AUTORADIOGRAPHIC ANALYSIS OF RNA SYNTHESIS BY SYMPATHETIC NEURONS IN A POPULATION CONTAINING A REDUCED NUMBER OF CELLS

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An autoradiographic analysis was made of the dynamics of synthesis of nuclear and cytoplasmic RNA by sympathetic neurons of presenile mice subjected to partial desympathization during the first days after birth by injection of guanethidine. Neurons from a population with a sharply reduced number of cells have lower rates of migration of newly synthesized RNA from nucleus into cytoplasm than in the corresponding age control. Neurons of partially desympathized animals of infantile, juvenile, and presenile ages are no larger than the cell bodies of control mice.
KEY WORDS: sympathetic neurons; guanethidine, [^{3}H]uridine.

Electron-microscopic analysis of the perikarya of sympathetic neurons from mice after partial immunosympathectomy has shown that if the nerve cells remain for a long time in a population with a greatly reduced number of cells ultrastructural changes take place, in particular, in the apparatus of protein synthesis [4].

This paper describes a comparative autoradiographic analysis of the dynamics of synthesis of nuclear and cytoplasmic RNA by sympathetic neurons of presenile mice undergoing chemical desympathization and the appropriate controls. The dimensions of the nerve cell bodies in sympathetic ganglia of animals of different ages were studied at the same time.

EXPERIMENTAL METHOD

Hybrid CBA × C57BL/6 mice aged 12 months, corresponding to the initial stages of the period of marked senile changes [1], were used. Desympathization was produced by injection of guanethidine (Isobarin, Pliva, Zagreb, Yugoslavia) into mice of the experimental group from the 1st through the 14th days after birth in a daily dose of 15 mg/kg. This series of injections caused death of 70-80% of neurons in the sympathetic ganglia. Starting from the age of 1 month, the experimental animals differed only a little in their rate of development from the controls. For instance, the weight of the control mice and of animals recieving guanethidine was 13 ± 1 and 15 ± 1 g at the age of 1 month, 25 ± 1 and 25 ± 1 g at 6 months, and 37 ± 2 and 40 ± 1 g at 12 months. When the mice of the control and experimental groups reached the age of 12 months they were given a subcutaneous injection of [^{3}H]uridine in a dose of 2 μCi/g (specific activity 40 Ci/mmole). The animals were killed 3, 6, 12, and 24 h after the injection of the labeled precursor. The superior cervical sympathetic ganglia were fixed in Carnoy's fluid and embedded in paraffin wax. Sections 7 μ thick were covered with type M nuclear emulsion and exposed for 1 month. The autoradiographs were analyzed visually by counting the number of grains of silver separately above the nucleus and cytoplasm of the perikarya of 100 neurons from each animal. At each time point ganglia from three or four mice were investigated. Depending on the intensity of the background, the results were corrected separately for each section by allowing for differences in the areas of cross section of the nucleus and cytoplasm in the plane of the section. Curves of disappearance of the label from the nucleus and its accumulation in the cytoplasm were plotted from the results of the count. Analysis of the character of the curves reflecting the dynamics of labeled RNA in the cell overcomes some of the difficulties associated with the functional assessment of results obtained by autoradiography [2].

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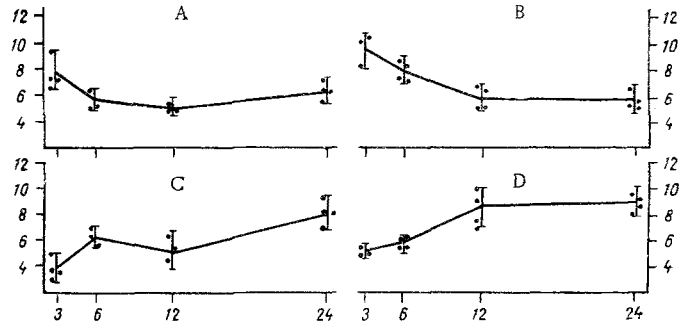

Fig. 1. Changes in intensity of labeling of nucleus (A and B) and cytoplasm (C and D) of sympathetic neurons of normally developing mice (A and C) and mice receiving guanethidine (B and D) at different times after injection of isotope. Abscissa, time after injection of [^{3}H]uridine (in h); ordinate, intensity of labeling (mean number of grains of silver above nucleus or cytoplasm) with 99% confidence interval. Each point is result for one animal.

