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EFFECT OF NERVE GROWTH FACTOR ON CHOLINERGIC NEURONS IN DISSOCIATED CULTURES OF THE SEPTUM PELLUCIDUM

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UDC 612.826.1.014.2:612.6].063:612.818.018

KEY WORDS: nerve growth factor, cholinergic neurons, neurons in culture.

In recent years investigations of trophic factors, with the primary aim of studying their importance for morphogenesis, viability, and regeneration of the neurons of the developing brain, have expanded greatly. Nerve growth factor (NGF), discovered as long ago as in the 1950s by Levi-Montalcini and Hamburger [10], and characterized by them as a trophic factor of peripheral and noradrenergic sympathetic and sensory neurons, has been studied particularly intensively in this respect.

It has now been shown that a comparatively large quantity of NGF is present in certain structures of the CNS (neocortex, hippocampus) [3, 8], and that if injected into the cortex, it is transported into cholinergic neurons (ChN) of the basal nuclei of the forebrain [9]. Studies of the whole brain and cell cultures have shown that NGF affects the viability of ChN and the level of activity of choline-acetyltransferase and acetylcholinesterase (AChE) [5-7, 11].

In the investigation described below the effects of NGF were studied on body size and on the number of ChN in dissociated cell cultures of the rat embryonic septum pellucidum.

Brain Institute, Academy of Medical Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR O. S. Andrianov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 3, pp. 305-306, March, 1991. Original article submitted September 11, 1990.

