The Effect of Nerve Growth Factor on a Selective Increase in the Cell Body Size of Rat Brain Septal Cholinergic Neurons In a Cell Culture

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UDC 616.831-091.81-092.4-092.9]-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 1, pp. 102-104, January, 1994
Original article submitted June 7, 1993

The addition of nerve growth factor to the culture medium increases the size of the bodies of three- and four-dendrite polygonal cholinergic neurons, but not of two-dendrite spindle-shaped neurons.

Key Words: *nerve growth factor; cholinergic neurons in culture*

There is a considerable body of evidence indicating that nerve growth factor (NGF), previously characterized as a trophic factor of peripheral, adrenergic, sympathetic, and some sensory neurons [12,13], exerts a potent effect on the development of cholinergic neurons of the basal nuclei of the forebrain. It has been found that NGF enhances the activity of acetylcholine transferase in the neurons of the cholinergic system of the forebrain basal nuclei [7-9,11,14], activates the growth of the dendrites of the septal cholinergic neurons [8,9], and stimulates morphogenesis of these cells [3].

We have tested the possibility of selective action of NGF on the development of cholinergic septal neurons in culture, taking into account the morphological heterogeneity of septal neurons [4,5].

MATERIALS AND METHODS

The septal areas were dissected from the brains of Wistar rats removed on day 18-19 of embryonal development and were used for the preparation of a cell suspension by modified enzyme dissociation. The cells were cultured on cover slips (22×22 mm) covered with collagen-poly-L-lysine and placed in plastic Petri dishes [1,8]. The initial density of the cell population was $80\text{-}130\times10^3$ cells/cm^2. Nerve growth factor (7S, final concentration 50 biological units/ml) was added to the culture medium on day 2 of culturing. Cells grown in NGF-free medium served as the control. The cells were cultured for 14 days with full replacement of the medium every 3-4 days to remove endogenous growth factors. Cholinergic neurons were visualized by acetylcholine esterase (AChE) staining [4]. Morphological changes in AChE-positive cells in dissociated cell cultures were evaluated by morphometry, using a Leitz-Ortolux microscope and drawing instrument [2]. The AChE-positive cells were precisely drawn at a magnification of 400. The area of the profile field of the neuron body (Sc) was measured in a semiautomatic Leitz-ACM analyzer. Four-hundred eighty cells were analyzed. The significance of differences was evaluated using Student's t test.

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RESULTS

Large AChE-positive ovoid, triangular, and polygonal neurons with 2-4 and occasionally 5 clearly

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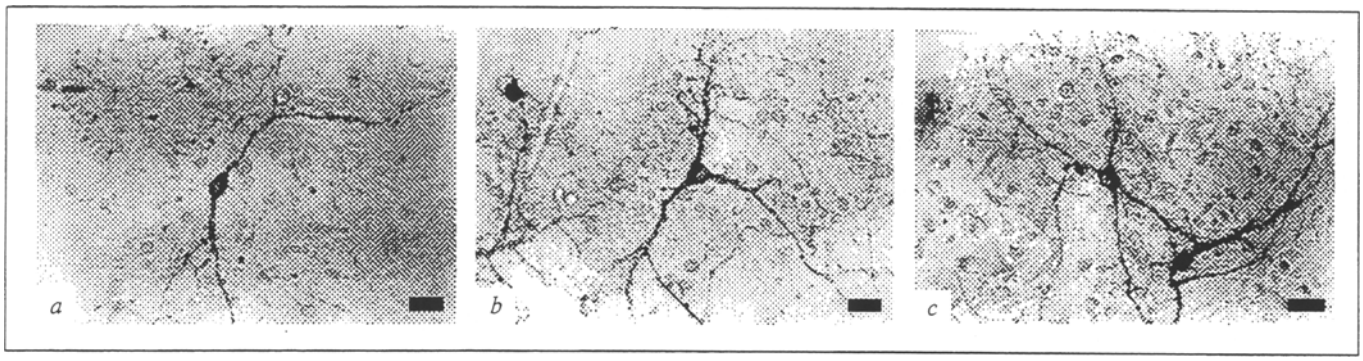

Fig. 1. AChE-positive neurons in 14-day dissociated cultures of the septal area of fetal rat brain. *a*) spindle-shaped two-dendrite neuron; *b*) polygonal three-dendrite neuron; *c*) polygonal four-dendrite neuron. Histochemical staining for AChE. Scale 20 μ.

stained dendrites were revealed in 14-day cultures (Fig. 1).

