Selective de Novo Synthesis of Tyrosine Hydroxylase in Organ Cultures of Rat Superior Cervical Ganglia after in Vivo Administration of Nerve Growth Factor

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Tyrosine hydroxylase is synthesized de novo in rat superior cervical ganglia in organ culture. The differential rate of synthesis is not increased significantly by the addition of nerve growth factor to the culture. Prior administration of nerve growth factor in vivo, however, leads to an augmented synthesis of tyrosine hydroxylase in ganglia subsequently cultured in vitro. The differential rate of tyrosine hydroxylase synthesis was increased by a factor of between 3 and 4. Increases in the differential rate of synthesis were detected within 6 h; the rate reached a maximum 24 to 36 h after a single injection of nerve growth factor. Administration of actinomycin D or of nerve growth factor antibody in vivo prevented the nerve growth factor-induced increase in the differential rate of tyrosine hydroxylase synthesis in vitro. However, the increase in the synthetic rate of tyrosine hydroxylase was not prevented by the addition of actinomycin D to the culture.

Nerve growth factor, a protein isolated from the submaxillary gland of the male mouse, causes hyper trophy and hyperplasia of the superior cervical ganglia when administered repeatedly to young rats or mice (1, 2). The accelerated growth of the ganglia is accompanied by increases in the total activities of the neuronal enzymes involved in the synthesis of noradrenaline. Of more significance for studies on the mechanism of action of NGF, however, is the observation that the activities of tyrosine hydroxylase and dopamine β-hydroxylase are selectively elevated by NGF treatment, i.e. there is a substantial increase in the specific as well as in the total activities of these two enzymes in vivo (3).

Only small increases in the specific activity of tyrosine hydroxylase have been observed in vitro when ganglia are maintained in the presence of NGF in a chemically defined organ culture system for 24 to 48 h (4). The total and specific activities of tyrosine hydroxylase are not increased to the same extent as that observed after the administration of NGF in vivo (5). The results obtained by measuring the de novo synthesis of tyrosine hydroxylase in organ culture (5) are consistent with those obtained by measuring enzyme activity changes, i.e. there is an increase in the total synthesis of tyrosine hydroxylase and of soluble protein in response to the presence of NGF, but at best only a small increase in the differential rate of tyrosine hydroxylase synthesis. It seems reasonable to conclude that the presence of NGF in vitro is not sufficient to initiate the subcellular events that precede the induction of tyrosine hydroxylase.

In order to obtain a system with which the effect of nerve growth factor on tyrosine hydroxylase synthesis could be studied, we have investigated the effect of the administration of NGF in vivo on the subsequent in vitro tyrosine hydroxylase synthesis. The results of such studies show that the administration of NGF as little as 6 h prior to sacrifice markedly increases the differential rate of tyrosine hydroxylase synthesis measured in superior cervical ganglia in organ culture. The characteristics of this induction are the subject of the present manuscript.

EXPERIMENTAL PROCEDURES

Methods - Young rats (1 to 6 days old) of the Sprague-Dawley strain (Zivic-Miller, Allison Park, Pa.) were injected subcutaneously with NGF or reconstituted NGF antiserum at concentrations of 10 μg/g and 4 mg/g of body weight, respectively. Actinomycin D (dissolved in 15% ethanol) was injected intraperitoneally at a concentration of 1 μg/g of body weight 1 h before the NGF injection. Hydrocortisone acetate (50% ethanol) and insulin (dissolved in 2.7 mM HCl) were injected subcutaneously at concentrations of 3.6 μg/g and 5 μg/g of body weight, respectively.

At various times after injection the rats were killed by a blow to the head. The superior cervical ganglia were removed and decapsulated by blunt dissection with the aid of a binocular microscope. The ganglia were cultured in 0.35 ml of BGJ media, Fitton-Jackson modification, without phenol red (Grand Island Biological Co.). The medium was supplemented with 0.1% bovine serum albumin, Fraction V, and an antibiotic-antimycotic mixture which included penicillin, streptomycin, and fungizone at 100 units/ml, 100 μg/ml, and 25 μg/ml, respectively. Actinomycin D (0.2 μg/ml) and 2.5 S NGF (1 μg/ml) were incubated with the ganglia for 1 h before the addition of either 250 or 750 μCi of [3H]leucine or 50 μCi of [3H]uridine.

