Nerve growth factor increases activity of ornithine decarboxylase in rat brain

(cranial nervous system/induction in glial cells/maturatation)

MICHAEL E. LEWIS, J. LAKSHMANAN, K. NAGAIAH, PAUL C. MACDONNELL, AND GORDON GUROFF

Section on Intermediary Metabolism, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014

Communicated by Elizabeth F. Neufeld, November 30, 1977

ABSTRACT Intraventricular administration of nanogram quantities of nerve growth factor to adult rats results in a marked increase in the activity of ornithine decarboxylase (L-ornithine carboxylase, EC 4.1.1.17) in the brain. The increase occurs in all major brain regions and the activity is maximal by 7.5 hr after administration. The enzyme response to nerve growth factor increases in magnitude during maturation; the relative increase in ornithine decarboxylase activity in adult animals is much greater than that in young. Neither insulin nor bovine growth hormone was able to increase ornithine decarboxylase activity to the same extent as did nerve growth factor. When brain was separated into neuronal- and glial-enriched fractions, induction of ornithine decarboxylase was found in both, but a greater increase was observed in the glial fraction.

Nerve growth factor (NGF) is a hormone-like protein that is necessary for the development and maintenance of peripheral sympathetic neurons (1). Some of the effects of NGF may be due to its ability to initiate the sequential biochemical events thought to participate in a trophic response (2). These include an increase in the intracellular level of cyclic AMP (3), an activation and translocation of the cytoplasmic protein kinase (M. W. Yu, N. Tolson, and G. Guroff, unpublished data), an induction of ornithine decarboxylase (L-ornithine carboxylase, EC 4.1.1.17) (4), and an increase in the activity of the RNA polymerases (K. Huff, J. Lakshmanan, and G. Guroff, unpublished data). Ornithine decarboxylase catalyzes the rate-limiting step in the biosynthesis of the polyamines, compounds extensively implicated in the regulation of cellular metabolism and growth (5), and also appears to activate RNA polymerase I (6).

The mature central nervous system is also sensitive to NGF. This has been demonstrated in studies of the regrowth of transected monoaminergic axons (7, 8), and in experiments designed to measure behavioral recovery after brain damage (9). It seemed reasonable to ask if NGF could initiate the biochemical components of a trophic response in the mature central nervous system.

In this report we present evidence that intraventricular administration of nanogram quantities of NGF to adult rats is followed by a marked increase in brain ornithine decarboxylase activity. In addition, we report that this response occurs in all major brain areas, is maximal at about 7 hr after administration, increases in magnitude with maturation, and is specific for NGF. Finally, fractions enriched with glia show a greater induction than those enriched with neuronal cell bodies.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Adult male rats and preweanling rats of either sex (Zivic—Miller, Allison Park, PA) were given bilateral injections of 2.5S NGF, NGF vehicle (0.05 M sodium acetate, pH 5), insulin, or bovine growth hormone in a total volume of 10 μl into the lateral ventricles. In adult rats, the injections were made 1.5 mm lateral to bregma and 3.7 mm ventral from the skull surface. In young rats, the injection sites were 1.5 mm lateral to bregma and 3.0 mm ventral from the skull surface. Prior to the experiments, dye was injected at these coordinates to verify the effective spread of the injected material through the ventricular system. The animals were decapitated at various times after the experimental injections, and the brains were frozen whole or after dissection into the following regions (10): limbic forebrain, striatum, rest of the hemispheres (including hippocampus), dienecephalon, brainstem, and cerebellum. Neuronal bodies and glia were separated as described by Sellinger et al. (11). The tissues were prepared as 20% homogenates with a buffer solution containing 50 mM Tris-HCl at pH 7.5, 5 mM dithiothreitol, and 40 μM pyridoxal phosphate. The homogenates were centrifuged at 27,000 × g for 15 min, and the supernatant fractions were used for enzyme assay.

The activity of ornithine decarboxylase was determined by measuring the formation of 14CO2 from DL-[1-14C]ornithine monohydrochloride essentially as described by Oka and Perry (12) and modified by MacDonnell et al. (4). Samples (250 μl) of the supernatant fraction were incubated for 1 hr at 37°C in a total volume of 0.5 ml in rubber-stoppered 15-× 100-mm glass tubes fitted with a hanging center well that contained 0.2 ml of Hyamine hydroxide. The reaction mixture contained 75 mM Tris-HCl at pH 7.5, 75 mM dithiothreitol, 60 μM pyridoxal phosphate, 6 mM EDTA, and 111 μM DL-[1-14C]ornithine monohydrochloride (2.5 μCi). After the reaction was terminated with 0.5 ml of 2.5 M H2SO4, the samples were allowed to stand at room temperature overnight. The overnight incubation ensures the complete liberation of 14CO2 when the reactions are performed in 15-× 100-mm tubes. The center wells of the reaction tubes were removed and the radioactivity of the trapped 14CO2 was measured in Liquifluor.

