AUTORADIOGRAPHIC ANALYSIS OF TRANSCRIPTION IN NUCLEI OF SYMPATHETIC NEURONS AFTER AXONAL INJURY

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The response of nerve cells to axonal injury is manifested regularly by morphological changes in the chromatophilic substance, nucleus, and nucleolus [9]. Quantitative cytochemical and autoradiographic methods in this situation reveal definite changes in the concentration and content of RNA [3, 8] and in some parameters of transcription [12, 13]. However, existing data on this problem are contradictory.

The aim of this investigation was an autoradiographic analysis of the nuclear chromatin of sympathetic neurons in order to discover any particular features of transcription in the cells at the height of development of morphological features of the axonal reaction.

EXPERIMENTAL METHOD

Experiments were carried out on six rabbits weighing 3 kg. Under pentobarbital anesthesia one trunk of the internal carotid nerve of the cranial cervical ganglion was divided. The animals were killed 8 days after the operation by air embolism. Ganglia from intact rabbits served as the control. The level of transcription was estimated by demonstrating activity of endogenous RNA-polymerases by an autoradiographic method [1, 10]. Longitudinal sections through the rabbit cranial cervical ganglion, 10 μ thick, were cut in a freezing microtome at −20°C, semidried in air, and fixed in a mixture of alcohol and acetone (1:1) for 5 min. Until required for use, the sections were kept at −20°C. Incubation mixture, made up in accordance with the formula given in [1], was then applied to the sections. Its essential component was a mixture of unlabeled nucleotide triphosphates (GTP, CTP, UTP) and one labeled nucleotide triphosphate ([^{3}H]-ATP, specific activity 27 Ci/mmole). The sections were incubated at 37°C for 30 min. The reaction was stopped by rinsing the sections in distilled water and they were postfixed in a mixture of ethanol and acetic acid (3:1) for 30 min at room temperature. Unincorporated triphosphates were removed by placing the sections in 5% TCA for 15 min, and then rinsing them in running water for 30 min. The sections were dried, coated with nuclear emulsion, exposed for 1.5 weeks, and developed. They were counterstained with 0.1% methylene blue solution.

The level of activity of endogenous RNA-polymerases was determined by counting the number of grains of silver above the nuclei, paying special attention to label above the nucleolus. A nucleolus with two grains of silver or more was considered to be labeled. The intensity of labeling of the nuclei, obtained by Moore's method, is proportional to the number of termination points of active genes, and it thus indicates the relative number of transcribed DNA sites [4, 10]. Altogether 50 cells were counted for each animal. The numerical data were subjected to statistical analysis by the Fisher–Student method [2].

EXPERIMENTAL RESULTS

After isolated division of one branch of the internal carotid nerve, the main concentration of retrogradely changed perikarya was found in longitudinal sections through the rabbit cranial cervical ganglion in the cranial region. Some of these neurons were mononuclear, others were binuclear (Fig. 1). Perikarya in a state of retrograde degeneration also were found in other zones of the ganglion, including the caudal region. Very few were found there.

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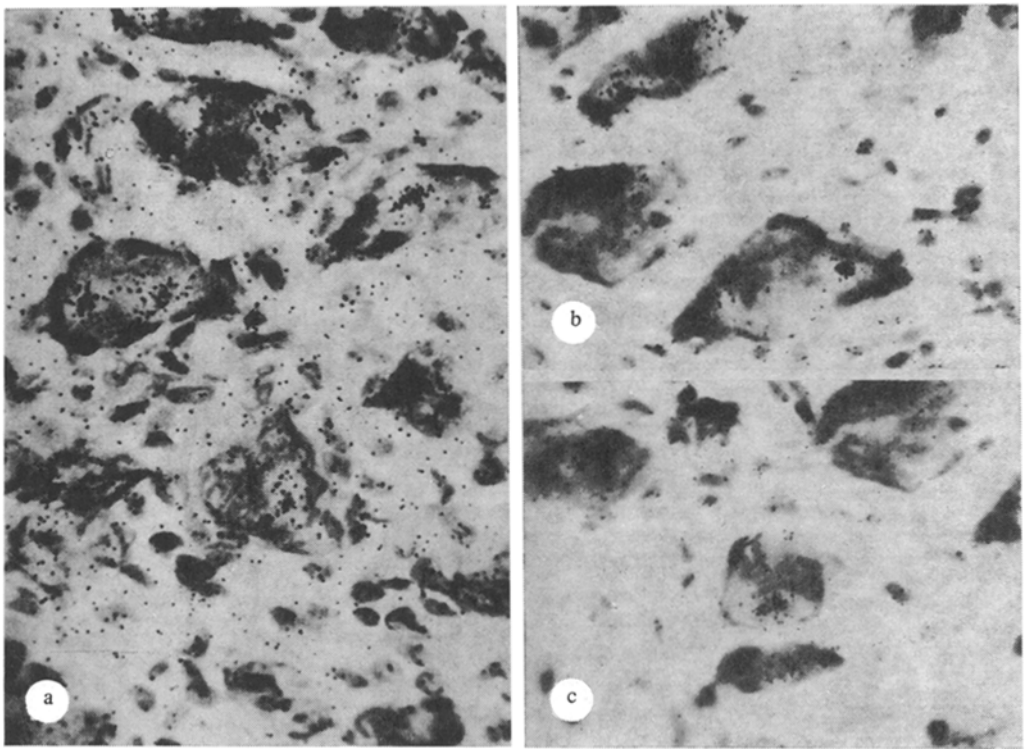

