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Experimental autoimmune model of nerve growth factor deprivation: Effects on developing peripheral sympathetic and sensory neurons

(immunosympathectomy/neuronal development/trophic factor)

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ABSTRACT **An experimental autoimmune model of nerve growth factor (NGF) deprivation has been used to assess the role of NGF in the development of various cell types in the nervous system. Adult rats immunized with 2.5S mouse NGF in complete Freund's adjuvant produced antibodies that crossreacted with their own NGF and that were transferred *in utero* to the fetus and in milk to the neonate. Cross-fostering experiments were carried out to separate the effects of exposure to anti-NGF *in utero* from those due to exposure through the milk. Anti-NGF transferred *in utero* and in milk resulted in the destruction of peripheral sympathetic neurons assessed by morphological methods (light microscopy) and biochemical methods (tyrosine hydroxylase activity, choline acetyltransferase activity, and protein content). No effects were observed on the adrenal medulla. Offspring of NGF-immunized females exposed to anti-NGF *in utero* had a decreased protein content in the dorsal root ganglia and were unable to transport ^{125}I-labeled NGF injected in the forepaw to the dorsal root ganglia. These results suggest that a subpopulation of sensory neurons is dependent on NGF for survival during some period of fetal development. This model offers the potential for determining the degree and time of dependence of various cell types on NGF.**

Nerve growth factor (NGF) is a protein that acts on peripheral neurons of neural crest origin in both avian and mammalian species (1). Much evidence suggests that NGF is a retrogradely transported trophic factor that regulates the development of immature sympathetic peripheral neurons and the maintenance of mature sympathetic neurons (2–4). Very little is known about the role of NGF for mammalian sensory neurons, although it has been shown that rat dorsal root ganglion (DRG) neurons retain the capability of retrograde axonal transport of NGF throughout postnatal development (5).

One means of assessing the physiological role of NGF on the diverse neuronal populations of the developing mammalian nervous system is to deprive cells of NGF. Because the normal source of NGF is not known, and specific NGF antagonists are not available, the only mechanism of producing a systemic deprivation of NGF is immunological. Previous studies (6–8) have demonstrated that antibodies to NGF can be prepared by repeated injections of animals with NGF derived from mouse. These and subsequent studies, using anti-NGF serum, demonstrated that mammalian sympathetic neurons of the para- and prevertebral ganglia die if deprived of NGF during the neonatal period. Other neural cell types (the short adrenergic neurons innervating the urogenital tract and brown adipose tissue, adrenal medullary chromaffin cells, peripheral sensory neurons of the DRG, and central adrenergic neurons) are not destroyed by anti-NGF serum administered in the neonatal period (9, 10).

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The usefulness of antiserum-induced NGF deprivation is limited by problems associated with its repeated administration—e.g., serum sickness. An an alternative approach, we have developed an experimental "autoimmune" model of NGF deprivation and have studied the prenatal and postnatal effects of NGF deprivation. The model is "autoimmune" in that rats immunized with 2.5S mouse NGF develop serum antibodies that crossreact with their own NGF and that are transferred to the offspring *in utero* and in milk.

MATERIAL AND METHODS

Animal Treatments. Adult Sprague–Dawley rats (200–250 g) were immunized with 100 μg of 2.5S mouse NGF (11) in complete Freund's adjuvant and were boosted at 4–6-week intervals with 10μg of NGF in complete Freund's adjuvant. Unimmunized rats and rats immunized with cytochrome *c* (Sigma)—a protein with physical properties similar to those of NGF—were used as controls.

Three types of treatment protocols were utilized: (*i*) Passive transfer experiments were carried out to test the crossreactivity of rat serum antibodies raised against mouse NGF with the rats' own NGF. (*ii*) Maternal transfer of anti-NGF *in utero* and through milk was assessed by evaluation of various tissues in offspring born to NGF-immunized females. (*iii*) Cross-fostering experiments were carried out to separate the effects of anti-NGF exposure *in utero* from those due to anti-NGF in milk.

Passive Transfer. In the passive transfer experiments 0.30 ml of sera from NGF-immunized adult rats (titer ≥ 500), sera from control-immunized rats, or sera from unimmunized rats were injected subcutaneously into neonatal rats at postnatal days 1 and 3. Rats were killed at 1 week of age. Superior cervical ganglia (SCGs) were removed for histological and biochemical analysis.