0007-4888/91/0003-0383$12.50

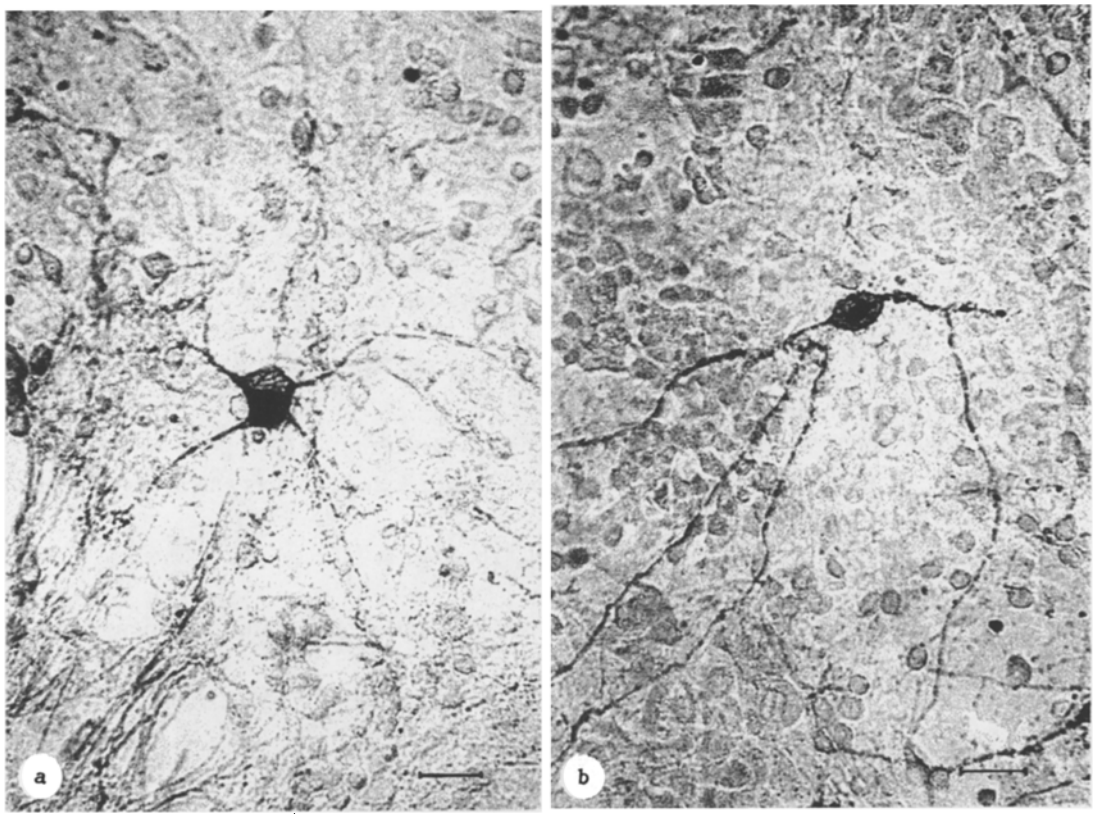

Fig. 1. ChN in 7-day dissociated cell culture of septum pellucidum from 18-19-day rat embryos: a) ChN developing in the presence of NGF (200 b.u./ml); b) ChN in control culture developing in absence of NGF. Histochemical staining for AChE by Karnovsky–Roots method. Scale: 20 μ.

EXPERIMENTAL METHOD

A cell suspension was obtained from tissue of the septum pellucidum (SP) of 18-19-day Wistar rat embryos, by a modified method of enzymic dissociation, and cultured on coverslips coated with polyethylenimine, in plastic Petri dishes [1]. The initial density of the cell population was $1 \cdot 10^5$-$1.5 \cdot 10^5$ cells/cm^2. The experimental cultures in culture medium were treated with ^{7}S NGF in a final concentration of 200 biological units (b.u.)/ml. Cultures grown in nutrient medium without NGF served as the control. Culture was carried out for 7 days with complete change of nutrient medium every 3rd-4th day of development of the cultures to remove endogenous growth factors. To detect ChN the histochemical method of staining for AChE was used [4]. Stained neurons were counted in the whole preparation and the results expressed as a percentage of the number of these neurons in the control. To determine the body size of ChN, the method of determination of the area of microscopic objects adopted in microtechnology was used [2]. The results of the measurements and calculations were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Histochemical testing for AChE revealed neurons differing in their intensity of staining in 7-day SP cell cultures, with round, oval, or fusiform bodies, giving off 2-4 or, less frequently 5 processes. In cultures developing in the presence of exogenous NGF these neurons were stained with the histochemical reaction product much more intensively than in cultures whose nutrient medium contained no added NGF (Fig. 1a, b), indicating a higher level of AChE activity in these neurons compared with the control.

The results of measurements of the area of the body of ChN showed that in neurons developing in the presence of NGF it averaged $230 \pm 10\ \mu^2$ ($n = 63$), whereas in the control this parameter reached only $167 \pm 6\ \mu^2$ ($n = 47$).

TABLE 1. Action of NGF on Number of AChE-Containing Neurons in 7-Day Cell Cultures from SP of 18-19-Day Rat Embryos ($p < 0.001$, $n = 3$-5)

Expt. No.	Number of AChE-containing neurons, percent of control	
	control cultures	exptl. cultures
1	100±5	205±18
2	100±10	146±10
3	100±9	192±10
4	100±13	197±14

Counting the AChE-containing neurons showed that in the experimental cultures the number of these neurons was 46-105% greater than in cultures to whose nutrient medium no exogenous NGF was added (Table 1). The variability of this parameter is due to a difference in the number of ChN in the initial cell suspension in the different experiments, and the different degree of injury to and death of these cells during dissociation, which inevitably leads to a difference between cultures with respect to the number of surviving neurons.

It was shown in [6], in an investigation conducted on 2-3-week dissociated SP cultures that the action of NGF increases the survival rate and body size of the ChN but only in cultures with low cell density ($1.5 \cdot 10^5$ cells/cm^2). The results of the present investigation, while confirming the conclusions of the study cited above on the whole, indicate that the effect of NGF on growth of ChN of SP is realized in the early stages of development of these neurons in vitro. These data are evidence that NGF, as a neurotrophic factor, is actively involved in development, regeneration, and survival of the ChN of the forebrain, and also in the maintenance of the necessary level of activity of the key enzymes of acetylcholine synthesis in them.

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