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ROLE OF NERVE GROWTH FACTOR IN THE DEVELOPMENT OF RAT SYMPATHETIC NEURONS IN VITRO

I. Survival, Growth, and Differentiation of Catecholamine Production

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ABSTRACT

To study the effect of nerve growth factor (NGF) on neuronal survival, growth, and differentiation, cultures of dissociated neonatal rat sympathetic neurons virtually free of other cell types were maintained for 3–4 wk. In the absence of NGF, the neurons did not survive for more than a day. Increased levels of NGF increased neuronal survival and growth (total protein and total lipid phosphate); saturation occurred at 0.5 μg/ml 7S NGF. Neuronal differentiation examined by measuring catecholamine (CA) production from tyrosine also depended on the level of NGF in the culture medium. As the NGF concentration was raised, CA production per neuron, per nanogram protein, or per picomole lipid phosphate increased until saturation was achieved between 1 and 5 μg/ml 7S NGF. Thus, NGF induces neuronal survival, growth, and differentiation of CA production in a dose-dependent fashion. Neuronal growth and differentiation were quantitatively compared in the presence of the high and low molecular weight forms of NGF; no significant functional differences were found.

KEY WORDS nerve growth factor · sympathetic neurons · cell culture · catecholamine production

Nerve growth factor (NGF) is essential for the survival and development of sympathetic neurons both in vitro and in vivo (15). With respect to survival, if NGF is excluded from the culture medium, dissociated sympathetic neurons die (14), and when neonatal mammals are injected with an antiserum to NGF, their sympathetic nervous systems undergo extensive degeneration (16). With respect to growth, addition of NGF to cultured sympathetic ganglia causes growth of neuronal fibers from the explants (13), and daily injections into neonatal mice result in marked hypertrophy of sympathetic ganglia with extensive hyperinnervation of peripheral organs and blood vessels (17). The in vitro outgrowth and in vivo hyperinnervation could have been due to increased process formation and/or increased neuronal survival. In adult mice, where neuronal survival is probably not a variable, NGF still induces hypertrophy of sympathetic ganglia (17), although the effect is not as marked as in the neonate.

In addition to promoting survival and fiber growth, there is evidence that NGF promotes neuronal differentiation. Thoenen et al. (32) found that injection of NGF into neonatal rats selectively induces the catecholamine (CA) synthesizing enzymes, tyrosine hydroxylase (TH) and dopamine-β-hydroxylase, in superior cervical ganglia (SCG). Similar NGF-induced increases in TH

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have subsequently been reported for SCG in organ culture (12, 23, 31). Interpretation of these results is complicated by the presence of ganglionic non-neuronal cells and by the fact that NGF increases neuronal survival (12) and fiber growth. Thus, the increase in TH may reflect an increased survival or growth of NGF-dependent neurons rather than selective biochemical changes within the neurons themselves.

To avoid these complications, we have studied the role of NGF in sympathetic development by using cultures of dissociated rat sympathetic neurons grown in the virtual absence of ganglionic non-neuronal cells (18). This system facilitates control of the fluid and cellular environment in which the neurons grow and are assayed. Our evidence indicates that NGF promotes the survival, growth and differentiation of CA production of rat sympathetic neurons in a dose-dependent fashion.

NGF can be isolated from the adult male mouse submaxillary gland as a high molecular weight complex, 7S NGF (35). Under mild acidic or basic conditions, 7S NGF dissociates to yield three subunits of similar molecular weight, α, β, and γ (34). Of these, only the β-subunit (36) elicits neurite outgrowth from sympathetic ganglia. Proteolytic derivatives of β, 2.5S NGF (2) and low molecular weight (LMW) NGF (21) are equally as effective. In addition, antiserum directed against the β-subunit blocks normal development of the sympathetic nervous system (16). Since the β-subunit of 7S NGF displays all the known biological activity, the significance of the complex containing the α and γ subunits is not understood. To determine whether we could discern functional differences between 7S and LMW NGF, their effects on neuronal growth and differentiation were compared. A preliminary report of some of these findings has appeared (5).

MATERIALS AND METHODS

Cell Preparation

Sympathetic neurons were mechanically dissociated from SCG of 1 to 3-day-old rats by a method similar to that previously described (18, 26). The animals were killed by a blow to the head and the SCG removed, cleaned, and teased apart with forceps. The suspension of cells was put through a 26-gauge needle attached to a 5-ml syringe five times, passed through a Nuclepore filter (Nuclepore Corp., Pleasanton, Calif., 12 μm pore size), and the filtrate centrifuged. The cells were resuspended, vibrated on a vortex mixer, and plated on collagen-coated modified culture dishes. L-15 Air growth medium (18) containing appropriate concentrations of NGF was used. The culture medium was changed every 2-3 days. Usually 80-90 dishes were prepared from 70 pups.