Maternal Transfer. NGF-immunized female rats, cytochrome *c*-immunized female rats, or unimmunized female rats were mated with unimmunized males. Offspring were killed within 12 hr of birth to assess the effects of exposure to anti-NGF *in utero*, and at 1 week or at 8 weeks of age to assess the effects of exposure to anti-NGF both *in utero* and in milk. Blood was obtained for titering. SCGs and adrenals were removed for biochemical analysis, and DRGs were removed for protein determination. SCGs and DRGs were removed for histology.

Cross-Fostering. For the cross-fostering experiments half of a litter born to an NGF-immunized female was exchanged at birth with half of a litter born to an unimmunized or cytochrome *c*-immunized control female. The cross-fostering technique produced two groups of offspring: rats exposed to anti-NGF only *in utero* and rats exposed to anti-NGF only in

Abbreviations: SCG, superior cervical ganglion; DRG, dorsal root ganglion; NGF, nerve growth factor.

milk. These groups were compared to offspring that were born to and nursed by an NGF-immunized female, and to offspring born to and nursed by a control female. Offspring were killed at 2 weeks of age (12 weeks for the retrograde transport experiment). Both SCGs were removed for enzyme analysis and five DRGs at the cervical level (C4–C8) were removed for protein determination.

Titering of Antisera. Serum titers of anti-NGF were monitored, using a modification of the embryonic chicken DRG bioassay (12, 13). Twenty-five microliters of antiserum mixed with NGF (diluted antiserum mixed with an equal volume of 2.5S NGF at 30 ng/ml) was added to 25 μl of chicken plasma (GIBCO), containing three or four DRGs from 9-day chicken embryos. Twenty-five microliters of basal Eagle's medium (1×, 25 mM Hepes buffer, GIBCO), containing kanamycin sulfate (Sigma) at 33 μg/ml and thrombin (Parke, Davis) at 2.5 mg/ml, was then added for a final volume of 75 μl. Titers are defined as being greater than or equal to the reciprocal of the highest serum dilution that blocked the outgrowth of neurites.

Biochemical Analysis. For enzyme measurements, SCGs and adrenals were homogenized in 5 mM Tris·HCl buffer, pH 7.4, containing 0.2% Triton X-100, at a dilution that ensured that the reaction was linear with enzyme concentration and time. Tyrosine hydroxylase (EC 1.14.16.2) activity was measured by a modification of the method of Phillipson and Sandler (14) in the presence of 40 μM tyrosine and 625 μM 6-methyl-5,6,7,8-tetrahydropterine dihydrochloride. Choline acetyltransferase (EC 2.3.1.6) activity was measured by the method of Schrier and Shuster (15) in the presence of 25 mM potassium phosphate, 50 mM choline chloride, 24 μM dithiothreitol, and 10 μM [^{14}C]acetyl-CoA. Proteins were determined by the method of Lowry *et al.* (16) with bovine serum albumin as the standard.

Histological Analysis. SCGs and DRGs were fixed in 10% (vol/vol) formol saline. After dehydration in graded ethanol solutions, ganglia were embedded in paraffin, cut in 7-μm sections, and stained with toluidine blue. Sections from comparable levels were examined under the light microscope for obvious changes in neuronal size or number. No quantitative analyses were performed.

Retrograde Transport. ^{125}I-Labeled NGF (^{125}I-NGF) (1.7 pmol, 3 × 10^6 cpm) was prepared by the lactoperoxidase method (17) and was injected into the forepaw (10 μl) in 12-week-old cross-fostered offspring and offspring of NGF-immunized and control females (see *Animal Treatments*). At 12 hr, the time of peak accumulation (5), animals were killed by decapitation and cervical DRGs C5–C8 were excised on both the ipsilateral and contralateral sides and their radioactivities were measured in a Beckman gamma counter. Both labeled and unlabeled NGF were kindly supplied by Nicholas Costrini and Ralph Bradshaw.

Statistical Methods. The Student's *t* test for independent groups was used for comparing the means of two groups. When there were more than two groups, analysis of variance was carried out, using either raw data or a logarithmic transformation, depending on which produced homogeneous variances (18).

