DNA SYNTHESIS ASSOCIATED WITH POLYPLOIDIZATION OF SYMPATHETIC NEURONS IN ADULT MICE RECEIVING NERVE TISSUE GROWTH FACTOR

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UDC 612.398.145.1-087.347

Investigations using autoradiography with thymidine-H^3 revealed DNA synthesis in the nuclei of hypertrophied sympathetic neurons of the stellate ganglia of adult mice receiving injections of nerve tissue growth factor. This synthesis takes place in the nuclei of differentiated cells and is associated, not with their preparation for mitotic division, but with their polyploidization.

The results of cytophotometric investigations of the state of the sympathetic neurons in mice receiving injections of nerve tissue growth factor (NTGF) suggested that the process of hypertrophy of the nerve cell bodies developing in this case is accompanied by polyploidization of their nuclei [1].

This paper describes the results of an autoradiographic study of DNA synthesis in the nuclei of sympathetic neurons of adult mice receiving definite doses of NTGF.

EXPERIMENTAL METHOD

Subcutaneous injections of NTGF isolated from the submandibular salivary glands of mice were given daily for 7 days to four albino mice weighing 21.0 ±1.0 g in a dose of 300 biological units/g body weight. Four animals of the same weight, reared under absolutely identical conditions, but receiving seven daily injections of physiological saline were used as the control. All the experimental and two control mice received two daily injections of thymidine-H^3 (specific activity 7 mCi/ml) in a total dose of 4 μCi/g. Half of the mice used in the experiment were killed 24 h after, and the remainder 7 days after the last injection. The stellate ganglia of the control and experimental mice were fixed in Carnoy's fluid and embedded in paraffin wax. The degree of hypertrophy obtained as the result of this cycle of NTGF injections was estimated from the change in dry weight of the nuclei and cytoplasm of the tested neurons. The dry weight was measured by means of an interference microscope. Sections for autoradiographic study were dewaxed, coated with type M liquid fine-grain emulsion and exposed in darkness at 4°C for 90 days. After development, these sections were counterstained with methyl green–pyronine.

EXPERIMENTAL RESULTS

The results given in Table 1 show that a 7-day course of injections of growth factor into adult mice leads to a marked increase in the dry weight of the perikaryons of the cells studied: the mass of the cytoplasm increased by more than 60% and the mass of the nuclei by more than 100%. Injection of NTGF thus definitely induces hypertrophy of the bodies of the sympathetic nerve cells of adult mice.

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TABLE 1. Changes in Concentration of Solids, Size and Dry Weight of Nuclei and Cytoplasm of Sympathetic Nerve Cells from Stellate Ganglia of Adult Mice after 7-day Cycle of NTGF Injections (M ± m)

	Nucleus		Cytoplasm	
	control	expt.	control	expt.
Phase shift ($\times 10^{-6}$ cm)	15±1,0	26±2,0*	47±2,0	51±2,0
Area of section (conventional units)	289±5,8	357±7,9*	810±30,2	1 240±35,9*
Dry weight (conventional units)	211±9,2	516±23,2*	2 115±88,8	3 513±123,0*

*Differ by statistically significant amount from the corresponding control (statistical analysis of the results by the Fisher–Student method).

