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Nerve growth factor increases activity of ornithine decarboxylase in superior cervical ganglia of young rats

(enzyme induction/cyclic AMP)

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ABSTRACT **Nerve growth factor produces a rapid increase in the activity of ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) in superior cervical ganglia of young rats *in vivo* and *in vitro*. Maximum activity occurs 6–7 hr after the addition of nerve growth factor. The nerve growth factor-mediated increase in ornithine decarboxylase activity *in vitro* can be prevented by the addition of cycloheximide, actinomycin D, or antibody to nerve growth factor. A number of other agents were tested for their ability to increase ornithine decarboxylase activity in the ganglia; only nerve growth factor, and, to a slight extent, insulin were able to raise the activity of the enzyme. High concentrations of dibutyryl cyclic AMP (10 mM) were able to mimic the effect of nerve growth factor.**

Nerve growth factor (NGF) is a protein that has been implicated in the growth and differentiation of the sympathetic nervous system (1, 2). Administration of NGF to young rats causes hypertrophy and hyperplasia of the sympathetic and sensory ganglia and, in superior cervical ganglia, increases in the specific activities of the norepinephrine biosynthetic enzymes, tyrosine hydroxylase (EC 1.14.16.2) and dopamine β-hydroxylase (EC 1.14.17.1) (3).

Growth and differentiation of a wide variety of cell and tissue types have been shown to be accompanied by polyamine synthesis (4). Ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) catalyzes the conversion of ornithine to putrescine and is the rate-limiting enzyme in polyamine biosynthesis. The fact that ornithine decarboxylase has been shown to be induced rapidly in a number of tissues by a variety of hormones suggested that it may also mediate the biological responses to NGF. Further, the fact that increases in ornithine decarboxylase activity frequently are preceded by rapid and transient increases in cyclic AMP (cAMP) levels (5, 6) made such a postulate more attractive, because we have previously observed an NGF-induced increase in intracellular cAMP levels in superior cervical ganglia *in vitro* (7). The present experiments show that NGF produces a rapid increase in ornithine decarboxylase activity both *in vitro* and *in vivo* in superior cervical ganglia from young rats and that this action is specific to NGF.

MATERIALS AND METHODS

Superior cervical ganglia were removed from young rats (Zivic–Miller, Allison Park, PA), desheathed, and cultured in 0.35 ml of BGJ_b medium, Fitton-Jackson modification, without phenol red. The medium was supplemented with 0.1% bovine serum albumin and an antibiotic-antimycotic mixture that included penicillin, streptomycin, and Fungizone at 100 units/ml, 100 μg/ml, and 25 μg/ml, respectively. Following culture, pairs of ganglia were frozen in ground-glass homogenizers at −20° overnight. The freezing of the tissue caused no discernible loss of enzyme activity. The next day the ganglia were homogenized in 0.35 ml of 50 mM Tris·HCl, pH 7.6/5 mM dithiothreitol/40 μM pyridoxal phosphate; after centrifugation at 35,000 × *g* for 15 min, ornithine decarboxylase activity was estimated in the supernatant fluid. In some experiments NGF was injected subcutaneously into 5-day-old rats at a dose of 10 μg/g of body weight; at the indicated times the animals were killed and the ganglia were treated as above.