RESULTS

Passive Transfer. Passive transfer of anti-NGF was demonstrated by the ability of antiserum from adult rats immunized with 2.5S mouse NGF to produce an immunosympathectomy in neonatal rats. SCGs from neonatal rats injected at 1 and 3 days of age with adult rat anti-NGF serum were markedly reduced in size and showed extensive neuronal loss in the SCG at 1 week of age (Fig. 1*B*). Control antiserum from adult rats

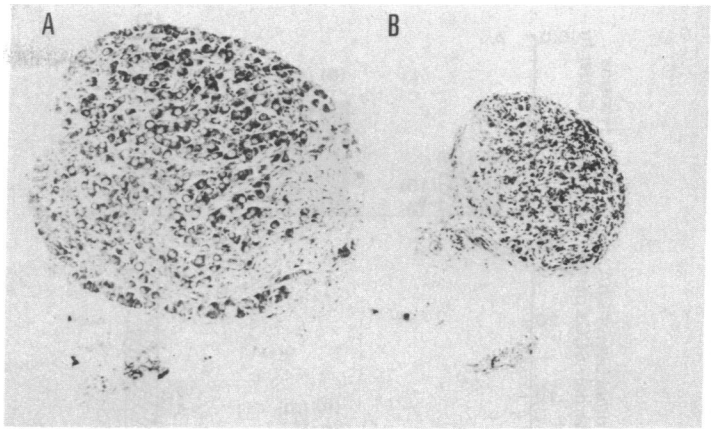

FIG. 1. SCG (×81) of neonatal rats injected with rat anti-cytochrome *c* serum (*A*) or rat anti-NGF serum (*B*). Neonates were injected subcutaneously with 0.3 ml of adult anti-NGF serum (titer ≥500) or anti-cytochrome *c* serum on postnatal days 1 and 3. Neonates were killed by decapitation at 1 week.

immunized with cytochrome *c* produced no morphological effects on neonatal SCG neurons (Fig. 1*A*).

Tyrosine hydroxylase was also measured in the SCG as an indication of destruction of sympathetic neurons in ganglia. Activity (nmol tyrosine hydroxylated per pair per hour) was 0.30 ± 0.03 (n = 6) in 1-week-old animals injected with anti-NGF serum, 4.05 ± 0.09 (n = 4) in animals injected with anti-cytochrome *c* serum, and 4.12 ± 0.27 (n = 5) in neonates injected with normal rat serum.

Evaluation of the Sympathetic Nervous System of Offspring Born to and Nursed by Female Rats Immunized with NGF. Tyrosine hydroxylase is the rate-limiting enzyme for catecholamine biosynthesis (19) and may be used as an index of maturation of postsynaptic neurons of the SCG and chromaffin cells of the adrenal medulla (20). It has been shown in the mouse SCG that choline acetyltransferase is a valid biochemical index of synapse formation of preganglionic cholinergic neuronal terminals on the principal ganglion cells (20). Tyrosine hydroxylase, choline acetyltransferase, and protein undergo developmental increases after birth in the normal rat SCG, reaching adult levels by 7 to 8 weeks (21). The changes in enzymes and protein seen at the various times are described below for SCG and adrenal. Because there were never significant differences between offspring of unimmunized females and offspring of cytochrome *c*-immunized females, in some cases only one type of control was used. No difference in size, appearance, or vitality was seen in the offspring of animals immunized against NGF or cytochrome *c*.

Tyrosine hydroxylase and choline acetyltransferase activities at birth in the SCGs of offspring exposed to anti-NGF *in utero* were reduced to one-third of those in offspring of control females (Fig. 2 *A* and *B*). Protein levels in the SCG were reduced to less than half of control levels at birth in offspring of NGF-immunized females (Fig. 2*C*). The decrease in enzyme activities and protein levels at birth in the offspring of NGF-immunized females reflected the marked neuronal atrophy and neuronal loss that were apparent at the light microscopic level (not shown).

Even greater reductions in ganglionic enzyme activities and protein content were observed in offspring of NGF-immunized females killed at 1 week and at 8 weeks of age. Tyrosine hydroxylase activity, choline acetyltransferase activity, and protein levels in the SCG at 1 week and at 8 weeks of age are shown in Fig. 2 *A*, *B*, and *C*, respectively.

