# ACTION OF NERVE GROWTH FACTOR ON DEVELOPMENT OF THE DENDRITIC SYSTEM OF CHOLINERGIC NEURONS OF THE RAT SEPTUM IN DISSOCIATED CULTURE

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The considerable interest in analysis of development of the nervous system has led to a substantial increase in the number of investigations into trophic factors with a view to elucidating their role in morphogenesis, viability, and regeneration of the neurons of the developing brain. At present, the best study of this class of biologically active substances is nerve growth factor (NGF), which is a trophic factor of peripheral, adrenergic, sympathetic, and certain sensory neurons [9, 10]. Recent work has shown that NGF has a significant effect on cholinergic neurons (CEN) of the basal nuclei of the forebrain. NGF plays the role of trophic factor of these neurons, it acts at the level of choline-acetyltransferase (CAT) and acetylcholesterase (ACE) activity, and it also affects the development of the cholinergic system of basal forebrain nuclei as a whole [1, 8, 11, 12]. Investigations likewise have shown that NGF actively influences the growth of septal cholinergic fibers of the forebrain [5, 6]. However, the question of the effect of NGF on morphogenesis of CEN of the basal forebrain nuclei remains open.

The aim of this investigation was to study the action of NGF on morphogenesis of developing cholinergic neurons in a dissociated culture of the rat brain septum.

## EXPERIMENTAL METHOD

A cell suspension was obtained from the septum of 18-19-day Wistar rat embryos by means of a modified method of enzymic dissociation and culture on coverslips (22 × 22 mm), covered with a combined collagen – poly-L-lysine substrate, in plastic Petri dishes [1, 7]. The initial density of the cell population was 80,000–130,000 cells per square centimeter. On the second day of culture 7S NGF was added to the experimental cultures in a final concentration of 50 biological units (b.u.)/ml. Cultures grown in nutrient medium without NGF served as the control. The cells were cultured for 14 days with complete change of nutrient medium every 3-4 days of growth of the cultures to remove endogenous growth factors. CEN in the cultures were demonstrated by the histochemical staining method for ACE [3]. During investigation of the morphologic changes in CEN in dissociated septal cell cultures a morphometric method of analysis of neurons with processes was used [2]. For this purpose, under the "Leitz Ortholux" microscope, with magnification of 400×, accurate drawings of CEN were obtained by means of a drawing apparatus from preparations of septal cell cultures stained for ACE. Next, the drawings of the neurons were analyzed by means of a "Leitz-AOM" automatic image analyzer, on the basis of four parameters: 1) the area of cross section of the neuron body; 2) the total length of the dendrites of a neuron; 3) the total ramification of the dendritic system of the neuron (the total number of all terminal segments of the dendritic system); 4) the area of the dendritic territory of the neuron. The significance of differences was determined by the Wilcoxon–Mann–Whitney nonparametric test (at the $p < 0.05$ level). In the course of this investigation 192 neurons were analyzed from two series of experiments. Throughout the duration of development of the cultures the neurons were observed under phase contrast conditions by means of a "Reichert" inverted microscope (Austria).