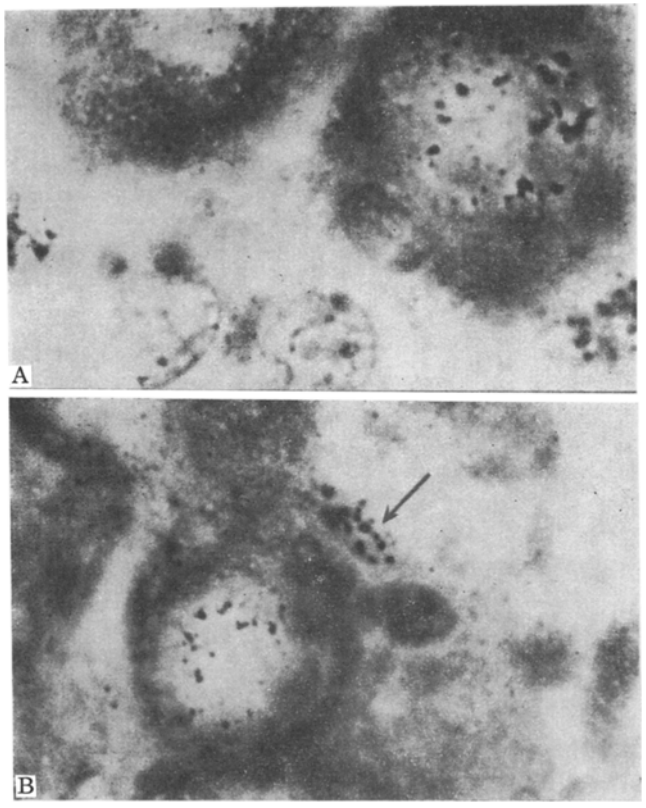

Fig. 1. Incorporation of thymidine-H^3 into DNA of nuclei of sympathetic neurons from adult mice receiving NTGF injections: A) labeled nucleus of nerve cell; B) labeled nucleus of nerve and glial cells (arrow). Counterstained with methyl green–pyronine, 1000×.

Examination of the autoradiographic sections showed that the nuclei of some neurons from the stellate ganglia of the experimental animals contained thymidine-H^3 label (Fig. 1), indicating the occurrence of DNA synthesis in them.

The accumulations of silver grains recorded in the autoradiographs indicate incorporation of the labeled precursor into DNA of adult neurons, because in work with sympathetic ganglia the main objections put forward usually against incorporation of thymidine-H^3 into the nuclei of nerve cells located in the central nervous system can largely be ruled out [2-4]. First, there is no germinative zone in the sympathetic ganglia of adult animals which could contain neuroblasts capable of division. Second, a special investigation showed that injection of NTGF into adult mice does not increase the number of nerve cells in their sympathetic ganglia [6]. This last finding rules out the possibility of the appearance of labeled neurons as a result of rapid differentiation of previously labeled single neuroblasts scattered among the ganglion cells. The probability that grains belonging to the nucleus of a gliocyte lying below or above the nucleus of a nerve cell could be taken for label actually in the latter nucleus was also unlikely in this case. Evidence of the improbability of this situation was given by the ratio between the dimensions of the sympathetic neurons and their nuclei, the dimensions of the satellite cells and the thickness of the section. A further argument to support the view that the precursor was incorporated into the nucleus of a nerve cell was the frequent coincidence between the distribution of silver grains and the chromatin structures in the labeled nucleus (Fig. 1). In addition, staining of the sections with methyl green–pyronine gave a predominantly green component in the color of the nuclei of the glial cells, and a violet component in the nuclei of the neurons, so that superposition of the nuclei would be clearly visible on examination of the sections.

In most cases the labeled nuclei belonged to medium-sized neurons. The well-developed tigroid in the cytoplasm of these cells indicates that they are fully matured. The intensity of labeling in the nuclei

of sympathetic cells is low. Neurons incorporating thymidine-H^3 were found in the stellate ganglia of experimental mice sacrificed both 24 h and 7 days after the last injection of NTGF. No labeled nuclei of nerve cells were found in the control animals.

The labeled precursor of DNA was also incorporated into the nuclei of some glial cells of the experimental and control mice. The intensity of the label above the nuclei of the gliocytes was usually much higher than above the nuclei of the neurons.

The results described in this paper are interesting because, first, they indicate that differentiated neurons are capable, in principle, of synthesizing DNA; second, by illustrating a temporary connection between polyploidization of the nerve cells of adult mice, recorded cytophotometrically, and incorporation of thymidine-H^3 into their nuclei, they demonstrate that DNA is being synthesized in order to increase the ploidy of the neurons, and third, the fact that polyploidization can be induced by the action of a substance which also leads to hypertrophy of the bodies of the sympathetic neurons and to hyperplasia of their processes [5] suggests a link between the phenomenon of polyploidy in neurons and growth processes in the nervous system.

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