Tyrosine hydroxylase activity was determined in the adrenal

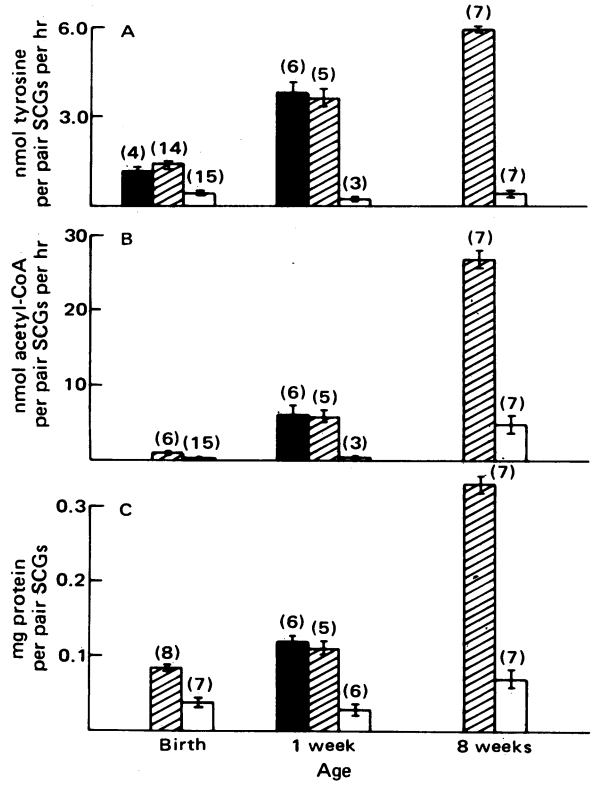

FIG. 2. Tyrosine hydroxylase (*A*), choline acetyltransferase (*B*), and protein levels (*C*) in the SCG of offspring born to and nursed by NGF-immunized or control female rats. Bars represent means ± SEM for offspring of unimmunized control females (solid bars), offspring of cytochrome *c*-immunized females (hatched bars), and offspring of NGF-immunized females (open bars). Number in parentheses at top of bar refers to number of animals in treatment group. In each case, means for the offspring of NGF-immunized rats were significantly different from the means for control groups ($P < 0.001$). There were no significant differences between the two control groups. Anti-NGF titers of offspring of NGF-immunized rats were ≥10 to <50 at birth, ≥50 to <100 at 1 week, and <10 at 8 weeks (4 weeks after weaning). Anti-NGF titers of mothers were ≥500.

glands of animals at birth, at 1 week of age, and at 8 weeks of age. At none of these times was there a difference in enzyme activity in adrenals of animals born to and nursed by anti-NGF producing rats when compared to offspring of either type of control rat (data not shown).

Comparison of Prenatal and Postnatal Effects of Anti-NGF on the Sympathetic Nervous System: Cross-Fostering Experiments. In order to separate the effects of anti-NGF exposure *in utero* from those due to anti-NGF in milk, cross-fostering experiments were carried out. In two different experiments results were similar, and Table 1 contains the pooled data of the two experiments. Significant reductions in tyrosine hydroxylase activity and protein levels in the SCG were seen in rats exposed to anti-NGF only *in utero*, only in milk, and in both situations (Table 1). Serum titers of neonates nursing from NGF-immunized females were higher than those of offspring at birth, exposed to anti-NGF only *in utero* (see legend to Table 1). All neonates nursed by control mothers had no serum titers of anti-NGF at 2 weeks.

Comparison of Prenatal and Postnatal Effects of Anti-NGF on Protein Content in the DRG: Cross-Fostering Experiments. In preliminary experiments it was noted that the protein content in DRGs of offspring of NGF-immunized females was significantly lower (35%) than control levels at birth, and it remained significantly lower (22%) in 8-week-old animals.