NGF Preparation

7S NGF was prepared from submaxillary glands of male mice through the Sephadex G-100 step of Bocchini and Angeletti (2) followed by the diethylaminoethyl (DEAE) step of Varon et al. (35). A final gel filtration over Sephadex G-150 was carried out in phosphate buffer, pH 6.8 (33). The pooled G-150 fractions were concentrated by pressure filtration to 1 mg/ml protein and stored at −20°C. The preparations were periodically checked for purity by acrylamide gel electrophoresis using the discontinuous Bistris-Tes system and one band containing >90% of the protein was observed as expected (30).

A low molecular weight form of NGF (LMW NGF) was purified by the rapid procedure of Mobley et al. (21). The preparations were periodically checked for purity by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (39) and one band containing >90% of the protein was observed as expected (21).

Serial dilutions of NGF were made into L-15 Air growth medium lacking Methocel (Dow Corning Corp., Midland, Mich.). To yield the desired final concentration of NGF, 0.1 ml of a dilution was added to each 1.9 ml of complete growth medium and thoroughly mixed. To minimize errors due to NGF adsorption, all dilutions were done with polystyrene pipettes and tubes.

Assays

Cultures were assayed after 3–4 wk of growth in the appropriate concentration of NGF, since at this age the ability of the neurons to synthesize and accumulate CA had matured (6). All the neurons in the dish were counted by scanning the entire well with a phase-contrast microscope. Soma diameters were obtained by photographing random fields and averaging the longest diameter and that perpendicular to it. Noncollagen protein was measured by the technique of Bonting and Jones (3) as modified by Patterson and Chun (26). Lipid phosphate was determined by inorganic phosphate assays of chloroform-extracted and Folch-washed cultures (26).

Isotopic Incubations

Stock solutions and buffers were prepared as follows: Buffer A, 0.47 M formic acid, 1.4 M acetic acid, pH 1.9 (11); buffer B, one-third strength buffer A diluted with H_2O; buffer C, 0.4 M Tris-HCl, 0.2% (wt/vol) Na_2EDTA, pH 8.5; buffer D, 0.1 M sodium phosphate, pH 6.5; solution E, 0.1% (wt/vol)

Na_2EDTA in H_2O; solution F, 60% (vol/vol) aqueous methanol; Solution G, 1 N HCl in 50% aqueous ethanol.

The rate of CA production was assayed by incubating the cultures for 8 h at 36°-37°C with [2,3-^{3}H]tyrosine (2.1-13.4 Ci/mmol, Amersham/Searle Corp., Arlington Heights, Ill.) in L15-CO_2 growth medium as previously described (18) with no NGF present. At the end of the incubation, 1 ml of warm plating medium containing 25 μg/ml ascorbic acid was added and the cultures were incubated for an additional 10 min. This medium, which contained the CA and deaminated metabolites excreted during the 8-h incubation, was decanted, added to 20 μl buffer A, and frozen for later analysis. The cultures were extracted at pH 1.9 in SDS (18). Total tyrosine incorporation was determined by counting an aliquot of the culture extract. The accumulation of synthesized CA was taken to be the radioactivity in norepinephrine (NE) and dopamine (DA), determined by high voltage electrophoresis of the culture extracts (18). The release of synthesized CA and deaminated metabolites was determined by putting the wash medium through a series of alumina and Dowex (Bio-Rad Laboratories, Richmond, Calif.) 50 pencil columns, following an elution protocol developed with the help of Dr. R. E. Mains (Department of Physiology, University of Colorado Medical School) and suggested by the work of Rutledge and Weiner (29) and Atack and Magnusson (1). The columns were made in Pasteur pipettes (5 mm inner diameter with a plug of glass wool at the constriction and filled with 100-200 μl resin. Alumina was prepared by the method of Crout (8). Dowex 50W-X8 (Bio-Rad Laboratories) was prepared either as the Na^+ or the H^+ form according to the manufacturer's instructions. A thawed sample of wash medium was mixed with 1 ml buffer C and applied to an alumina column. The column was washed twice with solution E and once with glass-distilled H_2O, and then placed piggyback above a Dowex-Na^+ pencil column which was in turn placed above a Dowex-H^+ pencil column. To elute the labelled deaminated metabolites, 3 ml of buffer B was passed through the columns and collected in 12 ml scintillation fluid. The Dowex-Na^+ column was then removed and washed twice with solution F, once with glass-distilled H_2O, thrice with buffer D, and once with glass-distilled H_2O. To elute the labelled CA, 3 ml of solution G was passed through the column and the eluate collected in 12 ml scintillation fluid. The amount of product was calculated from the specific activity of the radioactive tyrosine and subject to the qualifications outlined previously (18, 26).