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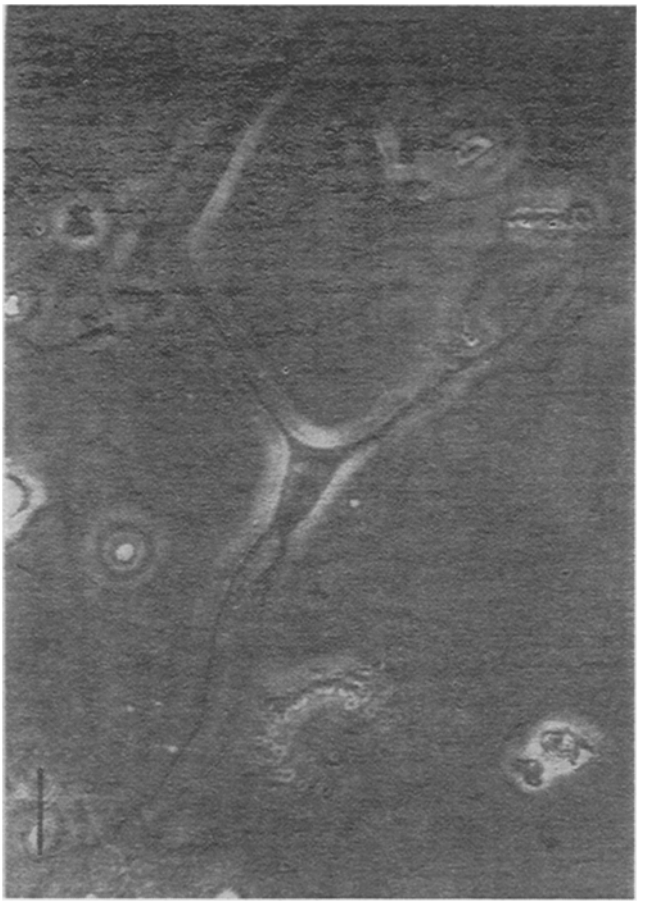

Fig. 1. Single large reticular neuron in 14-day dissociated septal culture of 18-19-day rat embryos. Living unstained cultures, phase contrast. Scale: 20 $\mu$.

## EXPERIMENTAL RESULTS

During dissociation of the septal tissue by enzymic and mechanical treatment most of the dissociated cells lost their processes. The cells became round, and only their bodies with a large nucleus and with a narrow rim of cytoplasm could be seen in a cell suspension located on a poly-L-lysine substrate. Only individual cells preserved the initial segments of their processes. By the 3rd day of culture the glia began to form a cellular supporting layer, which subsequently formed a confluent sheet. On the 14th day large neurons with a well marked dendritic system could be observed in these culture (Fig. 1).

Bodies of CEN and their dendrites, stained a varied intensity of brown, were detected by the method of histochemical staining for ACE in the cultures. In most cases the cell bodies had a brighter color than the dendrites (Fig. 2). In individual cases an axon was stained and had to be distinguished from dendrites.

The results of morphometric analysis of the two series (1 and 2) of the experiment showed that within each series bodies of cholinergic septal neurons in the experimental cultures were significantly larger than the controls (Fig. 3a). It was also shown under the influence of NGF, changes in the dendritic system of ACE-containing neurons took place in the septal culture under the influence of NGF: the total length of the dendrites, the degree of ramification of the dendritic system, and the area of dendritic territory all increased significantly (Fig. 3b, c, d).

Earlier studies [5, 6] showed that exogenous NGF causes an increase in total length of the processes of septal CEN without distinguishing between axons and dendrites. The authors cited also observed a tendency for the bodies of CEN to be enlarged. However, on the basis of our observations it was difficult to conclude to what extent NGF affects the development of the dendritic system of these neurons.