Tissues were maintained at 37°C in tissue culture clusters in a humidified atmosphere of 95% oxygen, 5% carbon dioxide for 20 to 24 h, except where noted in the tables. Tissues were removed from culture and rinsed in 0.25 M sucrose. Pairs of ganglia were then homogenized in ground glass homogenizers with 400 μl of 5 mM Tris buffer, pH 7.4, containing 0.1% Triton X-100 and centrifuged at 20,000 x g for 20 min. The supernatant fluids were used for tyrosine hydroxylase assay, immunoprecipitation of newly synthesized, radioactive tyrosine hydroxylase, and estimation of [3H]leucine incorporation into soluble protein.

For the measurement of total ribonucleic acid synthesis, portions of the homogenate were treated with 1 ml of 10% trichloroacetic acid; to measure the synthesis of soluble protein 500 μg of bovine serum...
albumin and 1 ml of 10% trichloroacetic acid were added to portions of the 20,000 × g supernatant fraction. In both cases, the samples were kept at 0° for 30 min and then filtered in a Yeda filtering apparatus over GF/C glass fiber paper (Whatman). The filters were washed five times with 0.5 ml of 5% trichloroacetic acid and dried. The precipitates were solubilized by heating the filters at 100° for 1 h in 1 ml of 1% sodium dodecyl sulfate and were counted in Aquasol. The efficiency of 12H counting was 26 to 30% in all experiments.

NGF (2.5 S) was prepared by the method of Bocchini and Angeletti (6) and stored at a concentration of 0.7 mg/ml at -20°C. Antiserum to NGF was raised in sheep as previously described (7). Protein was measured by the method of Lowry et al. (8), using bovine serum albumin fraction V as standard.

Monospecific tyrosine hydroxylase antibody was produced in sheep by repeated injections of a tyrosine hydroxylase fragment purified from a chymotryptic digest of bovine adrenal medulla chromaffin granules (9). The γ-globulin fraction of the immune serum was obtained by precipitation at 40% of saturation with ammonium sulfate, and was diluted to the original serum volume with 20 mM potassium phosphate buffer, pH 6.8, containing 0.3 mM glycine. Prior to use the serum was centrifuged at 12,000 × g for 4 min (Brinkmann microfuge) to remove insoluble protein.

Immunoprecipitation of the newly synthesized, radioactive tyrosine hydroxylase was performed in a volume of 1.0 ml in the presence of 1% Triton X-100, 1% freshly prepared sodium deoxycholate, 200 μl of supernatant fluid from the ganglia homogenate, and sufficient carrier tyrosine hydroxylase from the 150,000 × g supernatant fluid of rat adrenal glands to adjust the final concentration of tyrosine hydroxylase in the reaction mixtures to 2.5 units. Enough tyrosine hydroxylase antiserum was added to precipitate quantitatively twice this amount of enzyme. Samples were incubated at 30°C for 1 h and then at 4°C for 16 h. The antigen-antibody precipitates were collected using a modification of the procedures of Rbohsa et al. (10) and of Sippel et al. (11) as follows. The samples were layered onto a 0.5-ml sucrose cushion (1 M sucrose, 150 mM NaCl, 1% Triton X-100, 10 mM sodium phosphate, pH 7.0) and centrifuged at 16,000 × g for 15 min in an HR-4 Sorvall rotor. The top of the sucrose cushion was gently rinsed twice with phosphate-buffered saline (10 mM NaPO₄, pH 7.0, containing 140 mM NaCl); the pellet itself was then washed twice to three times with phosphate-buffered saline, each time being vigorously blended on a Vortex mixer and microfuged for 4 min. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed using 7.5% gels by the procedure of Weber et al. (12). The antigen-antibody precipitates were solubilized by boiling for 15 min in 100 μl of a solution containing 1% sodium dodecyl sulfate, 1% 2-mercaptoethanol, 0.009% bromphenol blue tracking dye, and 50% glycerol. Electrophoresis was carried out for 5 h at 8 mA/gel. Gels were sliced into 1.2-mm sections and the slices were dissolved by heating at 55°C overnight in 1 ml portions of hydrogen peroxide containing 5% ammonium hydroxide. The samples were counted and tyrosine hydroxylase synthesis was quantitated by determining the counts in the slices representing the tyrosine hydroxylase peak.