The activity of ornithine decarboxylase was determined by measuring the formation of $^{14}CO_2$ from DL-[*1*-^{14}C]ornithine monohydrochloride essentially as described by Oka and Perry (8). The enzyme reaction was carried out in rubber-stoppered, 15- × 100-mm glass tubes that were fitted with a hanging center well which contained 0.2 ml of Hyamine hydroxide. The reaction mixture (total volume, 0.5 ml) consisted of 75 mM Tris at pH 7.6, 7.5 mM dithiothreitol, 60 μM pyridoxal phosphate, 6 mM EDTA, 111 μM DL-[*1*-^{14}C]ornithine monohydrochloride (2.5 μCi), and an aliquot of the supernatant fluid equivalent to 1.5 ganglia. Samples were incubated at 37° for 1 hr and then the reaction was terminated by injecting 0.5 ml of 2.5 M H_2SO_4. The samples were allowed to stand overnight at room temperature and then the center well was removed and the radioactivity of the trapped $^{14}CO_2$ was measured in Liquifluor. The activity of ornithine decarboxylase is expressed as pmol of $^{14}CO_2$ produced per hr. Due to the low levels of ornithine decarboxylase activity in the ganglia, the enzyme was routinely assayed in the presence of 55.5 μM L-ornithine. This is probably less than a saturating concentration of substrate because the K_m for liver ornithine decarboxylase has been reported to be about 0.1 mM (9). It was shown, however, that, under the present conditions, the enzyme reaction was linear with respect to volume of extract up to at least 500 pmol of $^{14}CO_2$ per hr and with respect to incubation time for at least 60 min.

DL-[*1*-^{14}C]Ornithine monohydrochloride (specific activity: 45 mCi/mmol), Hyamine hydroxide, and Liquifluor were purchased from New England Nuclear Corp. Adenosine, pyridoxal phosphate, and dithiothreitol were obtained from CalBiochem. Decadron (dexamethasone) was from Merck, Sharp, and Dohme and 3-isobutyl-1-methylxanthine (IBMX) from Aldrich Chemical Co. Bovine growth hormone was obtained from Martin Rodbell. Media and antibiotics were from Grand Island Biological Co. Nerve growth factor, 2.5S form, was prepared by the method of Bocchini and Angeletti (10). Antibody to nerve growth factor was purified from sheep antiserum by the method of Stoeckel *et al.* (11). All other chemicals were obtained from Sigma Chemical Co.

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Abbreviations: NGF, nerve growth factor; cAMP, cyclic AMP; Bt_2cAMP, N^6,$O^{2'}$-dibutyryl cAMP; cGMP, cyclic GMP; Bt_2cGMP, N^2,$O^{2'}$-dibutyryl cGMP; IBMX, 3-isobutyl-1-methylxanthine.

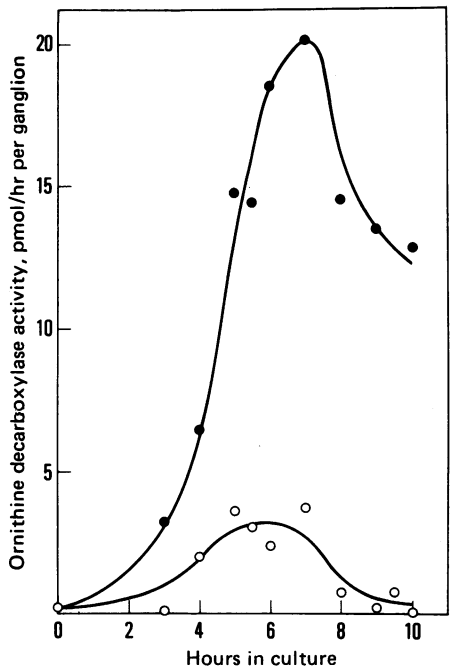

FIG. 1. The *in vitro* time course of the increase in ornithine decarboxylase activity in superior cervical ganglia. Ganglia from 6-day-old rats were cultured either with or without NGF (1 μg/ml). Values represent the mean of two experiments. ○, −NGF; ●, +NGF.