RESULTS

Effect of NGF on Neuronal Survival

As found by Bray (4) in the absence of ganglionic non-neuronal cells, dissociated sympathetic neurons from neonatal rat have an absolute requirement for NGF. Without exogenous NGF the neurons exhibited virtually no process formation and degenerated within a day after plating. Our results indicate in concentrations as low as 0.5-1 ng/ml some neurons had thin processes 1-2 days after plating, but the neurons did not survive beyond 3-4 days. Under our culture conditions the lowest concentration of 7S NGF which sustained neurons for 3-4 wk in vitro was 10 ng/ml. As the 7S NGF concentration was increased, survival increased until saturation was achieved at 0.5 μg/ml (Fig. 1). Concentrations up to 10 μg/ml maintained the same level of survival (Fig. 1), but by 50 μg/ml some loss of cells, ~30%, was observed (data not shown).

Effect of NGF on Neuronal Growth

Morphological differences in cell soma size and in the extent of process outgrowth were seen when neurons were grown in various concentrations of NGF. After growth for 3 wk in 10 ng/ml 7S NGF (Fig. 2 *a*), cell somas had a mean diameter of 20.2 μm and extended relatively few processes; at this concentration, the neurons were never in isolation but always in contact with one of the few non-neuronal cells in the dish. At higher NGF concentrations, cell body diameters increased as did neurite outgrowth (Fig. 2 *b-d*). As illustrated in Fig. 3, the mean soma diameter was 40% larger for cells grown

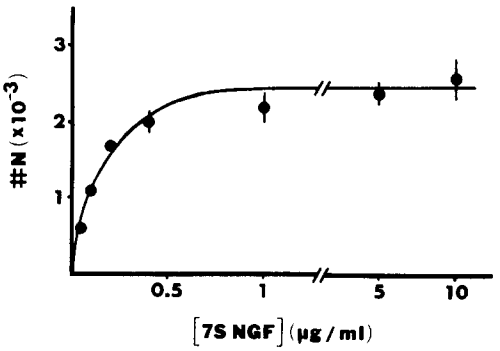

FIGURE 1 Neurons were grown in the indicated concentrations of 7S NGF and after 30 days neuronal somas were counted. In this and in all subsequent figures the results are expressed as the mean of at least three sister cultures ± SEM. Data presented in Fig. 1 and 4-8 were obtained from the same experiment and are therefore directly comparable. Similar results were obtained in many other experiments. *N*, neuron.

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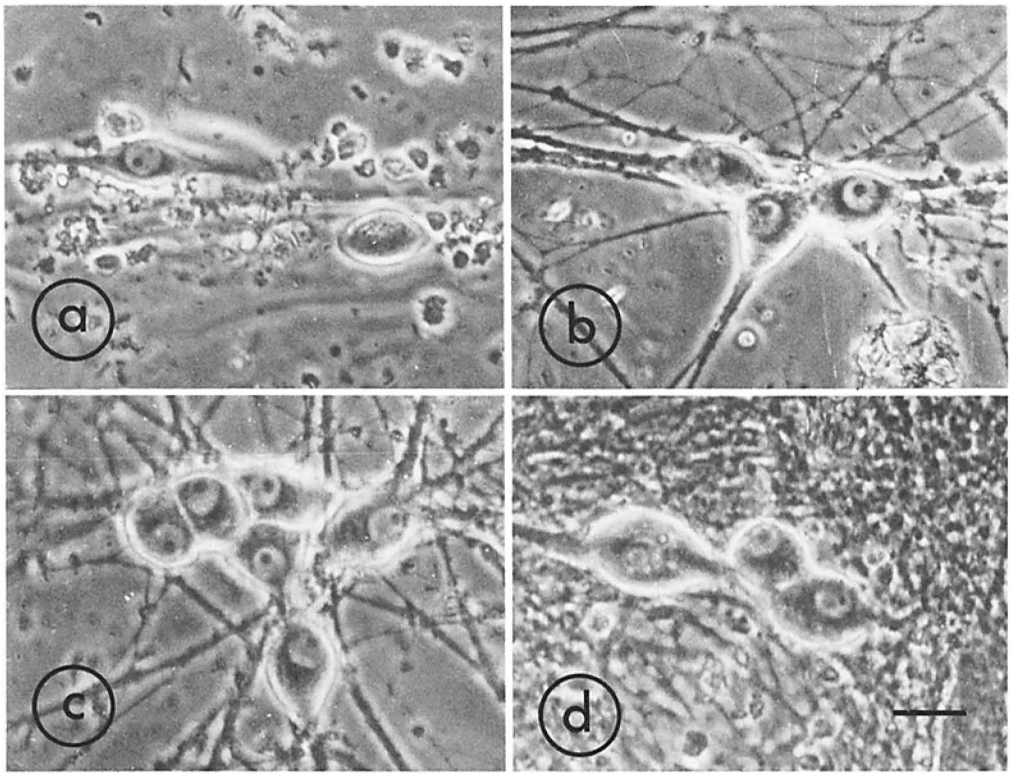