Table 1. Comparison of prenatal and postnatal effects of anti-NGF in rat SCG: Cross-fostering experiments

Exposure to anti-NGF*		Number	Protein,† mg	Tyrosine hydroxylase activity,† nmol tyrosine hydroxylated per pair
In utero	Milk	in group	per pair SCGs	SCGs per hr
−	−	8	0.201 ± 0.011 (100 ± 5%)	4.91 ± 0.30 (100 ± 6%)
+	−	6	0.075 ± 0.013‡ (37 ± 6%)	1.81 ± 0.37‡ (37 ± 7%)
−	+	8	0.055 ± 0.006‡ (27 ± 3%)	0.221 ± 0.033‡ (5 ± 1%)
+	+	5	0.044 ± 0.010‡ (22 ± 5%)	0.020 ± 0.020‡ (0.4 ± 0.4%)

* Anti-NGF titers in offspring at time of sacrifice (2 weeks of age) were <10 for the *in utero* group (≥10 to <50 at birth), ≥100 to <500 for the milk group, and ≥100 to <500 for the *in utero* plus milk group. Anti-NGF titers of NGF-immunized mothers at time of sacrifice of offspring were ≥100 to <1000.

† Number in parentheses is the mean ± SEM expressed as percent of control. Results were similar for two different experiments and data within each treatment group were pooled.

‡ Significant differences ($P < 0.001$) resulted from exposure to anti-NGF *in utero* or in milk.

The separation of effects of anti-NGF exposure *in utero* from those due to anti-NGF in milk on protein content in the DRG of 2-week-old rats cross-fostered at birth is shown in Table 2. Protein levels in the DRG were significantly reduced in both groups exposed to anti-NGF *in utero*. Similar reductions in protein (20–30%) were seen in rats killed at 8 weeks or at 12 weeks (data not shown). The group exposed to anti-NGF in milk alone showed no significant reduction in DRG protein. Serum titers of nursing offspring of NGF-immunized females were higher than those of offspring exposed to anti-NGF only *in utero*, as described previously (Table 1).

Effect of Exposure to Anti-NGF on Retrograde Axonal Transport in the DRG. The decrease in protein content in DRGs from animals exposed to anti-NGF *in utero* suggested the possibility that a population of sensory neurons had not survived. Preliminary experiments were carried out in 8-week-old animals born to and nursed by anti-NGF-producing mothers to determine if these animals retained the ability to

Table 2. Comparison of prenatal and postnatal effects of anti-NGF in rat DRG: Cross-fostering experiments

Exposure to anti-NGF*		Number	
In utero	Milk	in group	Protein,† mg/DRG
−	−	8	0.047 ± 0.002 (100 ± 5%)
+	−	6	0.035 ± 0.002‡ (74 ± 2%)
−	+	8	0.043 ± 0.003 (91 ± 6%)
+	+	5	0.033 ± 0.001‡ (70 ± 2%)

* Anti-NGF titers in offspring at time of sacrifice (2 weeks of age) were <10 for the *in utero* group (≥10 to <50 at birth), ≥100 to <500 for the milk group, and ≥100 to <500 for the *in utero* plus milk group. Anti-NGF titers of NGF-immunized mothers at time of sacrifice of offspring were ≥100 to <1000.

† Number in parentheses is the mean ± SEM expressed as percent of control. Results were similar for two different experiments and data within each treatment group were pooled.

‡ Only *in utero* exposure to anti-NGF resulted in significant differences ($P < 0.01$).

Table 3. Comparison of prenatal and postnatal effects of anti-NGF on retrograde transport of ^{125}I-NGF in sensory neurons of adult rats: Cross-fostering experiments

Exposure to anti-NGF*		Number	Transport,† cpm/four DRGs	
In utero	Milk	in group	Ipsilateral	Contralateral
−	−	8	1637 ± 127	83 ± 18
+	−	7	391 ± 58‡	50 ± 32
−	+	6	1273 ± 94§	80 ± 40
+	+	3	256 ± 83¶	80 ± 61

* At the time of the transport experiment (12 weeks of age) no animals had serum titers of anti-NGF. At time of weaning (4 weeks of age) anti-NGF titers were <10 for the *in utero* group (≥10 to <50 at birth), ≥100 for the milk group, and ≥100 for the *in utero* plus milk group. Anti-NGF titers of NGF-immunized mothers at time of weaning were ≥100 to <1000.

† Mean ± SEM of DRGs (C5–C8) from animals injected in the forepaw with 3 × 10^6 cpm of ^{125}I-NGF (1.7 pmol) and killed 12 hr later.

‡ Significant difference ($P < 0.0001$) resulted from *in utero* exposure to anti-NGF.

§ Significant difference ($P < 0.05$) resulted from in milk exposure to anti-NGF.