The activity of tyrosine hydroxylase was determined by measuring the formation of 3H10 from L-[3,5-3H]tyrosine as described by Nagatsu et al. (13) and modified by Oesch et al. (14). A unit is that amount of enzyme which will produce 1 nmol of 3H10 at 30°C.

Materials—L-[4,5-3H]Leucine (specific activity: 57.4 Ci/mmol), [5-3H]jaridine (specific activity: 28.7 Ci/mmol), and L-[3,5-3H]tyrosine (specific activity: 80.3 Ci/mmol) were obtained from New England Nuclear Corp. Actinomycin D, hydrocortisone acetate, and insulin were purchased from Sigma Chemical Co. The culture media and the antibiotic-antimycotic mixture were purchased from Grand Island Biological Co.; tissue culture clusters were obtained from Microbiological Associates.

RESULTS

Superior cervical ganglia in organ culture are able to synthesize tyrosine hydroxylase de novo. The sodium dodecyl sulfate-polyacrylamide gel electrophoretic profile of immunologically precipitated tyrosine hydroxylase from ganglia cultured in the presence of [3H]leucine shows one major peak and the protein represented by that peak has an apparent molecular weight of approximately 60,000 to 65,000 (5). Repeated injection of 2.5 S nerve growth factor markedly increases the total amount of tyrosine hydroxylase that is subsequently synthesized in vitro when compared to control ganglia cultured under similar conditions. In addition to the increase in the total tyrosine hydroxylase synthesis caused by prior administration of NGF, the differential rate of tyrosine hydroxylase synthesis is increased 3- to 4-fold (Table I). This is accompanied by a substantial increase in the activity of the enzyme. It should be noted that NGF is present in the culture medium of both experimental and control ganglia in order to avoid the possibility of variations in the survival of sympathetic neurons in vitro.

The increases in enzyme activity and enzyme biosynthesis are abolished when actinomycin D is administered 1 h before NGF in vivo (Table I). However, once the animals have been treated with NGF, the subsequent synthesis of tyrosine hydroxylase in vitro is not decreased by the presence of actinomycin D in culture. There may even be some increase in the differential rate of tyrosine hydroxylase synthesis, as well as a slight increase in the activity of tyrosine hydroxylase under these conditions. In control experiments it was shown that 0.2 μg of actinomycin D/ml of medium inhibits the incorporation of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>In vivo</th>
<th>In vitro</th>
<th>Soluble protein (μg/ganglion)</th>
<th>Tyrosine hydroxylase-Specific activity (units/mg protein)</th>
<th>Soluble proteins (cpm × 10^-4/mg protein)</th>
<th>Tyrosine hydroxylase synthesis (μg/ganglion)</th>
<th>Differential rate of tyrosine hydroxylase synthesis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>NGF</td>
<td>27.9 ± 4.1</td>
<td>7.32 ± 1.68</td>
<td>184,965 ± 13,438</td>
<td>203 ± 59</td>
<td>0.11 ± 0.03</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>NGF</td>
<td>32.6 ± 7.3</td>
<td>16.6 ± 1.72</td>
<td>140,702 ± 1,223</td>
<td>588 ± 86</td>
<td>0.42 ± 0.06</td>
<td>0.21 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>NGF + actinomycin D</td>
<td>21.7 ± 3.4</td>
<td>6.43 ± 1.57</td>
<td>153,628 ± 4,951</td>
<td>227 ± 56</td>
<td>0.15 ± 0.03</td>
<td>0.31 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>NGF + actinomycin D</td>
<td>29.0 ± 4.4</td>
<td>17.47 ± 1.26</td>
<td>113,069 ± 2,230</td>
<td>708 ± 71</td>
<td>0.63 ± 0.07</td>
<td>0.73 ± 0.08</td>
<td></td>
</tr>
</tbody>
</table>
of tritiated uridine into ribonucleic acid in the ganglia by over 90%.