FIGURE 2 Phase-contrast micrographs of neurons grown for 21 days in various concentrations of 7S NGF. (*a*) 0.01 μg/ml, (*b*) 0.05 μg/ml, (*c*) 0.1 μg/ml, (*d*) 10 μg/ml. Bar, 25 μm. × 400.

in 1 μg/ml 7S NGF than for those grown in 0.01 μg/ml.

To test whether the increase in process outgrowth at higher NGF levels was due simply to the increased neuronal survival, overall growth per neuron was measured by assaying total neuronal protein and lipid phosphate. Such measurements were possible because these cultures were virtually free of non-neuronal cells (26). Both protein and lipid phosphate per neuron increased about two-fold over a concentration range of 0.05 to 0.5 μg/ml 7S NGF (Fig. 4). Thus, NGF also increased neuronal growth in a dose-dependent fashion. No inhibition of fiber outgrowth was apparent at higher NGF concentrations.

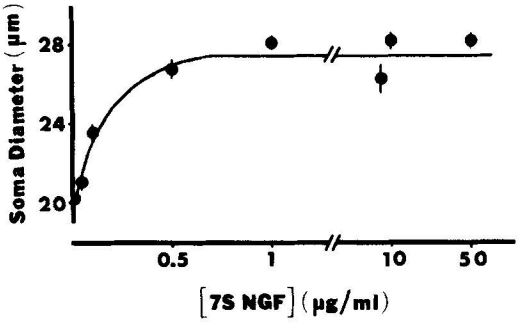

FIGURE 3 After growth in the indicated concentrations of 7S NGF for 21 days, phase-contrast micrographs were taken, and soma diameters determined.

Effect of NGF on Differentiation of CA Production

One measure of neuronal differentiation is the ability to produce neurotransmitters. We determined the total amount of CA and deaminated metabolites synthesized from [^{3}H]tyrosine in an 8-h incubation period (total CA). Since previous work indicated that 3,4-dihydroxyphenylalanine does not accumulate in these cultures (18) and the major metabolite of NE is the deaminated product (27), this synthetic rate is a measure of the actual tyrosine hydroxylase (TH) activity in

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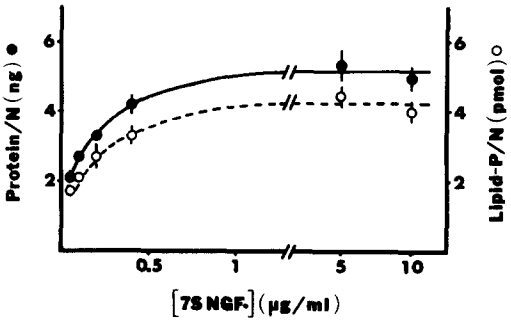

FIGURE 4 Cultures were grown in the indicated concentrations of 7S NGF for 30 days. Neuronal protein (●) and lipid phosphate (*Lipid-P*) (○) were measured (26) and expressed per neuron (*N*).

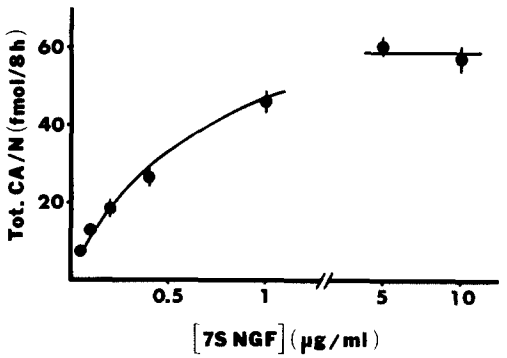

FIGURE 5 Cultures were grown in the indicated concentrations of 7S NGF. After 30 days they were incubated for 8 h with [^{3}H]tyrosine. Radioactivity in CA and deaminated metabolites was determined and expressed per neuron (*N*).