Fig. 1. Perikarya of mono- and binuclear neurons of control rabbits (a) and animals with isolated division of internal carotid nerve (b, c). Method in [10]. Objective 40, ocular 10.

The number of grains of silver was counted above the nuclei of neurons with a typical axonal reaction, in cells from the cranial region in the immediate vicinity of the zone of concentration of retrogradely changed neurons, but preserving their normal morphology, cells in the caudal region of the ganglion near the point of departure of the sympathetic trunk, and neurons of uninjured ganglia in intact animals.

Table 1 gives the results of estimation of the intensity of total, nucleolar, and nucleoplasmic labeling in the groups of neurons mentioned above. The decrease in labeling of cells located in the caudal region of the injured ganglia compared with neurons in intact rabbits will be noted ($P < 0.05$). These changes may be connected with the stimulating effect of the manipulations involved in isolation of the ganglion. Retrogradely changed neurons were characterized by a decrease by half in the intensity of total labeling of the nuclei compared with cells from ganglia of intact animals ($P < 0.001$) and by a one-third less intensity of labeling compared with cells of the caudal region of the ganglia of rabbits undergoing the operation ($P < 0.01$). Neurons from the cranial region of the ganglia of animals undergoing the operation, which preserved a relatively normal structure of their Nissl's substance, were distinguished by a higher level of total nuclear labeling than cells exhibiting an axonal reaction ($P < 0.01$). With respect to the value of this parameter they closely resembled neurons from the caudal region ($P > 0.05$).

By contrast with total nuclear labeling, the mean level of nucleolar labeling was comparable in neurons of all cell groups studied in ganglia from rabbits undergoing the operation ($P > 0.05$). At the same time, a higher intensity of labeling was recorded in the case of this structure in neurons of ganglia of intact animals ($P < 0.01$). This confirms the view that the experimenter's manipulations on the ganglion in the course of the operation may have affected the total nuclear labeling.

Trauma to the axon thus causes a marked decrease in total and nucleoplasmic labeling of the nuclei of cells with well-marked morphological features of an axonal reaction. The level of nucleolar labeling is unchanged under these circumstances.

The axonal reaction is a complex process consisting of several stages, which differ in the character of the cytological changes and which do not coincide in time for different

TABLE 1. Intensity of Labeling of Nuclei of Rabbit Neurons after Isolated Division of a Branch of the Internal Carotid Nerve (number of grains of silver above the structure, M ± m)