The elevation in the in vitro synthesis of tyrosine hydroxylase produced by NGF administration in vivo is specific to NGF. Neither the administration of insulin nor of hydrocortisone to young rats, nor the addition of these hormones to the cultures had any effect on the rate of tyrosine hydroxylase synthesis in vitro. The administration of NGF antiserum to rats 24 h before sacrifice virtually prevented the in vitro synthesis of tyrosine hydroxylase while inhibiting synthesis of supernatant protein in the ganglia by less than 35% and lowering tyrosine hydroxylase activity by less than 50%.

It was of interest to attempt to find the maximal rate of tyrosine hydroxylase synthesis. For this purpose newborn rats were injected with NGF each day for 3 days (Table II). The specific activity of tyrosine hydroxylase was substantially higher than that found after a single administration of NGF. The differential rate of the synthesis of tyrosine hydroxylase in cultured ganglia from these animals, however, was only slightly higher than after a single injection.

The effect of nerve growth factor in elevating the synthesis of tyrosine hydroxylase was evident as early as 6 h after it was administered to the rats (Table III). A small increase in tyrosine hydroxylase activity and some increase in total protein synthesis was also observed at that time. The differential rate of tyrosine hydroxylase synthesis reached a maximum 24 to 36 h after NGF injection. It appears that the increase in the rate of tyrosine hydroxylase synthesis begins with no substantial lag after the administration of NGF.

Since, in most of the present studies, the ganglia were exposed to nerve growth factor, in vivo and in vitro, for a total of almost 48 h, it seemed appropriate to inquire whether a 48-h exposure in vitro would produce the same increase in the selective synthesis of tyrosine hydroxylase. As seen in Table IV, even long term exposure to NGF under culture conditions did not result in an increase in the differential rate of tyrosine hydroxylase synthesis. The synthesis of tyrosine hydroxylase was the same in the presence or the absence of NGF for the first 22 h of culture. If the ganglia were kept in culture with NGF for 44 h and [3H]leucine added for the last 22 h, the rate of synthesis observed was the same as that seen in the first 22 h. In the absence of NGF the rate of tyrosine hydroxylase synthesis declined after the first 22 h in culture.

Although it appears likely that nerve growth factor is, in fact, inducing increased synthesis of tyrosine hydroxylase, other explanations are possible. In order to rule out decreased

### Table II

**Effect of repeated administration of nerve growth factor on the in vitro synthesis of tyrosine hydroxylase**

Conditions were the same as in Table I except that newborn rats were used. The animals were injected with 2.5 S NGF (10 μg/g of body weight) every day for 3 days and killed 24 h after the last injection.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>In vivo</th>
<th>In vitro</th>
<th>Soluble protein</th>
<th>Tyrosine hydroxylase activity</th>
<th>Tyrosine hydroxylase specific activity</th>
<th>Radioactivity in</th>
<th>Differential rate of tyrosine hydroxylase synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>μg/ganglion</td>
<td>unit/mg protein</td>
<td>cpm x 10^-14/mg protein</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>NGF</td>
<td>27.7 ± 5.3 (6)</td>
<td>0.167 ± 0.062 (3)</td>
<td>6.18 ± 2.61 (3)</td>
<td>2.217 ± 411 (3)</td>
<td>2.75 ± 0.33 (3)</td>
<td>0.13 ± 0.02 (3)</td>
</tr>
<tr>
<td>NGF</td>
<td>49.2 ± 8.4 (5)</td>
<td>1.063 ± 1.587 (3)</td>
<td>12.76 ± 514 (3)</td>
<td>17.10 ± 3.50 (3)</td>
<td>0.50 ± 0.03 (3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table III