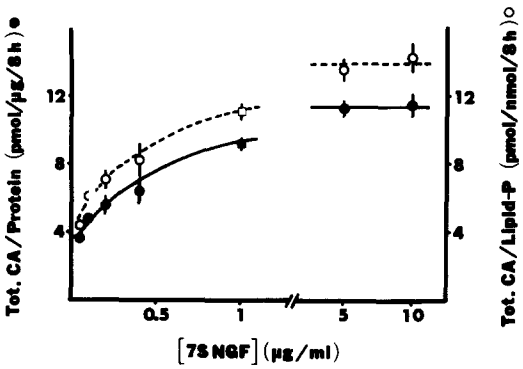

FIGURE 6 Cultures grown for 30 days were incubated for 8 h with [^{3}H]tyrosine. Radioactivity in CA and deaminated metabolites was determined and expressed either per nanogram protein (●) or per picomole lipid phosphate (*Lipid-P*) (○). Since protein and lipid phosphate were not measured for cultures grown in 1 μg/ml 7S NGF, these values were obtained graphically from Fig. 4. The data points which are plotted as squares here were obtained by determining values of total (*Tot.*) CA for cultures in 1 μg/ml 7S NGF as described above and dividing these by values of either nanogram protein (■) or picomole lipid phosphate (□) obtained graphically.

the living cell. (This may be different from the potential TH activity which could be assayed in homogenates under conditions where concentrations of substrate and cofactors and their access to the enzyme are optimized.) NGF induced a six-fold increase in total CA synthesis per neuron over the concentration range studied, and saturation was reached between 1 and 5 μg/ml 7S NGF (Fig. 5).

It is clearly of interest to know whether this increase in total CA synthesized per neuron was simply proportional to overall neuronal growth produced by increasing concentrations of NGF (Fig. 4) or whether NGF caused the neurons to produce specialized proteins such as TH faster than other cellular proteins. If CA production were simply commensurate with overall neuronal growth, total CA/protein and total CA/lipid phosphate should remain constant as a function of NGF concentration. Fig. 6 shows that this was not the case; both ratios increased as the NGF concentration was raised, and saturation occurred by 5 μg/ml. Thus, NGF induced differentiation of the neuronal functions involved in total CA production and did so at a concentration 10-fold higher than that required for either maximal survival or growth.

Another measure of neuronal differentiation in increasing concentrations of NGF was the rate at which the neurons accumulated [^{3}H]DA and [^{3}H]NE synthesized during an 8-h incubation period (accumulated CA). Accumulated CA presumably reflects the ability of the neuronal storage mechanisms to compete with the deaminating mechanisms for newly synthesized CA. Fig. 7 shows that, as with total CA per neuron (Fig. 5), NGF induced a dose-dependent increase in accumulated CA per neuron, and saturation occurred at 2–5 μg/ml 7S NGF. Although both rates, total CA per neuron and accumulated CA per neuron, increased as the NGF concentration rose, newly synthesized CA was stored most efficiently when the NGF concentration was 0.1–0.2 μg/ml as shown in Fig. 8. At higher NGF concentrations, a smaller percentage of synthesized CA was stored. In fact, by 1 μg/ml, <20%

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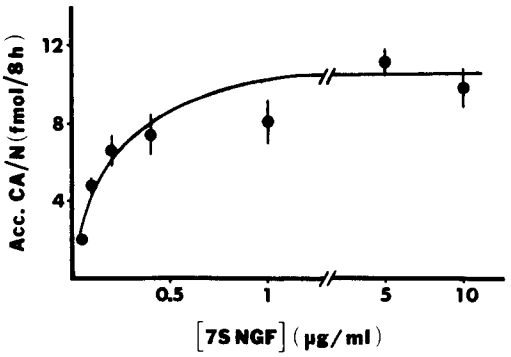

FIGURE 7 After growth for 30 days, cultures were incubated for 8 h with [^{3}H]tyrosine. Radioactivity accumulated (*Acc.*) in the cells as DA and NE was determined (18) and expressed per neuron (*N*).

of the newly synthesized CA was stored in the neurons; the remainder appeared as deaminated metabolites in the medium.

Comparison of 7S and LMW NGF

To determine if the α and γ subunits of 7S NGF directly influence the development of sympathetic neurons, the effectiveness of the 7S and LMW NGF in stimulating neuronal growth and differentiation was studied. The rate of protein synthesis which was taken to reflect neuronal growth was assayed as the radioactive tyrosine accumulated by the cells in an 8-h incubation (accumulated tyrosine), since Mains and Patterson (19) have shown that in these cultures, labeled tyrosine is largely present as protein. In the presence of both forms of NGF, tyrosine accumulation increased four-fold over the concentration ranges studied, as shown in Fig. 9. If the difference in molecular weights of the two forms is taken into account, there was virtually no difference in tyrosine accumulation. Moreover, as shown in Fig. 10, there was little difference in the ratio of accumulated CA to accumulated tyrosine in the presence of the two forms of NGF; in both cases this ratio, a reflection of neuronal differentiation (20), increased about 25-fold. Thus, with respect to the two parameters studied, no significant difference between 7S and LMW NGF was discernible.

