGF RNA was present only in ganglia infected with pHSVngf virus. Moreover, Northern analysis demonstrated that NGF RNA was abundantly expressed in pHSVngf-infected ganglia. In a second experiment, pHSVngf virus was injected into a SCG and allowed to express NGF for 4 days prior to axotomy. Ten days after axotomy, both the infected and the control SCG were harvested, and TH activity was quantitated. pHSVngf virus infection and subsequent NGF expression prevented the decrease in TH caused by axotomy. To control for nonspecific effects due to the defective HSV-1 vector system, we performed the same experiment with another defective HSV-1 vector, pNFlac (23), which expresses *E. coli* β-galactosidase instead of NGF. Infection with pNFlac virus did not prevent the decline in TH levels produced by axotomy.

Since a SCG contains both neurons and non-neuronal cells, direct injection of pHSVngf virus into a SCG could result in the infection of both neurons and non-neuronal cells. Our studies with defective HSV-1 vectors in cell culture indicate that they can infect a variety of different neuronal types from both the peripheral and central nervous systems, including sympathetic neurons from SCG (21, 22) and numerous non-neuronal cell types (32). pHSVngf virus also appears to have a wide host range in cultured cells; our data indicate that NIH 3T3 fibroblasts, and other cell types (unpublished data), are capable of efficient transcription, RNA processing, and translation of NGF following infection with pHSVngf virus. Efficient expression of NGF may also occur in SCG neurons, since infection of pure cultures of neonatal SCG neurons with pHSVngf virus promotes their survival in the absence of exogenous NGF (unpublished data). Other investigators have shown that many cell types possess the requisite machinery to synthesize and secrete bioactive NGF (33). We do not know which cell types were infected following injection of pHSVngf virus into a SCG. Although the contribution made by each infected cell type to the total amount of NGF produced is not known, this does not affect our conclusions regarding the efficacy of pHSVngf virus.

The ability of attenuated (34) or defective (refs. 21–23 and 35; unpublished data) HSV-1 vectors to transfer genes into neurons in the normal or pathologic nervous system provides a potential approach to analyzing the function of the nervous system, as well as a potential method of human gene therapy. In particular, expression of neurotrophic factors in cells that do not normally produce them has great potential. We have begun to explore these possibilities with a defective HSV-1 vector carrying a NGF minigene. The use of pHSVngf virus to study other NGF-responsive neuronal populations, such as those in the basal forebrain (12–14, 36), is now conceivable. In addition, construction of defective HSV-1 vectors

that express other neurotrophic factors, such as BDNF (37, 38), NT3 (39, 40), or CNTF (41), should broaden this approach. The ability to use such viruses to augment neurotrophic factor concentrations in specific regions of the brain will contribute to our understanding of the functions of these factors in the normal nervous system and perhaps allow us to explore their potential therapeutic role in the diseased nervous system.