We analyzed the NGF-induced changes in the cell body size for the totality of AChE-positive neurons. There was a significant increase in the body size of AChE-positive cells grown in the presence of NGF: 271±6 versus 209±6 μ² in control cultures. On the basis of the number of dendrites and the cell body shape, AChE-positive neurons were then divided into 3 populations: two-dendrite spindle-shaped, three-dendrite triangular or ovoid, and four- or five-dendrite polygonal cells. Analysis of these populations showed that there was no statistically significant difference in the Sc of neurons of the control populations, whereas the cell body size was significantly increased in neurons with 3 or more primary dendrites. The effect was similarly pronounced (48% compared with the control) in three- and four-dendrite neurons. There were no differences in the population of two-dendrite neurons as compared with the control (Fig. 2).

Thus, these observations and our previous data [3] on the progressive differentiation of the dendrite system of AChE-positive neurons under the influence of NGF (without division into morphological subpopulations) led us to conclude that NGF is an epigenetic factor that stimulates the development of cholinergic neurons of the basal parts of the forebrain at the early stages of development. However, NGF produces highly selective effects on various subpopulations of cholinergic neurons composing one system of nuclei.

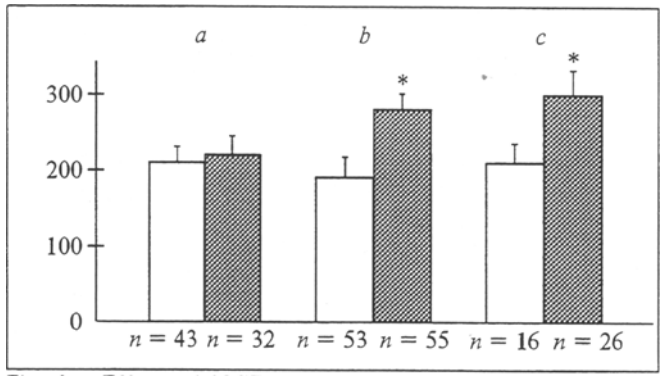

Fig. 2. Effect of NGF on the size of the bodies (Sc) of AChE-positive neurons in 14-day dissociated cultures of the setal area of fetal rat brain. Ordinate: mean Sc value (μ). The asterisk indicates $p<0.0001$; *n*: number of neurons. White bars: control; shaded bars: cells grown in the presence of NGF. Other notation as in Fig. 1.

REFFERENCES

1. I. V. Viktorov, N. A. Andreeva, and N. K. Isaev, *Tsitologiya*, **32**, № 7, 81-84 (1990).
2. T. A. Leontovich, in: *Neuronal Organization of Subcortical Structures of the Forebrain* [in Russian], Moscow (1978).
3. E. G. Markova and N. K. Isaev, *Byull. Eksp. Biol. Med.*, **113**, № 3, 318-320 (1992).
4. E. G. Marcova, *Microscop. Sci.*, **25**, 397-400 (1990).
5. K. Brauer, W. Schober, G. Werner, *et al., J. Hirnforschung*, **29**, № 1, 43-47 (1988).
6. A. El-Badavi and E. A. Schenc, *J. Histochem. Cytochem.*, **15**, 10-13 (1967).
7. H. Gnahn, F. Hefti, R. Heuman, *et al., J. Neuron. Neurosci.*, **9**, 45-52 (1988).
8. J. Hartikka and F. Hefti, *Ibid.*, **8**, 2967-2985 (1988).
9. J. Hartikka and F. Hefti, *J. Neurosci. Res.*, **21**, 352-354 (1988).
10. E. Hawrot, *Dev. Biol.*, **74**, 136-151 (1980).
11. F. Hefti, J. Hartikka, *et al., Neuroscience*, **14**, № 1, 55-68 (1985).
12. R. Levi-Montalciny and P. U. Angeletti, *Physiol. Rev.*, **8**, 534-539 (1968).
13. R. Levi-Montalciny, *TMBO J.*, **6**, № 5, 1145-1154 (1987).
14. W. C. Moblei, J. L. Putkowski, G. I. Tennekoon, *et al., Mol. Brain Res.*, **1**, 53-62 (1986).