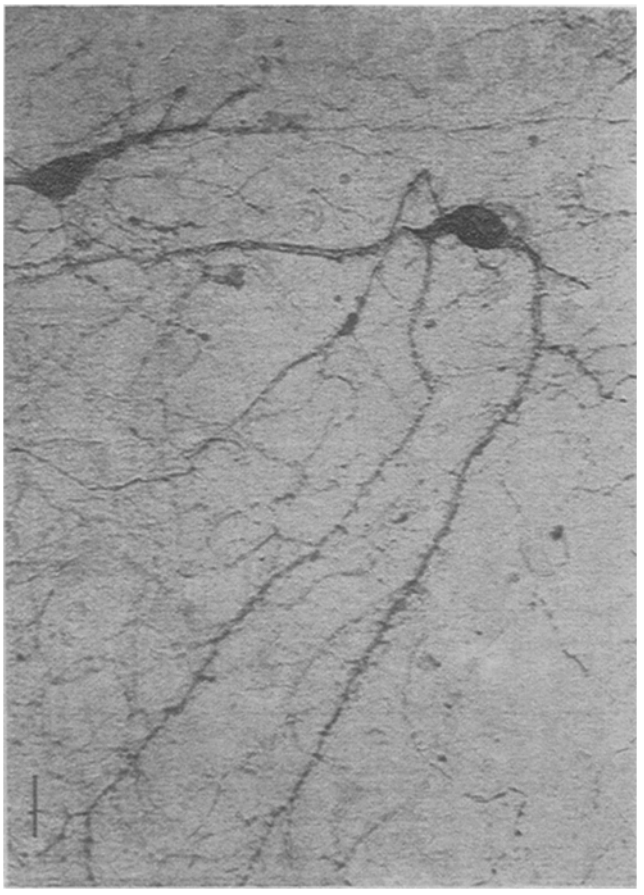

Fig. 2. ACE-positive neuron in 14-day dissociated septal culture from 18-19-day rat embryos. Histochemical staining for acetylcholinesterase. Scale: 20 $\mu$.

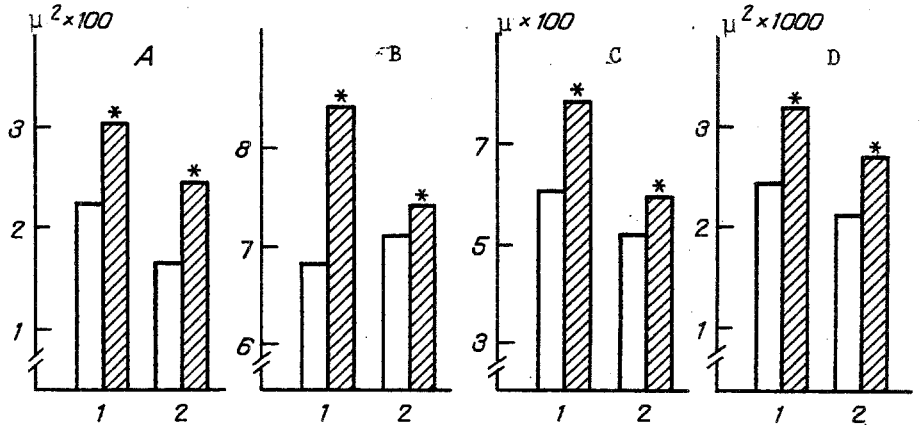


Fig. 3. Effect of NGF on morphologic differentiation of ACE-positive neurons in 14-day dissociated septal cultures from 18-19-day rat embryos (shaded columns) compared with control (unshaded columns) in two experimental series: 1 and 2 respectively. Ordinate: a) mean area of cross section of bodies (in $\mu^2$); b) mean total ramification of dendritic system of a neuron, c) mean total length of dendrites of a neuron (in $\mu$); d) mean area of dendritic territory of a neuron (in $\mu^2$), $p < 0.05$.

Our investigation showed that NGF actively influences the morphogenesis of the ACE-positive septal neurons in dissociated cultures of the rat brain. There is a significant increase in size of the neuron bodies, which is accompanied by progressive differentiation of the dendritic system. Similar morphological changes have been described at the qualitative level on sympathetic adrenergic neurons of the superior cervical ganglia of the peripheral nervous system of rats [13]. It can be postulated on the basis of the results of that investigation and of our own findings that there is a universal character of action of NGF on NGF-sensitive neurons of the central and peripheral nervous system, although not thereby ruling out the selectivity of its action on different types of cholinergic neurons.

It can thus be concluded from the results that nerve growth factor is one of the most active epigenetic factors capable of stimulating development of cholinergic neurons in the basal regions of the forebrain in the early stages of development, by increasing the total length and degree of ramification of their dendritic system, but preserving the basic phenotypic features characteristic of one particular type of neuron.

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