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THE PERMISSIVE EFFECT OF CORTICAL STEROIDS ON THE INDUCTION OF BRAIN ORNITHINE DECARBOXYLASE BY NERVE GROWTH FACTOR

Takeyuki Ikeno, Paul C. MacDonnell, K. Nagaiah,
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.

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Summary

The intraventricular administration of nerve growth factor causes a marked increase in the activity of ornithine decarboxylase in rat brain. This increase is much smaller in adrenalectomized rats. Dexamethasone and corticosterone, administered either systemically or intraventricularly, are able to restore the ability of nerve growth factor to induce ornithine decarboxylase. The steroids must be given at least three hours before the nerve growth factor to be fully effective.

Introduction

Nerve growth factor (NGF) is a protein that is necessary for the growth and maintenance of sympathetic and certain sensory neurons (1,2). Many metabolic effects of NGF have been observed and some progress has been made in understanding the mechanism of action of NGF on sympathetic neurons. Ornithine decarboxylase (L-ornithine carboxylase, EC 4.1.1.17) (ODC), a key enzyme in the biosynthesis of polyamines, appears to serve an important regulatory function in the control of cell division and growth. This enzyme is induced by nerve growth factor in sympathetic and sensory ganglia of the rat (3).

In previous studies from this laboratory, a marked increase in the activity of ODC was also observed in the central nervous system of adult rats

Abbreviation: NGF, nerve growth factor.

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following the intraventricular administration of NGF (4). In subsequent experiments (5) it was observed that the NGF-mediated increase in brain ODC activity was smaller in adrenalectomized rats than in sham-operated controls. These results suggested that the induction of ODC in brain might be influenced by adrenal steroids.

In this report, we show that cortical steroids stimulate the induction of brain ODC by NGF in the adrenalectomized rat. Several steroids were investigated for their ability to induce brain ODC with NGF. Dexamethasone and corticosterone were effective and aldosterone had a slight effect on the induction of brain ODC by NGF. To induce brain ODC to its normal level it was necessary to administer the steroid at least three hours before the NGF.

Materials and Methods

Male rats (150-200 g) of the Sprague-Dawley strain were used. Some animals were bilaterally adrenalectomized by Zivic-Miller Laboratories, Inc., Allison Park, PA, one week before use. Animals were anesthetized with ether and NGF was injected intraventricularly as previously described (4). Rats were killed 4.5 hours after the injection of NGF. Brains were homogenized in 4 volumes of a buffer containing of 50 mM Tris, pH 7.5, 5 mM dithiothreitol, and 40 μM pyridoxal-5'-phosphate. The homogenate was centrifuged at 27,000 g for 15 minutes and the supernatant fraction was used for enzyme assay as previously described (3,6,7). The results are expressed as nanomoles $^{14}CO_2$ liberated/h per g tissue. Steroids were administered intraperitoneally or intraventricularly as described in the Figure and the Tables.

Nerve growth factor (2.5 S form) was purified from the submaxillary glands of mature male mice by the procedure of Bocchini and Angeletti (8). L-[1-^{14}C]-Ornithine monohydrochloride (specific activity: 60 mCi/mmol) was purchased from Amersham Corporation. Liquifluor and Hyamine hydroxide were obtained from New England Nuclear Corp. Dithiothreitol was purchased from Calbiochem. Decadron (dexamethasone sodium phosphate) was obtained from Merck, Sharpe, and Dohme. Other steroids and pyridoxal-5'-phosphate were purchased from Sigma Chemical Co. Center wells and rubber stoppers were from Kontes Glass Co.

Results

The intraventricular administration of nerve growth factor leads to a marked increase in the activity of brain ornithine decarboxylase. There is approximately a 20-fold increase in ODC activity in the brain of sham-operated rats within 4.5 hours. In adrenalectomized rats comparable injections of NGF produced only a six-fold increase (Table 1). In order to investigate the

Table 1. Effect of NGF and dexamethasone on brain ornithine decarboxylase activity in adrenalectomized and sham-operated rats.

Treatment	Sham	Adrenalectomized
	Ornithine decarboxylase Activity (nmol/h per g tissue)	
Buffer	0.31 ± 0.03 (18)	0.29 ± 0.03 (16)
NGF	5.99 ± 0.43 (29)	1.77 ± 0.22 (24)
Dexamethasone	0.84 ± 0.05 (5)	1.08 ± 0.18 (8)
NGF, Dexamethasone	6.68 ± 1.14 (4)	5.96 ± 0.39 (19)

Rats were adrenalectomized or sham-operated one week before use. Ten µl of acetate buffer (0.05M, pH 5.0) or NGF in acetate buffer (0.20 nmoles, equivalent to 4.68 µg) were injected intraventricularly. Dexamethasone (0.2 ml, 15 mg/ml in absolute alcohol) was injected intraperitoneally 3 hours before NGF administration. Rats were killed 4.5 hours after the administration of NGF. Results are expressed as the mean ± SEM with the number of animals in parentheses.

mechanism of this effect, intraperitoneal injections of dexamethasone were given to adrenalectomized rats three hours before the administration of NGF. Such injections completely restored the ability of NGF to induce ornithine decarboxylase (Table 1). Dexamethasone did not increase the effect of NGF in sham-operated controls and had only a small effect by itself in either adrenalectomized animals or sham controls.

To determine the specificity of the stimulation, several steroids were investigated for their ability to induce brain ODC in conjunction with NGF (Table 2). Corticosterone was as effective as dexamethasone; estradiol, testosterone, and progesterone had no effect on ODC induction. Aldosterone was

Table 2. Effect of various steroids on brain ornithine decarboxylase activity in adrenalectomized rats treated with NGF.