¶ Significant difference ($P < 0.0001$) resulted from exposure to anti-NGF both *in utero* and in milk.

retrogradely transport NGF in the peripheral sensory ganglia. After it had been determined that these animals no longer had circulating anti-NGF antibodies, ^{125}I-NGF was injected into a forepaw. The animals were killed 12 hr later (time of maximal accumulation), and the radioactivity in ipsilateral and contralateral DRGs (C5–C8) was determined. The accumulation of retrogradely transported ^{125}I-NGF in the ipsilateral DRGs was reduced by 90% (data not shown). The experiment was then repeated in cross-fostered animals in order to assess the relative importance of prenatal and postnatal exposure to anti-NGF. The data in Table 3 demonstrate a pattern of sensitivity to anti-NGF quite different from that seen in sympathetic ganglia (Table 1) but entirely consistent with the DRG protein data in Table 2. Exposure to anti-NGF *in utero* reduced retrograde transport of ^{125}I-NGF (ipsilateral minus contralateral) by 80–90%, as seen in the preliminary experiment. Despite the much higher titers of anti-NGF achieved postnatally, exposure to anti-NGF in milk alone produced only a small (23%) decrease in retrograde transport of ^{125}I-NGF.

DISCUSSION

Experiments have been reported (22–24) in which attempts have been made to determine the effect of injections of heterologous anti-NGF serum into pregnant mice on the sympathetic nervous systems of the fetus. The results have produced conflicting data and in no case was the titer of antisera in either the mother or offspring determined. Levi-Montalcini and Angeletti (22) failed to see an effect on offspring when heterologous (rabbit) anti-NGF serum was injected into pregnant mice. Administration of the same antiserum to lactating mice resulted in destruction of sympathetic neurons in the suckling offspring. Klingman and Klingman (23, 24) injected heterologous anti-NGF serum into pregnant mice during different periods of gestation and analyzed cell numbers in the SCG and tissue catecholamines in the offspring at 1–7 months of age. The decreases observed were ascribed to *in utero* transfer of anti-NGF. However, because offspring were evaluated 1–7 months after birth (rather than at birth), and because cross-fostering experiments were not used to exclude the distinct possibility that the effects were due to antibody reaching the neonate through the milk, the conclusions of Klingman and Klingman are subject to doubt.

The work described in this paper utilizes an experimental "autoimmune" model in which 2.5S mouse NGF is administered to rats. The demonstration of passive transfer of homologous serum anti-NGF in rats (Fig. 1) is consistent with a similar demonstration in rabbits in the original paper describing immunosympathectomy (8). The presence of serum titers of anti-NGF that disappear within a few days of birth in cross-fostered animals, histological changes in the SCG, and the reduction in tyrosine hydroxylase activity in the SCG at birth demonstrate that offspring born to NGF-immunized female rats were exposed *in utero* to anti-NGF (presumably of maternal origin). In general, there was a correlation between serum titer in the mother and amount of tyrosine hydroxylase reduction in the neonate at birth. An exception was one litter (out of nine litters examined) in which there were normal levels of tyrosine hydroxylase activity in the SCGs of the offspring despite a moderate titer (≥100 to <500) in the mother. At no time was anti-NGF activity detected in serum of offspring born to control females. The bioassay used to determine serum titers detects antibodies that react with mouse NGF. The precise titers against rat NGF are not known, because rat NGF is not available. It is possible that a number of subclasses of antibodies directed against mouse NGF are produced by the mother and that the various subclasses differ in their affinity for rat NGF and in their availability to the developing embryo.

Effects of Prenatal and Postnatal Anti-NGF on the Peripheral Sympathetic Nervous System. The failure of tyrosine hydroxylase to reach control levels by 8 weeks indicates that prenatal or neonatal (or both) exposure to anti-NGF results in interference with the normal biochemical development of adrenergic neurons in the SCG. The persistence of reduced tyrosine hydroxylase activity and reduced ganglion size into adulthood, coupled with the histological observations in the SCG at birth, suggest that there is an extensive loss of SCG neurons in offspring born to NGF-immunized females. Morphometric analysis of the SCG is required to exclude the unlikely possibility that neuronal atrophy, rather than neuronal death, accounts for these changes. The failure of choline acetyltransferase to reach control levels by 8 weeks probably represents a permanent retrograde transynaptic effect on presynaptic neurons. Retrograde degenerative changes in preganglionic neurons have been demonstrated in neonatally sympathectomized rats (25–27).