RESULTS

Ornithine decarboxylase activity can be increased *in vitro* within a few hours by the addition of NGF to the ganglia; the maximum activity of ornithine decarboxylase occurs within approximately 6–7 hr (Fig. 1). Ganglia cultured in the absence of NGF exhibit a spontaneous increase in ornithine decarboxylase activity that also occurs 6–7 hr after the ganglia are placed in culture. The increase in the activity of ornithine decarboxylase in ganglia cultured with NGF is usually 4- to 5-fold higher than in corresponding ganglia cultured without NGF. When ganglia were cultured for 4 hr in the absence of NGF, the addition of NGF still produced a marked increase in ornithine decarboxylase activity 6–7 hr later (data not shown). It is thus possible to separate the NGF-dependent and the NGF-independent elevations in ornithine decarboxylase activity. Nerve growth factor also can increase ornithine decarboxylase activity *in vivo* (Table 1), ruling out any possibility that the NGF-mediated increase observed *in vitro* is artifactual and caused only by the manipulation of the tissues. The optimum amount of NGF needed to increase ornithine decarboxylase activity *in vitro* was between 0.5 and 1.0 μg/ml, corresponding to a concentration of 20–40 nM (Fig. 2). No differences in ornithine decarboxylase activity due to the presence of NGF were observed in nodose ganglia, adrenal medulla, or explants of rat liver and kidney when these tissues were cultured under comparable conditions (data not shown).

Table 1. *In vivo* increase in ornithine decarboxylase activity in superior cervical ganglia

Treatment	Ornithine decarboxylase activity, pmol/hr per ganglion
Saline, 6 hr	0.63 ± 0.25
NGF, 6 hr	17.52, 12.47
Saline, 8 hr	0.05 ± 0.09
NGF, 8 hr	7.68, 4.03

Six-day-old rats were injected subcutaneously with saline or with NGF (10 μg/g of body weight), and ornithine decarboxylase activity was estimated 6 and 8 hr later. Values represent the mean ± SD of three experiments or, where individual values are given, of separate experiments.

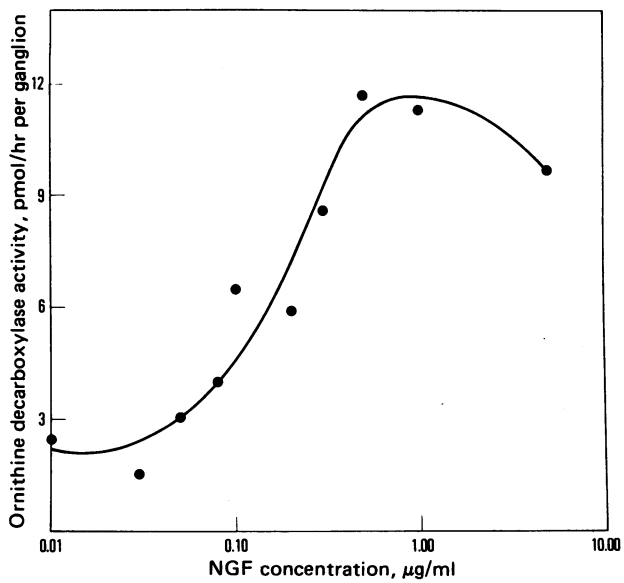

FIG. 2. The NGF dose–response curve of the increase in ornithine decarboxylase activity in superior cervical ganglia *in vitro*. Ganglia were cultured with the indicated concentrations of NGF (2.5 S) for 5.5 hr. Values represent the mean of duplicate experiments. Ornithine decarboxylase activity in ganglia cultured without NGF was 2.16 pmol/hr per ganglion.

A number of other substances were tested for their ability to raise ornithine decarboxylase activity in the superior cervical ganglia. Of the agents tested (Table 2), only NGF was capable of substantially increasing the activity of the enzyme. In view of the similarity in structure of NGF and insulin (12), it is not surprising that insulin was found to elevate ornithine decarboxylase activity slightly. Even at 4000 nM the effect of insulin was only slightly greater than at 40 nM, and less than 20% of the effect of NGF. Neither dexamethasone (Table 2) nor any of the other hormones tested (data not shown) enhanced the effect of NGF. Both the NGF-independent and the NGF-dependent increase in enzyme activity can be abolished by culturing the ganglia with cycloheximide (Table 3). However, actinomycin D and NGF antibody prevent only the NGF-mediated increase in ornithine decarboxylase, having no effect

Table 2. Specificity of the effect of nerve growth factor on ornithine decarboxylase activity in superior cervical ganglia