Animals	Treatment	Ornithine decarboxylase activity
		(nmol/h per g tissue)
Sham	NGF	5.99 ± 0.43 (29)
Adrenalectomized	NGF	1.77 ± 0.22 (24)
"	NGF, Dexamethasone	5.96 ± 0.39 (4)
"	NGF, Corticosterone	6.82 ± 0.73 (4)
"	NGF, Estradiol	1.22 ± 0.17 (4)
"	NGF, Testosterone	1.85, 1.33
"	NGF, Progesterone	1.89 ± 0.24 (4)
"	NGF, Aldosterone	3.02 ± 0.43 (4)

Steroids (0.2 ml, 15 mg/ml in absolute alcohol) were injected intraperitoneally 3 hours before the intraventricular administration of NGF (4.68 μg). Rats were killed 4.5 hours after the administration of NGF. Results are expressed as the mean ± SEM with the number of animals in parentheses, except in the testosterone-treated group where only two animals were used.

about half as effective as corticosterone.

To provide evidence that the steroid is acting directly on the brain, it seemed desirable to determine the effectiveness of small amounts given intraventricularly. In such experiments it was shown that the intraventricular administration of small amounts of steroid is as effective as the administration of much larger amounts by the intraperitoneal route (Table 3).

Finally, in order to understand the nature of the effect of the steroid, the time course of its action was determined. It was found essential that the

Table 3. Effect of intraventricular administration of NGF and Decadron on brain ornithine decarboxylase activity in adrenalectomized rats.

Treatment	Ornithine decarboxylase activity
	(nmol/h per g tissue)
Buffer	0.22 ± 0.04 (5)
Decadron	0.90 ± 0.08 (5)
NGF	1.59 ± 0.07 (5)
NGF, Decadron	5.64 ± 0.50 (5)

Rats were adrenalectomized one week before use. Five µl portions of acetate buffer (0.05 M, pH 5.0), NGF (0.20 nmoles, equivalent to 4.68 µg), or Decadron (17 µg) were injected intraventricularly. Decadron was injected 3 hours before NGF administration. The animals injected with Decadron itself were killed 7.5 hours later. The other animals were killed 4.5 hours after NGF and buffer injection. Results are expressd as the mean ± SEM with the number of animals in parentheses.

steroid be administered at least three hours before NGF for the induction of maximal ODC activity (Fig. 1). When dexamethasone was injected at the same time as the NGF it had no additional effect on ODC induction; injected one or two hours before NGF, dexamethasone was partly effective.

Discussion

The presence of receptors for glucocorticoids in the brain is well documented (9,10). They are widely distributed in various brain regions and seem to be localized to the neurons (11,12). There is evidence that the steroid-receptor complex is translocated to the nucleus in brain as in other

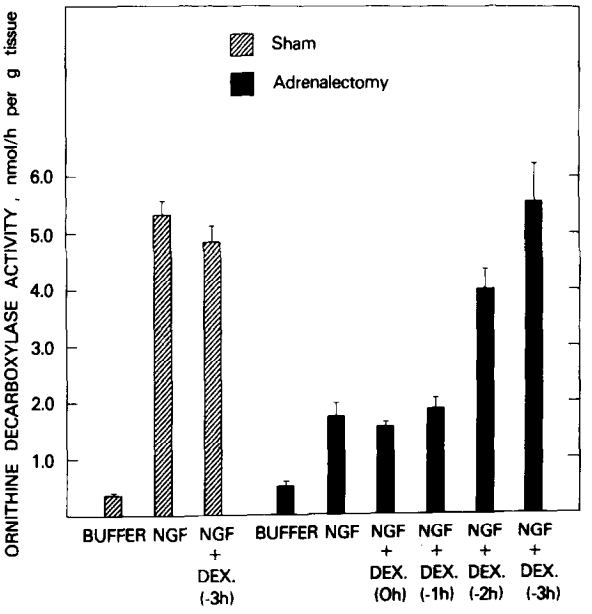

Fig. 1 Ornithine decarboxylase activity in the brain following the intraperitoneal administration of dexamethasone (Dex) and the intraventricular administration of nerve growth factor (NGF). Dexamethasone (3 mg in 0.2 ml of absolute alcohol) was injected either 3, 2, or 1 hour(s) before, or together (0 hour) with NGF (4.68 µg). Animals were killed 4.5 hours after NGF administration. Results are expressed as the mean ± SEM; five animals were used in each group.

tissues. The biochemical actions which such receptors mediate in the brain are not as well known. This report indicates that the glucocorticoids participate in, or permit, the induction of brain ornithine decarboxylase by nerve growth factor.

The participation of cortical steroids in some of the actions of NGF in the sympathetic nervous system is now thoroughly accepted. The induction of tyrosine hydroxylase in sympathetic neurons by NGF has been shown to be faster and of greater magnitude in the presence of these steroids both *in vivo* and *in vitro* (13-15). The mechanism by which the steroids participate in the

induction is not known, but the observations are similar to those in other systems in which the steroid is considered "permissive."

The mechanism by which the steroid acts in the brain is also not known. The requirement that the steroid be administered several hours before NGF suggests that the steroid is required for the synthesis of some ancillary factor which is necessary for the action of NGF. It is possible to speculate that some portion of the nerve growth factor-initiated sequence of messengers is depleted in adrenalectomized animals and is resynthesized in response to the glucocorticoids.

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