DISCUSSION

Cultures of dissociated rat sympathetic neurons offer certain advantages over neurons in vivo for studying the role of NGF quantitatively. Since the cultures are virtually free of other cell types, (*a*) the concentration of NGF to which the cells are exposed is under experimental control, (*b*) any observed effect may be attributed to a direct action of NGF on the neurons themselves, (*c*) influences of non-neuronal cells which may complement or compete with NGF are absent, and (*d*) biochemical analyses are not complicated by non-neuronal cells. Therefore, it is possible to distinguish an effect of NGF on neuronal differentiation from an effect on neuronal survival and growth.

Effect of NGF on Neuronal Survival and Growth

In the absence of exogenous NGF, neurons did not survive for more than 1 day. In 10 ng/ml 7S NGF, the lowest concentration which permitted survival, the surviving neurons were attached to the few non-neuronal cells in the dish. This is consistent with the idea that ganglionic non-neuronal cells can provide NGF-like support, as proposed by Varon et al. (37, 38), and that only those neurons closely applied to a non-neuronal cell obtained enough NGF for survival. As the concentration of 7S NGF was increased, greater numbers of neurons survived until saturation was reached at 0.5 μg/ml 7S NGF. It is interesting to consider why certain cells survived and

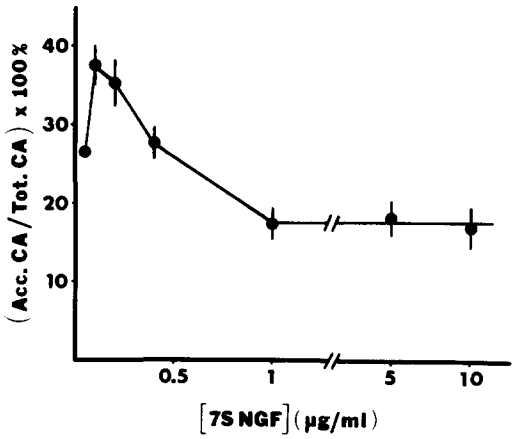

FIGURE 8 Cultures were grown for 30 days in the indicated concentrations of 7S NGF and the fraction of total CA derived from [^{3}H]tyrosine in an 8-h incubation which accumulated in the neurons is plotted as a function of 7S NGF concentration. The data were derived by dividing the value for each culture in Fig. 7 by the value for the same culture in Fig. 5, and the mean was taken for each concentration.

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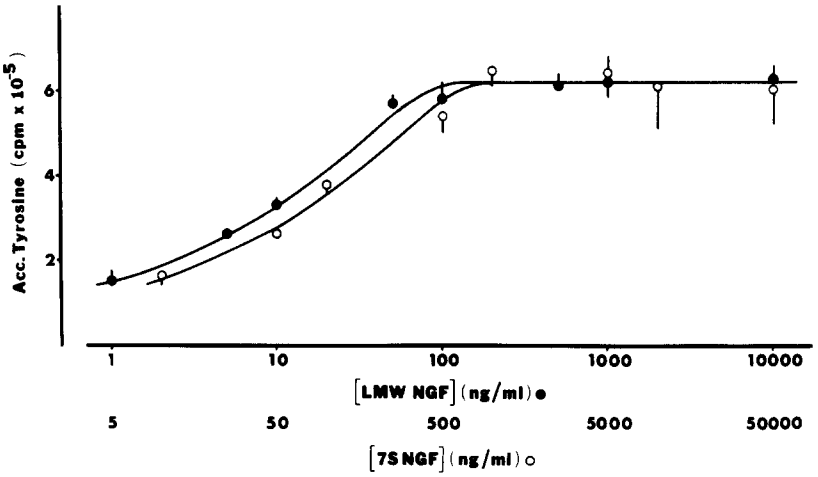

FIGURE 9 Cultures grown in NGF for 21 days were incubated for 8 h with [^{3}H]tyrosine, and the total radioactivity accumulated in the cells was determined and plotted as a function of the concentrations of LMW NGF (●) and 7S NGF (○). In this and Fig. 10, the scales on the abscissa are logarithmic and are adjusted to take into account the five-fold difference in molecular weight between the two protein forms.