The results of the cross-fostering experiments confirm that rat anti-NGF is transferred both *in utero* and in milk. The finding of a prenatal period of susceptibility to NGF deprivation for SCG neurons (shown by decreases in tyrosine hydroxylase activity and protein levels at 2 weeks) is consistent with recent *in vitro* findings with SCG explants from mouse embryos. Coughlin *et al.* (28) demonstrated that ganglia from 14-day mouse fetuses showed abundant neurite outgrowth and normal developmental increases in tyrosine hydroxylase activity *in vitro* in the presence of anti-NGF. Ganglia from 18-day fetuses, which showed neurite outgrowth with exogenous NGF, had no neurite outgrowth and reduced levels of tyrosine hydroxylase activity when cultured without exogenous NGF or in the presence of anti-NGF. The finding of transfer of anti-NGF in milk is consistent with the morphological observations of Levi-Montalcini and Angeletti (22) in neonatal mice nursed by mothers injected with rabbit anti-mouse NGF serum. Future fetal studies using the *in vivo* autoimmune model should be able to determine precisely the onset of NGF dependence in SCG and other peripheral sympathetic neurons and determine unambiguously whether migration, differentiation, and neuronal survival are NGF dependent.

Our finding of normal levels of adrenal tyrosine hydroxylase

in offspring exposed to anti-NGF *in utero* and during the first few weeks of postnatal life via mothers' milk suggests that the adrenal is insensitive to anti-NGF. The relative resistance of the adrenal compared to sympathetic ganglia may be due to locally higher concentrations of NGF in the adrenal. It has been shown in organ cultures that mouse adrenal medullary cells are capable of NGF secretion (29). The lack of biochemical evidence of adrenal medullary degeneration in animals exposed to anti-NGF *in utero* is inconsistent with the recent morphological findings of Aloe and Levi-Montalcini (30). These workers injected heterologous anti-NGF into rats at day 17 *in utero* and for the first 8 days of postnatal life and saw degeneration of adrenal medullary cells. It is possible that this discrepancy is due to differences in levels of anti-NGF attained with maternal transfer of anti-NGF vs. exogenous administration of anti-NGF.

Effects of Prenatal and Postnatal Anti-NGF on Peripheral Sensory Neurons. Retrograde axonal transport of ^{125}I-NGF can be used as a functional test for the presence of sensory neurons that are NGF dependent at some stage of development, if it is assumed that the sensory neurons capable of retrogradely transporting NGF (5) are the putative NGF-dependent cells. The present findings that *in utero* exposure to anti-NGF results in a 20–30% decrease in protein levels in the adult DRG and that adult rats exposed to anit-NGF *in utero* have a markedly reduced ability to retrogradely transport ^{125}I-NGF to the DRG suggest that NGF is required for the survival of a subpopulation of DRG sensory neurons in the rat. Definitive proof that cell death occurs will require morphometric analysis of the DRG. The period of susceptibility to anti-NGF (and presumably dependence on NGF) appears restricted to a period prior to birth. This is consistent with findings in the chicken embryo which show that DRG explants require NGF for survival and neurite outgrowth (1). Herrup and Shooter (31) have shown that the disappearance of NGF-stimulated neurite outgrowth *in vitro* between 14 and 16 days of embryonic life is coincident with loss of the ability to bind NGF to specific cell surface receptors in the chicken DRG. Thus a subpopulation of avian sensory neurons appears to be sensitive to NGF *in vitro* only during a circumscribed period of development. A potential role for NGF in the development of sensory neurons in mammals is supported by *in vitro* studies in which dissociated DRGs (but not intact ganglia) from newborn mice and rats show enhanced survival and neurite outgrowth in the presence of NGF (32, 33).

The experimental autoimmune model of NGF deprivation presented in this report should prove to be a useful tool in addressing some of the critical questions concerning the role of NGF in neuronal development. Which cell types are dependent on NGF at any stage of development for survival or maintenance? At what stage in the life of a cell population is NGF required? In addition to elucidating basic trophic mechanisms in neuronal development, the experimental autoimmune model of NGF deprivation may be helpful in elucidating pathophysiological mechanisms in developmental abnormalities of the peripheral nervous system, such as those manifested in familial dysautonomia and the hereditary sensory neuropathies.