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1. Rosenberg, S. A., Aebersold, P., Cornetta, K., Kasid, A., Morgan, R. A., Moen, R., Karson, E. M., Lotze, M. T., Yang, J. C., Topalian, S. L., Merino, M. D., Culver, K., Miller, A. D., Blaese, R. M. & Anderson, W. F. (1990) *N. Engl. J. Med.* **323,** 570–578.
2. Barde, Y. (1989) *Neuron* **2,** 1525–1534.
3. Snider, W. D. & Johnson, E. M. (1989) *Ann. Neurol.* **26,** 489–506.
4. Thoenen, H. & Barde, Y. A. (1980) *Physiol. Rev.* **60,** 1284–1335.
5. Thoenen, H., Bandtlow, C. & Heuman, R. (1987) *Rev. Physiol. Biochem. Pharmacol.* **109,** 146–178.
6. Kessler, J. A. & Black, I. B. (1980) *Brain Res.* **189,** 157–168.
7. Johnson, E. M., Gorin, P. D., Brandeis, L. D. & Pearson, J. (1980) *Science* **210,** 916–918.
8. Hendry, I. A. (1975) *Brain Res.* **90,** 235–244.
9. Gorin, P. D. & Johnson, E. M. (1980) *Brain Res.* **198,** 27–42.
10. Dibner, M. D. & Black, I. B. (1976) *Brain Res.* **103,** 93–102.
11. Kessler, J. A. & Black, I. B. (1979) *Brain Res.* **171,** 415–424.
12. Hefti, F. (1986) *J. Neurosci.* **6,** 2155–2162.
13. Gage, F. H., Wictorin, K., Fischer, W., Williams, L. R., Varon, S. & Bjorklund, A. (1986) *Neuroscience* **19,** 241–256.
14. Gage, F. H., Armstrong, D. M., Williams, L. R. & Varon, S. (1988) *J. Comp. Neurol.* **269,** 147–155.
15. Apfel, S., Lipton, R. B., Arezzo, J. C. & Kessler, J. A. (1991) *Ann. Neurol.* **29,** 87–97.
16. Hellweg, R. & Hartung, H.-D. (1990) *J. Neurosci. Res.* **26,** 258–267.
17. Schmidt, R. E., Modert, C. W., Yip, H. K. & Johnson, E. M. (1983) *Diabetes* **32,** 654–663.
18. Hefti, F., Hartikka, J. & Knusel, B. (1989) *Neurobiol. Aging* **10,** 515–533.
19. Goedert, M., Otten, U. & Thoenen, H. (1978) *Brain Res.* **148,** 264–268.
20. Wolf, D., Richter-Landsberg, C., Short, M. P., Cepko, C. & Breakefield, X. O. (1988) *Mol. Biol. Med.* **5,** 43–59.
21. Geller, A. I. & Breakefield, X. O. (1988) *Science* **242,** 1667–1669.
22. Geller, A. I. & Freese, A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1149–1153.
23. Federoff, H. J., Geller, A. I. & Lu, B. (1990) *Soc. Neurosci. Abstr.* **16,** 353.
24. Selby, M. J., Edwards, R., Sharp, F. & Rutter, W. J. (1987) *Mol. Cell. Biol.* **7,** 3057–3064.
25. Scott, J., Selby, M., Urdea, M., Quiroga, M., Bell, G. I. & Rutter, W. J. (1983) *Nature (London)* **302,** 538–540.
26. Seeburg, P. (1982) *DNA* **1,** 239–249.
27. Geller, A. I., Keyomarsi, K., Bryan, J. & Pardee, A. B. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8950–8954.
28. Lindsay, R. M., Thoenen, H. & Barde, Y. (1985) *Dev. Biol.* **112,** 319–328.
29. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
30. Federoff, H. J., Grabczyk, E. & Fishman, M. C. (1988) *J. Biol. Chem.* **263,** 19290–19295.
31. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–12.
32. Boothman, D. A., Geller, A. I. & Pardee, A. B. (1989) *FEBS Lett.* **258,** 159–162.
33. Edwards, R. H., Selby, M. J., Mobley, W. C., Weinrich, S. L., Hruby, D. E. & Rutter, W. J. (1988) *Mol. Cell. Biol.* **8,** 2456–2464.
34. Dobson, A., Margolis, T. P., Sedarati, F., Stevens, J. & Feldman, L. T. (1990) *Neuron* **5,** 353–360.
35. Freese, A., Geller, A. I. & Neve, R. (1990) *Biochem. Pharmacol.* **40,** 2189–2199.
36. Rosenberg, M. B., Friedmann, T., Robertson, R. C., Tuszynski, M., Wolff, J. A., Breakefield, X. O. & Gage, F. H. (1988) *Science* **242,** 1575–1578.
37. Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H. & Barde, Y. A. (1989) *Nature (London)* **341,** 149–152.
38. Hofer, M. M. & Barde, Y. (1988) *Nature (London)* **331,** 261–262.
39. Rosenthal, A., Goeddel, D. V., Nguyen, T., Lewis, M., Shih, A., Laramee, G. R., Nikolics, K. & Winslow, J. W. (1990) *Neuron* **4,** 767–773.
40. Hohn, A., Leibrock, J., Bailey, K. & Barde, Y. (1990) *Nature (London)* **344,** 339–341.
41. Stockli, K. A., Lottspeich, F., Sendtner, M., Masiakowski, P., Carroll, P., Gotz, R., Lindholm, D. & Thoenen, H. (1989) *Nature (London)* **342,** 920–922.
42. Sabel, B. A., Martin, C., Waldman, C., Freese, A. & Geller, A. I. (1989) *Soc. Neurosci. Abstr.* **15,** 9.