DL-[1-14C]Ornithine monohydrochloride (specific activity: 45 mCi/mmol), Liquifluor, and Hyamine hydroxide were purchased from New England Nuclear Corp. Pyridoxal phosphate and dithiothreitol were obtained from Calbiochem. Bovine growth hormone (NIH-GH-B17) was generously supplied by Martin Rodbell. Nerve growth factor was prepared in the 2.5S form according to the method of Bocchini and Angeletti (13). All other chemicals were obtained from Sigma Chemical Co.

Abbreviation: NGF, nerve growth factor.
Table 1. Regional distribution of ornithine decarboxylase induction in adult rat brain

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Ornithine decarboxylase activity, nmol/hr per g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Limbic forebrain</td>
<td>0.99 ± 0.13</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.71 ± 0.10</td>
</tr>
<tr>
<td>Cerebral hemispheres</td>
<td>0.98 ± 0.14</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.84 ± 0.29</td>
</tr>
<tr>
<td>Diencephalon</td>
<td>1.45 ± 0.23</td>
</tr>
<tr>
<td>Brainstem</td>
<td>1.56 ± 0.17</td>
</tr>
</tbody>
</table>

Adult male rats were injected intraventricularly with 2.3 μg of NGF in 10 μl of 0.05 M acetate, pH 5.0, or with acetate buffer alone. The animals were killed 4.5 hr later and ornithine decarboxylase activity was measured. Values represent the mean ± SEM for four animals in each group.

RESULTS

The effect of intraventricular administration of NGF on the activity of ornithine decarboxylase in various brain regions of adult rats is shown in Table 1. Four hours after the injection of NGF, the activity was increased in all regions, although the extent of response varied. Because the increase in activity was not specific to a particular brain region, further characterization of the enzyme response was carried out with whole brains.

The time course of the increase in ornithine decarboxylase activity after NFG administration was then examined (Fig. 1). The activity was maximal 7.5 hr after administration, and had returned to baseline levels within 18 hr. At 7.5 hr, the activity of ornithine decarboxylase in NFG-treated brains was increased at least 7-fold. No increase was seen in brains from vehicle-treated animals compared to brains from untreated animals.

The ontogeny of the enzymatic response to NGF was determined (Table 2). As previous investigators have shown, the activity of brain ornithine decarboxylase falls sharply during maturation (14, 15). As the baseline level of the enzyme approaches adult levels, NGF becomes increasingly effective in stimulating activity.

The specificity of the action of NGF was explored by determining the effects of two other trophic hormones, insulin and growth hormone, on adult brain ornithine decarboxylase activity (Table 3). Although bovine growth hormone has been reported to stimulate ornithine decarboxylase activity in the brain of immature rats (15), its effect in adult brain under the present conditions is small compared to that of NGF. Insulin, a hormone with some structural similarity to NGF (16) and capable of stimulating ornithine decarboxylase activity in liver (17) and cultured mammary tissue (18), was ineffective in adult brain. In other experiments, not reported here, it has been shown that comparable doses of cytochrome c, bovine serum albumin, glucagon, or thyroxine had no effect on ornithine decarboxylase activity in brain. As little as 468 ng of NGF gave a maximal response. A partial response was obtained with 234 ng and no response was seen upon injection of 46- or 23-ng doses.

In order to determine which cells in the brain are stimulated by NGF, brains were fractionated into neuronal-enriched and glial-enriched populations. As shown in Fig. 2, both fractions showed a substantial induction of ornithine decarboxylase, but the glial-enriched fraction was more responsive to NGF.

DISCUSSION

The present results show that intraventricular administration of nanogram quantities of NGF to adult rats caused a marked

Table 2. Induction of ornithine decarboxylase during development

<table>
<thead>
<tr>
<th>Age, days</th>
<th>Ornithine decarboxylase activity, nmol/hr per g tissue</th>
<th>NGF as % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>19th fetal</td>
<td>31.52 ± 2.61 (4)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12.27 ± 0.95 (4)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.58 ± 0.13 (3)</td>
<td>178</td>
</tr>
<tr>
<td>8</td>
<td>5.42 ± 0.30 (13)</td>
<td>149</td>
</tr>
<tr>
<td>12</td>
<td>1.55 ± 0.11 (6)</td>
<td>185</td>
</tr>
<tr>
<td>16</td>
<td>0.42 ± 0.06 (3)</td>
<td>661</td>
</tr>
<tr>
<td>20</td>
<td>0.28 ± 0.03 (9)</td>
<td>1835</td>
</tr>
<tr>
<td>90</td>
<td>0.58 ± 0.09 (17)</td>
<td>1507</td>
</tr>
</tbody>
</table>