Additions to media	Concentration, nM	Ornithine decarboxylase activity, pmol/hr per ganglion
None	—	1.68 ± 0.22
NGF	40	14.40 ± 2.44
Bovine growth hormone	40	1.91 ± 0.05
Thyroxine	40	1.16 ± 0.18
Insulin	40	3.34 ± 1.44
Glucagon	40	1.53 ± 1.52
Dexamethasone	100	0.99 ± 0.56
Dexamethasone + NGF	100 40	11.03 ± 4.30

Ganglia from 6-day-old rats were cultured for 6 hr with the appropriate hormones. Values represent the mean ± SD of three experiments.

Table 3. Effect of inhibitors on the increase in ornithine decarboxylase activity in superior cervical ganglia

Additions	Concentration, μg/ml	Ornithine decarboxylase activity, pmol/hr per ganglion
None	—	3.39
NGF	1	19.24
Actinomycin D	1	3.02
Actinomycin D + NGF	1 + 1	4.84
Cycloheximide	10	0
Cycloheximide + NGF	10 + 1	0
NGF antibody	330	2.73
NGF antibody + NGF	330 + 1	4.50

Ganglia from 10-day-old rats were cultured for 5.5 hr; the inhibitors and/or NGF were added simultaneously at the beginning of the experiment.

on the spontaneous increase in the enzyme activity. This effect of actinomycin D is similar to that reported by Oka and Perry (8) in their studies on ornithine decarboxylase induction in mouse mammary gland explants.

The effect of NGF on ornithine decarboxylase can be mimicked by high concentrations of $N^6,O^{2'}$-dibutyryl cAMP (Bt_2cAMP) (Table 4). The addition of $N^2,O^{2'}$-dibutyryl cyclic GMP (Bt_2cGMP), butyrate, or adenosine to cultured ganglia was ineffective, as were lower concentrations of Bt_2cAMP. Ganglia cultured with IBMX, a phosphodiesterase inhibitor, had an increased level of ornithine decarboxylase activity (Table 4). The combination of IBMX and NGF was much more active than either alone, as was the combination of NGF and Bt_2cAMP. IBMX plus Bt_2cAMP, however, gave no higher activity than either IBMX or Bt_2cAMP separately.

Table 4. Effect of cyclic nucleotides and phosphodiesterase inhibitors on the NGF-mediated increase in ornithine decarboxylase activity in superior cervical ganglia

Additions to culture	Concentration	Ornithine decarboxylase activity, pmol/hr per ganglion	% of control
None	—	1.95 ± 0.26 (3)	100
NGF	1 μg/ml	8.77 ± 2.93 (4)	450 ± 150 (4)
Bt_2cAMP	10 mM	6.26 ± 1.55 (3)	321 ± 80 (3)
Bt_2cGMP	10 mM	0.68	35
Adenosine	10 mM	1.68	86
Butyrate	10 mM	1.99	102
IBMX	0.5 mM	5.75 ± 2.74 (4)	295 ± 110 (4)
IBMX + Bt_2cAMP	0.5 mM + 10 mM	4.30 ± 0.62 (4)	221 ± 32 (4)
NGF + Bt_2cAMP	1 μg/ml + 10 mM	25.92 ± 2.00 (3)	1329 ± 103 (3)
NGF + IBMX	1 μg/ml + 0.5 mM	20.84 ± 6.25 (4)	1069 ± 321 (4)
NGF + Bt_2cAMP + IBMX	1 μg/ml + 10 mM + 0.5 mM	30.28 ± 7.04 (4)	1553 ± 361 (4)

Ganglia from 6-day-old rats were cultured for 6 hr with the indicated concentrations of the compounds. Ornithine decarboxylase activity is expressed as the mean ± SD, with the number of experiments in parentheses, except where single experiments were performed. The ornithine decarboxylase value from uncultured ganglia ("0" time) is 0.42 ± 0.43 (16).