Experimental conditions	Neurons from control ganglia	Neurons from caudal region of ganglion	Unchanged neurons from cranial region of ganglion	Retrogradely changed neurons from cranial region of ganglion
Total nuclear labeling	21,3 22,0 22,5	15,7 18,4 18,9	14,8 15,7 14,5	11,8 11,9 11,0
Mean	21,9±0,42	17,6±1,21	15,0±0,43	11,5±0,35
Nucleolar labeling	4,5 4,3 3,8	2,9 2,8 2,8	2,8 2,8 2,5	3,1 2,4 2,6
Mean	4,2±0,26	2,8±0,04	2,7±0,12	2,7±0,25
Nucleoplasmic labeling	16,8 17,7 18,7	12,8 15,6 16,1	12,0 12,9 12,0	8,7 9,5 8,4
Mean	17,7±0,66	14,8±1,25	12,3±0,36	8,8±0,40

types of nerve cells. This evidently explains the disagreement between the results of investigation of incorporation of RNA precursors into the perikarya obtained by different workers [7, 11]. Imperfection of the method of routine autoradiography may play a definite role: dependence of the end result on the state of permeability of the surrounding tissues and cell membrane, on the effectiveness of utilization of the exogenous precursor by the cell, and on the rate of processing and of nucleo-cytoplasmic transport of the labeled RNA. For instance, various workers have demonstrated increased incorporation of labeled nucleotides into RNA during the first few days after trauma to the processes of neurons [8, 14] or later [11]. It has been shown, however, that division of the main nerve trunks of the rat cranial cervical ganglion was accompanied by hydration of the ganglion tissues [6] and initially increased transport of RNA into the cytoplasm of neurons of certain brain-stem nuclei, which fell more rapidly than in the control [14]. Meanwhile definite changes in DNA-dependent RNA synthesis in nuclei of neurons evidently take place in the early stages of the axonal reaction. This is shown by delay of the corresponding morphological changes to the perikarya as a result of administration of actinomycin D to the animals before, during, or immediately after the operation [12, 13]. An indirect indicator of the level of DNA-dependent RNA synthesis is the quantity of labeled actinomycin D bound with chromatin. This parameter increased after axotomy performed on neurons of the rat hypoglossal nerve [15]. Moore's method is carried out *in vitro* on fixed material. Incorporation of the precursor takes place in the course of elongation of the synthesized RNA molecule, and with the completion of synthesis, the RNA remains on the template [10]. This largely eliminates the limitations of routine autoradiography. Allowing for the particular features of the method used, it can be concluded from our data that the state of well-marked morphological manifestations of the axonal reaction is characterized by a decrease in the number of transcribed extranucleolar DNA sites. This stage of the axonal reaction of rat sympathetic neurons is distinguished by a substantial decrease in synthesis of mediator and enzymes concerned in the metabolic basis of specific cell functions, but by maintenance of the biosynthesis of structural proteins of axons at a high level [5]. The decrease in nucleoplasmic incorporation, while nucleolar is preserved, in this situation may reflect a switch of metabolism to synthesis of a more restricted group of proteins than during normal cell function.

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PROLIFERATIVE ACTIVITY OF LYMPHOCYTES IN CULTURE AFTER EXPOSURE TO THE MUTAGENIC ACTION OF THIOPHOSPHAMIDE *IN VIVO* AND *IN VITRO*

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The effect of chemicals with mutagenic activity on cell proliferation has been studied as a rule incidentally to the main investigation of cytogenetic effects of mutagens. The research workers concerned were interested chiefly in the degree of correlation between frequencies of cytogenetic effects and changes in cell proliferation [4-6]. No work has been done on the study of dependence of proliferative activity of cells on the mutagenic dose *in vivo* or *in vitro*. Such investigations are important for choosing the conditions under which the results of cytogenetical analysis of mutagenic action *in vitro* can be extrapolated directly to the living organism.

In this investigation the proliferative activity of lymphocytes was studied after exposure to the mutagenic action of thiophosphamide *in vivo* and *in vitro*.

EXPERIMENTAL METHOD

The effect of dose of mutagenic action on proliferative activity of the cells was studied on rabbit blood lymphocytes treated with thiophosphamide *in vivo* and *in vitro* [1]. The term "exposure dose" of thiophosphamide *in vitro* was taken to mean the product of concentration and duration of exposure (60 min), whereas *in vivo* it was taken to be the integral of the change in blood thiophosphamide concentration from the time of injection of the compound to the time of taking the blood sample. The method of culture of the lymphocytes in experiments *in vivo* and *in vitro* was the same [1]; the cells were fixed after 56-60 h. To distinguish between the 1st, 2nd, and 3rd mitoses, 5-BUdR was added to the cultures and preparations were stained by the modified method of differential staining of sister chromatids [3].

Dependence of the change in proliferative activity was analyzed by the use of the parameter T, standing for the mean number of divisions through which the cells passed after

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