Adult male rats and preweaning (<21 days old) rats of both sexes were injected intraventricularly with 2.3 μg of NGF in 0.05 M acetate, pH 5.0, and were killed 7 hr later. Control animals were injected with acetate buffer only and killed 7 hr later along with the experiments. Values represent the mean ± SEM, with the number of animals used given in parentheses.

Table 3. Specificity of the effect of nerve growth factor on ornithine decarboxylase induction in adult rat brain

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ornithine decarboxylase activity, nmol/hr per g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.62 ± 0.10 (9)</td>
</tr>
<tr>
<td>NGF (0.468 μg)</td>
<td>9.60 ± 2.38 (3)</td>
</tr>
<tr>
<td>NGF (2.34 μg)</td>
<td>10.08 ± 0.85 (9)</td>
</tr>
<tr>
<td>Insulin (2.5 μg)</td>
<td>0.98 ± 0.14 (3)</td>
</tr>
<tr>
<td>Bovine growth hormone (20 μg)</td>
<td>1.35 ± 0.46 (3)</td>
</tr>
</tbody>
</table>

Adult male rats were injected intraventricularly with the various materials. The animals were killed 7 hr later. Values represent the mean ± SEM, with the number of animals used given in parentheses.

FIG. 1. Time course of the induction of ornithine decarboxylase by nerve growth factor in adult rat brain. Adult male rats were injected intraventricularly with 2.3 μg of 2.5S nerve growth factor in 10 μl of 0.05 M acetate, pH 5.0. Control animals were injected with 10 μl of the acetate buffer vehicle and killed at the same time as the experiments. The vertical bars indicate the standard error of the mean.
increase in the activity of brain ornithine decarboxylase. Presumably the increase in activity is followed by an increase in the content of polyamines in the brain. Be that as it may, this paper reports a biochemical alteration in the central nervous system due to physiological levels of NGF.

It is not completely surprising that NGF has a biochemical effect on the mature central nervous system. As mentioned before, others have reported potent effects of NGF on axonal regrowth (7, 8) and behavioral recovery (9) after brain damage in adulthood. Small amounts of NGF have been found in the brains of 5-week-old mice (19), and NGF antiserum has been reported to impair axonal regrowth of damaged noradrenergic neurons in adult rat brain (20). Such findings may be indicative of an endogenous role of NGF, or an NGF-like protein in brain.

Another line of research that is consistent with the present observations are the studies on NGF receptors in the brain (21–24). In these experiments, receptors for NGF have been found in the brains of rats and chickens, and these receptors appear to be much like those found in sympathetic and sensory ganglia, the recognized target tissues for NGF. Indeed, even the increasing sensitivity of the brain to NGF with maturation is consistent with the observation of Frazier et al. (21) that a large increase occurs in the specific binding of 125I-labeled NGF to brain tissue of rats as they grow from 6 weeks to 4 months of age.

It must be noted that Roger et al. (15), in a study of the effect of growth hormone on brain ornithine decarboxylase, observed an effect of NGF also. The amount they used (about 400 μg), however, made it possible that their results were due to either a contaminant in the NGF preparation or to some less specific action of NGF. The present results, in which effects are seen at concentrations of NGF comparable to those that are effective in vitro in sympathetic ganglia (3, 4, 25), and to those used in studies on axonal regrowth (7, 8) and on behavioral recovery (9) do not permit such alternate interpretations.

The general anatomical distribution of the ornithine decarboxylase response makes it unlikely that the NGF effect is localized to the catecholaminergic neurons or to any other particular class of neurons. Indeed, the present data show the response is not localized to the neurons at all. What functions the ornithine decarboxylase induction subserves in glia is yet another question.

The results reported in this paper, then, present a new avenue for exploring the effects of NGF in the mature brain. Because increased activity of ornithine decarboxylase appears to be a key event in the elicitation of the trophic response (2), a search for biochemically related events in brain is appropriate. But more important would seem to be an inquiry into the functional end result of the ornithine decarboxylase induction in adult brain.

We would like to thank Dr. O. Z. Sellinger for the hospitality extended to one of us (J.L.) and for instructing us in the cell separation technique used in this study. We also appreciate the assistance of Dr. R. M. Brown with the brain dissections.