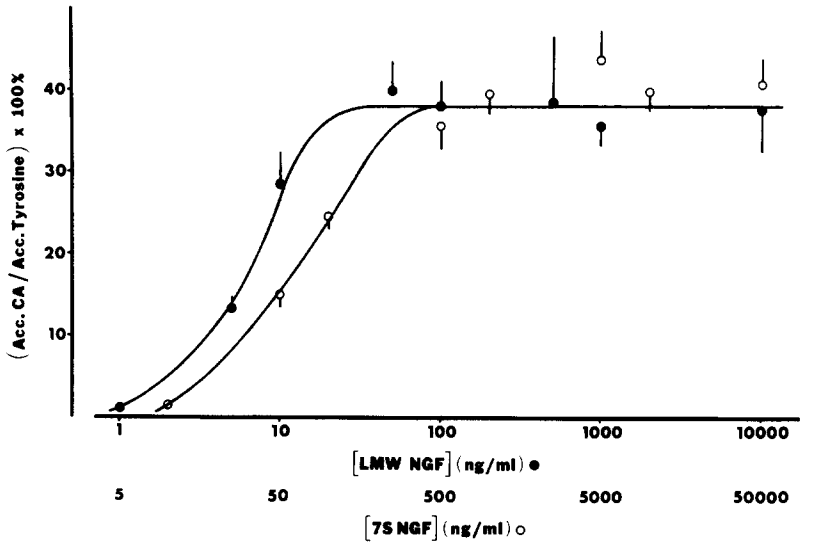

FIGURE 10 Cultures were grown for 21 days in the indicated concentrations of NGF. The fraction of the total radioactivity derived from [^{3}H]tyrosine accumulated in an 8-h incubation which is found in CA is plotted as a function of LMW NGF (●) and 7S NGF (○) concentration. The data were derived by dividing the cpm in accumulated CA for each culture by the cpm in accumulated tyrosine for the same culture, and the mean was taken for each concentration.

others did not at submaximal levels of NGF; perhaps the neurons were at different stages of maturity and had different affinities for NGF (10). In concentrations above 50 μg/ml, some inhibition of neuronal survival was observed. It is not known whether this effect is due to NGF or to an impurity in the protein preparation.

Although the initial formation of a neuronal process may be taken to reflect differentiation, we consider continued extension of a process as neuronal growth. As the NGF concentration was raised, the extension of processes, the soma

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diameter, and the total neuronal protein and lipid phosphate all increased. This is reminiscent of the hypertrophy of sympathetic neurons produced by injection of NGF in vivo (17). Levi-Montalcini and Booker (17) also reported hyperplasia of neurons in vivo, but in our cultures, the number of cell somas did not increase with time (6), nor did neurons incorporate [^{3}H]thymidine when grown with supramaximal levels of NGF (18). In any case, the measured increases in neuronal protein and lipid phosphate were expressed per neuron, using cell counts made on each dish before biochemical assay.

There was no evidence of inhibition of fiber outgrowth at NGF concentrations above saturation either morphologically or as measured by nanogram protein and picomole lipid phosphate. Levi-Montalcini and Angeletti (13) reported that fiber outgrowth from explants decreased in high concentrations of NGF, but they suggested that this apparent inhibition was due to the growth of fibers around the circumference of explants instead of radially outward as observed with lower NGF concentrations (15). However, Hill and Hendry (12) have reported a similar decrease of fiber outgrowth from cultured rat sympathetic ganglia with no evidence of fibrillar capsules around the ganglia. High NGF levels also inhibited fiber growth from dissociated chick sympathetic cells (9).

Effect of NGF on Differentiation of CA Production

By measuring CA synthesized per cell, per nanogram protein, or per picomole lipid phosphate, it was possible to distinguish neuronal differentiation from survival or growth in these neuron-alone cultures. Since both total CA per neuron and accumulated CA per neuron increased as a function of NGF concentration, the increased synthetic rates were not due simply to increased neuronal survival. In addition, although the growth per neuron was sensitive to NGF levels, it did not increase as much as total CA per neuron over the same concentration range. Therefore, the specific activity of TH measured as total CA per nanogram protein was increased by NGF in a dose-dependent fashion; this confirms the conclusions drawn from earlier studies of NGF effects on sympathetic ganglia in vivo (32) and in organ culture (12, 23, 31). Thus, NGF is not only important for survival and growth but also induces sympathetic neuronal differentiation in a dose-dependent fashion.