DISCUSSION

The present work shows that nerve growth factor increases the activity of ornithine decarboxylase in superior cervical ganglia of young rats. The inhibition seen with actinomycin D strongly suggests that this increase is a true enzyme induction. It appears that, as in other systems, the induction is mediated by the adenylate cyclase system. We have previously shown that nerve growth factor produces a rapid increase in cAMP levels in superior cervical ganglia *in vitro* (7). The present data show that a phosphodiesterase inhibitor, IBMX, and NGF act synergistically to increase ornithine decarboxylase activity. Similarly, Bt_2cAMP has a synergistic effect with NGF, but does not potentiate the effect of IBMX. This latter observation suggests that Bt_2cAMP is acting as an inhibitor of phosphodiesterase (13), and not as a precursor or substitute for cAMP.

Although the *in vitro* control ganglia were cultured in the absence of NGF, it seems unlikely that neuronal survival is involved in the NGF response for the following reasons. First, NGF produces a substantial response 6–7 hr after addition even if the ganglia are kept for 4 hr in its absence. Second, we have shown that the levels (14) and, even further, the differential rate of the synthesis (15) of tyrosine hydroxylase, a neuronal marker, are unchanged for the first 22 hr in culture under the present conditions. Finally, the *in vivo* response confirms the *in vitro* work and would appear to obviate the need for such considerations.

It would be of interest to know if the increase in ornithine decarboxylase activity produced by NGF is obligatory for the subsequent NGF-mediated induction of tyrosine hydroxylase (15, 16). Some of our data are at least consistent with such a relationship. First, the concentration of NGF needed to increase ornithine decarboxylase activity maximally in the ganglia is quite similar to that previously found to be maximal for tyrosine hydroxylase induction (14) and much higher than that needed to promote neurite outgrowth in the same system (17). Second, the increase in ornithine decarboxylase activity follows an increase in the activity of nuclear protein kinase (M. W. Yu, N. Tolson, and G. Guroff, unpublished data), and precedes an increase in RNA polymerase activity (K. Huff, J. Lakshmanan, and G. Guroff, unpublished data). Both of these responses appear to be involved in the induction of tyrosine hydroxylase in other systems (18). However, direct proof of the involvement of ornithine decarboxylase in the induction of tyrosine hydroxylase must await the design of experiments in which either ornithine decarboxylase induction or ornithine decarboxylase activity is inhibited and the subsequent effect on tyrosine hydroxylase induction is evaluated.

One point of interest is that the addition of dexamethasone to the cultures did not influence the action of NGF on ornithine decarboxylase activity. We have recently shown (19) that dexamethasone markedly potentiates the induction of tyrosine hydroxylase by NGF *in vivo*. The present results indicate either that the increase in ornithine decarboxylase activity is not linked to the induction of tyrosine hydroxylase or that dexamethasone influences some step in the sequence of intracellular events subsequent to ornithine decarboxylase.

It is appropriate to ask whether the NGF-mediated increase in ornithine decarboxylase activity is important for the more general actions of NGF, i.e., growth and differentiation in non-sympathetic systems. Our recent demonstrations that NGF increases ornithine decarboxylase activity in both dorsal root ganglia and the central nervous system (unpublished data) suggest that, indeed, an increase in ornithine decarboxylase

activity is a consequence of the action of NGF on cells other than sympathetic neurons.

From this work, then, it can be concluded that an increase in ornithine decarboxylase activity intervenes between the binding of nerve growth factor to its receptor and the final biological response of the tissue. This observation confirms and strengthens our previous findings on the effect of nerve growth factor on the levels of cAMP (7) and suggests that NGF acts at least partly through the cascade of events suggested by Russell *et al.* (20). Unpublished work in this laboratory indicates that such a cascade may be a consequence of the action of NGF in all its target tissues and not just a function of its action on tyrosine hydroxylase induction in superior cervical ganglia. This observation may shed important light on our understanding of the basic mode of action of NGF.

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