**Time course of action of NGF in vivo on the in vitro synthesis of tyrosine hydroxylase**

Animals were injected with vehicle or with NGF (10 μg/g of body weight). Ganglia were cultured simultaneously for 22 h in media containing NGF (1 μg/ml) and 250 μCi of [3H]leucine as described under "Methods." Enzyme activity was determined in separate cultures from which [3H]leucine had been omitted. Other conditions were as specified in Table I.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>In vivo</th>
<th>In vitro</th>
<th>Soluble protein</th>
<th>Tyrosine hydroxylase specific activity</th>
<th>Radioactivity in</th>
<th>Differential rate of tyrosine hydroxylase synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>μg/ganglion</td>
<td>unit/mg protein</td>
<td>cpm x 10^-14/mg protein</td>
<td>%</td>
</tr>
<tr>
<td>Vehicle</td>
<td>NGF</td>
<td>28.3 ± 4.9 (13)</td>
<td>4.83 ± 1.43 (9)</td>
<td>114,517 ± 13,874 (3)</td>
<td>166 ± 25 (3)</td>
<td>0.15 ± 0.01 (3)</td>
</tr>
<tr>
<td>6-h NGF</td>
<td>NGF</td>
<td>23.7 ± 3.6 (9)</td>
<td>7.88 ± 0.70 (3)</td>
<td>168,624 ± 8,575 (3)</td>
<td>346 ± 33 (3)</td>
<td>0.21 ± 0.03 (3)</td>
</tr>
<tr>
<td>12-h NGF</td>
<td>NGF</td>
<td>30.4 ± 3.4 (8)</td>
<td>8.42 ± 0.60 (6)</td>
<td>162,928 ± 21,680 (3)</td>
<td>396 ± 54 (3)</td>
<td>0.24 ± 0.01 (3)</td>
</tr>
<tr>
<td>24-h NGF</td>
<td>NGF</td>
<td>38.4 ± 2.6 (7)</td>
<td>13.27 ± 0.35 (3)</td>
<td>146,075 ± 8,491 (3)</td>
<td>496 ± 60 (3)</td>
<td>0.32 ± 0.06 (3)</td>
</tr>
<tr>
<td>36-h NGF</td>
<td>NGF</td>
<td>36.4 ± 3.8 (6)</td>
<td>11.96 ± 1.60 (3)</td>
<td>118,146 ± 14,362 (3)</td>
<td>462 ± 68 (3)</td>
<td>0.39 ± 0.02 (3)</td>
</tr>
</tbody>
</table>

### Table IV

**Effect of nerve growth factor in vitro on tyrosine hydroxylase synthesis**

Groups of ganglia from 5-day-old rats were cultured for either 22 h or 44 h in the presence or absence of NGF. Isotope (250 μCi of [3H]leucine) was present for the full 22 h in the shorter cultures or for the last 22 h of a 44 h culture. Other conditions were as described in Table I.

<table>
<thead>
<tr>
<th>NGF</th>
<th>Length of culture</th>
<th>3H pulse time</th>
<th>Soluble protein</th>
<th>Radioactivity in</th>
<th>Differential rate of tyrosine hydroxylase synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h</td>
<td>h</td>
<td>μg/ganglion</td>
<td>cpm x 10^-14/mg protein</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>0-22</td>
<td>28.4 ± 3.5 (5)</td>
<td>113,357 ± 21,588 (4)</td>
<td>163 ± 10 (4)</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>23-44</td>
<td>31.9 ± 2.7 (3)</td>
<td>108,512 ± 9,335 (3)</td>
<td>47 ± 6 (3)</td>
</tr>
<tr>
<td>+</td>
<td>22</td>
<td>0-22</td>
<td>36.1 ± 6.4 (12)</td>
<td>111,540 ± 9,683 (6)</td>
<td>158 ± 28 (5)</td>
</tr>
<tr>
<td>+</td>
<td>44</td>
<td>23-44</td>
<td>47.0 ± 2.0 (3)</td>
<td>138,211 ± 20,597 (3)</td>
<td>226 ± 56 (3)</td>
</tr>
</tbody>
</table>
Induction of Tyrosine Hydroxylase Synthesis by Nerve Growth Factor

TABLE V
Effect of in vivo administration of nerve growth factor on the in vitro synthesis of tyrosine hydroxylase in 3-h cultures

Animals were injected with vehicle or with NGF (10 μg/g body weight) and killed 24 h later. Ganglia were cultured for 3 h with 750 μCi of [3H]leucine and NGF (1 μg/ml). Other conditions are as described in Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soluble protein (μg/gan-glion)</th>
<th>Radioactivity (cpm × 10^4/mg protein)</th>
<th>Differential rate of tyrosine hydroxylase synthesis (μg/mg protein) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>28.4 ± 2.0</td>
<td>60,116 ± 2,962</td>
<td>32 ± 7 0.05 ± 0.01</td>
</tr>
<tr>
<td>NGF</td>
<td>30.6 ± 5.5</td>
<td>58,622 ± 5,196</td>
<td>38 ± 3.5 0.15 ± 0.03</td>
</tr>
</tbody>
</table>