If one takes into account the five-fold difference in molecular weight between 7S and LMW NGF, the NGF concentration needed to obtain the maximal neuronal survival and growth in our cultures (0.5 μg/ml) was 200-fold higher than that needed to obtain the maximal number of fiber-bearing neurons in cultures of dissociated chick sympathetic ganglia (9). In addition, it is 50-fold greater than the concentration needed to elicit optimal fibrillar outgrowth from explants of chick sensory ganglia (33). In both studies on chick neurons, the cultures were exposed to NGF for <24 h, whereas we assayed fiber outgrowth after 3–4 wk of culture. Hill and Hendry (12) indicated that the concentration of LMW NGF needed for optimal fiber density from rat SCG explants shifts from 10 ng/ml to 100 ng/ml as the ganglia are cultured for longer times; these results suggest that there is a difference in the NGF requirement for fiber growth between short- and long-term cultures (see below).

To obtain maximal neuronal differentiation, 1–5 μg/ml 7S NGF was required in our cultures, a concentration 2- to 10-fold higher than that required to elicit maximal survival and growth. This is reminiscent of the observation that when rat SCG is organ-cultured in 3 μg/ml 7S NGF, the fiber outgrowth and monoamine oxidase (MAO) activity are comparable to those in 0.3 μg/ml, but the TH activity is 3-fold higher (31). High levels of 2.5S NGF (23) and LMW NGF (12) are also required to elicit maximum tyrosine hydroxylase activity in these ganglia. Thus, neurons require higher concentrations of NGF for maximal differentiation of CA production than for either maximal survival or growth.

It is not surprising that survival, growth, and differentiation of CA production in long-term cultures (3–4 weeks) reported here required higher levels of NGF than does survival and fiber outgrowth in short-term cultures (<24 h; 9, 12, 33) since (*a*) at short times the neurons might be influenced by factors which in vivo might lower the concentration of NGF necessary for survival (e.g., glucocorticoids; 23), factors which are lacking at later times in culture, or (*b*) there might be a changing importance of high and low affinity binding sites for NGF. For example, high affinity sites might be associated with the cell soma and related to initial fiber

outgrowth, whereas low affinity sites might be associated with nerve endings and important at later developmental stages. The latter sites might be involved with the retrograde transport of NGF, which has been correlated with the induction of TH (24).

Although both accumulated CA per neuron and total CA per neuron increased as the NGF concentration was increased, with saturation of both parameters at 1–5 μg/ml, the efficiency of accumulation of newly synthesized CA was optimal at 0.1–0.2 μg/ml 7S NGF and decreased at higher NGF levels. This may have been due to an increase in the CA turnover rate at higher NGF concentrations; for example, if MAO activity increased as a function of NGF, CA would have been deaminated at a faster rate, since vesicular and cytoplasmic CA exchange rapidly (see reference 27). Alternatively, if NGF induced the neurons to synthesize CA faster than they could store it, the cytoplasmic CA would have been deaminated and excreted. A better understanding of the decreased efficiency for storage of newly synthesized CA will require vesicle counts and measurements of endogenous CA at different NGF concentrations.

Comparison of 7S and LMW NGF

Although NGF can be isolated from submaxillary glands as a high molecular weight complex, the biological significance of this complex is not understood. It has been suggested that the complex serves to protect the β-subunit from proteolytic degradation by salivary gland enzymes (22). To determine whether the α and γ subunits act directly on the development of sympathetic neurons, the effects of 7S NGF were compared to those of the LMW form. No significant differences between the two forms were found with respect to stimulation of neuronal growth or differentiation. Recent work by Young et al. (40) suggests that 7S NGF dissociates in dilute solution. However, Pattison and Dunn (28) reported that Zn^{++} is important for maintaining the integrity of the 7S complex. Since no attempt was made to include Zn^{++} in our experiments, it is possible that the 7S complex dissociated at the concentrations used. However, whether the α and γ subunits were complexed or dissociated, they exerted no detectable influence on neuronal growth or differentiation in this study.

Mains and Patterson (20) have shown that cultured SCG neurons increase their capacity to synthesize and accumulate CA during a 3-wk period, after which a steady-state level is achieved. The effects of NGF reported here were studied at that 3- to 4-wk-old stage. The present results raised the question whether the smaller cell size and lower CA production at submaximal NGF concentrations represent a stable state or whether the neurons grown in low NGF will reach a large size and fully differentiated state along a slower time-course. This question is considered in the next paper of this series (6). It is also of interest to know whether synthesis and accumulation of acetylcholine by these neurons, when they are grown in a medium permissive for cholinergic functions (25–27) is dependent on the level of NGF as is the synthesis and accumulation of CA; this is the subject of the third paper of this series (7).

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