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1. Levi-Montalcini, R. & Angeletti, P. U. (1968) *Physiol. Rev.* **48,** 534–569.
2. Mobley, W. C., Server, A. C., Ishii, P. N., Riopelle, R. J. & Shooter, E. M. (1977) *N. Engl. J. Med.* **297,** 1096–1104.
3. Mobley, W. C., Server, A. C., Ishii, D. N., Riopelle, R. J. & Shooter, E. M. (1977) *N. Engl. J. Med.* **297,** 1149–1188.
4. Black, I. B. (1978) *Annu. Rev. Neurosci.* **1,** 183–214.
5. Stoeckel, K., Schwab, M. & Thoenen, H. (1975) *Brain Res.* **89,** 1–14.
6. Cohen, S. (1960) *Proc. Natl. Acad. Sci. USA* **46,** 302–311.
7. Levi-Montalcini, R. & Cohen, S. (1960) *Ann. N.Y. Acad. Sci.* **85,** 324–341.
8. Levi-Montalcini, R. & Booker, B. (1960) *Proc. Natl. Acad. Sci. USA* **46,** 373–384.
9. Levi-Montalcini, R. (1972) in *Immunosympathectomy,* eds, Steiner, G. & Schönbaum, E. (Elsevier, Amsterdam), pp. 55–78.
10. Konkol, R. J., Mailman, R. B., Bendeich, E. G., Garrison, M., Mueller, R. A. & Breese, G. R. (1978) *Brain Res.* **144,** 277–285.
11. Bocchini, V. & Angeletti, P. U. (1969) *Proc. Natl. Acad. Sci. USA* **64,** 787–794.
12. Levi-Montalcini, R., Meyer, H. & Hamburger, V. (1954) *Cancer Res.* **14,** 49–57.
13. Fenton, E. L. (1970) *Exp. Cell Res.* **59,** 383–392.
14. Phillipson, O. T., & Sandler, M. (1975) *Brain Res.* **90,** 283–296.
15. Schrier, B. K. & Shuster, L. (1967) *J. Neurochem.* **14,** 977–985.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
17. Marchalonis, J. J. (1969) *Biochem. J.* **113,** 299–305.
18. Snedecor, G. W. & Cochran, W. G. (1967) *Statistical Methods* (Iowa State Univ. Press, Ames, IO).
19. Levitt, M., Spector, S., Sjoerdsma, A. & Udenfriend, S. (1965) *J. Pharmacol. Exp. Ther.* **148,** 1–8.
20. Black, I. B., Hendry, I. A. & Iversen, L. L. (1971) *Brain Res.* **34,** 229–240.
21. Thoenen, H., Kettler, R. & Saner, A. (1972) *Brain Res.* **40,** 459–468.
22. Levi-Montalcini, R. & Angeletti, P.U. (1961) *Q. Rev. Biol.* **36,** 99–108.
23. Klingman, G. I. (1966) *Int. J. Neuropharmacol.* **5,** 163–170.
24. Klingman, G. I. & Klingman, J. D. (1967) *Int. J. Neuropharmacol.* **6,** 501–508.
25. Black, I. B., Hendry, I. A. & Iversen, L. L. (1972) *J. Physiol.* **221,** 149–159.
26. Aguayo, A. J., Peyronnard, J. M., Terry, L. C., Romine, J. S. & Bray, G. M. (1976) *J. Neurocytol.* **5,** 137–155.
27. Johnson, E. M., Caserta, M. T. & Ross, L. L. (1977) *Brain Res.* **140,** 1–10.
28. Coughlin, M. D., Boyer, D. M. & Black, I. B. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3438–3442.
29. Harper, G. P., Pearce, F. L. & Vernon, C. A. (1976) *Nature (London)* **261,** 251–253.
30. Aloe, L. & Levi-Montalcini, R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1246–1250.
31. Herrup, K. & Shooter, E. M. (1975) *J. Cell Biol.* **67,** 118–125.
32. Burnham, P., Raiborn, C. & Varon, S. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 3556–3560.
33. Varon, S., Raiborn, C. & Tyszyka, E. (1973) *Brain Res.* **54,** 51–63.