The protocol adopted for these experiments is one approach to the investigation of the effect of nerve growth factor on tyrosine hydroxylase synthesis. The use of organ culture conditions for the actual measurement of tyrosine hydroxylase synthesis appears mandatory. A more direct approach, i.e. the in vitro administration of radioactive amino acid, is, for all practical purposes, precluded since the size of the ganglia does not permit the incorporation of significant radioactivity into tyrosine hydroxylase. The alternate approach, that of incubating NGF with ganglia in vitro, is not acceptable either since it is shown (5) that NGF alone does not induce tyrosine hydroxylase in vitro to the same extent that it does in vivo. Thus, the present protocol seems the only currently available strategy.

The data resulting from this study are consistent with that presented by Thoenen et al. (3) who showed that NGF administration produces a selective increase in tyrosine hydroxylase activity. The present work shows that NGF administration produces a selective increase in tyrosine hydroxylase synthesis. The further observation that enhanced in vitro synthesis of tyrosine hydroxylase proceeds even in the absence of the synthesis of ribonucleic acid suggests that the de novo formation of the enzyme in culture is dependent only upon the relative concentration, in the ganglia, of preformed tyrosine hydroxylase templates. Thus, this work indicates that NGF in vivo induces the selective synthesis of tyrosine hydroxylase by increasing the selective accumulation of tyrosine hydroxylase messenger RNA. The maximum differential rate of such synthesis appears to be about 0.50%, and cannot be raised even with repeated administration of NGF.

The above data, then, suggests a role for DNA-directed RNA synthesis in the regulation of tyrosine hydroxylase synthesis by NGF in vivo. If messenger RNA synthesis is necessary for tyrosine hydroxylase induction then the data also show that within 6 h after NGF treatment in vivo the synthesis of tyrosine hydroxylase messenger RNA is specifically elevated above the synthesis of other mRNAs. Indeed, an inspection of the time course after NGF treatment shows that the increase in tyrosine hydroxylase synthesis appears without a perceptible lag, although a lag of a few hours might not be apparent in these experiments.

That the increased incorporation into tyrosine hydroxylase is actually a reflection of increased synthesis is shown by the short term cultures. The increase in the differential rate of tyrosine hydroxylase synthesis due to NGF pretreatment during the first 3 h of the culture is as great as that seen in 22-h cultures. If the increased radioactivity were due to a decreased proteolytic destruction of tyrosine hydroxylase, for example, it would seem that the observed increase in the differential rate of synthesis should not be seen, or at least not be of the same magnitude, during a shorter incubation.

Overall, a comparison of the present study with our previous one (5) strongly suggests that the mechanism of NGF action on tyrosine hydroxylase synthesis in vitro is different than it is in vivo. The earlier study showed that NGF in culture did not enhance the differential rate of tyrosine hydroxylase synthesis to any great extent. We concluded that the major action of NGF in vitro was to allow neuronal survival. It should be noted that NGF was added to all the cultures in the present work, making differential neuronal survival an unlikely explanation of the present data.

If the mechanism of action of NGF in vitro is different than that in vivo, the current system can be used to try to uncover the biochemical bases of the difference. If some permissive factor intervenes between NGF and the synthesis of tyrosine hydroxylase messenger RNA, the elucidation of such a relationship could illuminate the basic mechanism of NGF action.

Note Added in Proof—It has recently been demonstrated (Brain Res. (1976) 111, 438–441) that dexamethasone modulates the NGF-mediated increase in the specific activity of tyrosine hydroxylase in organ cultures of rat superior cervical ganglia. We have further shown (Biochem. Biophys. Res. Commun. (1977) 75, 822–837) that dexamethasone in combination with NGF in vitro causes an increase in tyrosine hydroxylase synthesis that approaches that obtained by the in vivo administration of NGF.

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Induction of Tyrosine Hydroxylase Synthesis by Nerve Growth Factor

(1951) J. Biol. Chem. 193, 265-275

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