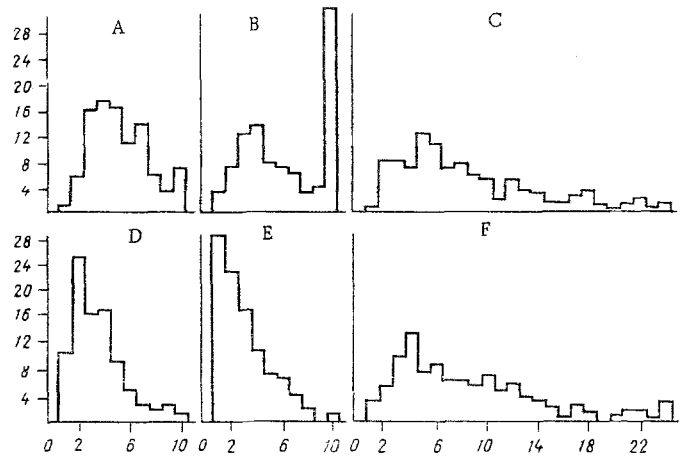

Fig. 2. Distribution of volume of perikarya of sympathetic neurons of control (A, B, C) mice and mice receiving guanethidine (D, E, F) aged 1 month (A, D), 6 months (B, E), and 12 months (C, F). Abscissa, volume of bodies of neurons (in conventional units); ordinate, number of perikarya (in %).

Considering the possibility of development of compensatory changes in the neurons in a population containing a reduced number of cells [3, 4], a special study was made of the distribution of sympathetic nerve cells of the control and experimental mice based on the size of the perikarya. These were approximated by ellipsoids of rotation [5]. The volume was calculated by the formula $V = (\pi/6)\, AB^2$, where A is the major and B the minor diameter of cross section of the perikaryon measured by means of a screw-operated ocular micrometer. The dimensions of the bodies of the neurons were determined for the superior cervical sympathetic ganglia of mice aged 1, 6, and 12 months.

EXPERIMENTAL RESULTS

Curves showing the change in intensity of label above the nucleus (A and B) and cytoplasm (C and D) of neurons of the superior cervical ganglion of control (A and C) and experimental (B and D) mice aged 12 months are given in Fig. 1. The course of the curves of disappearance of label in nucleus and its accumulation in the cytoplasm was, in principle, similar in type of neurons of the control and experimental animals.

The intensity of label above the nucleus, having reached a maximum after 3 h, decreased with the further passage of time after injection of [^{3}H]uridine. As the level of the labeled nucleus fell, accumulation of the label was observed above the cytoplasm. Meanwhile, distinct differences are evident between the curves. For instance, the level of label above the nuclei of the control neurons (Fig. 1A) flattens on a plateau as early as 6 h after injection of [^{3}H]uridine, whereas in the experimental animals (Fig. 1B) this does not happen until 12 h. A further rise in the intensity of labeling of the cytoplasm in the control neurons was observed 6 h after injection of [^{3}H]uridine (Fig. 1C) and in neurons of the experimental animals after 12 h (Fig. 1D). These differences in the dynamics of uridine labeling of nucleus and cytoplasm indicate that sympathetic neurons from superior cervical ganglia of mice aged 12 months, containing a reduced number of cells, differ from the corresponding neurons of normally developing animals of the same age by their lower rate of migration of newly synthesized RNA from nucleus into cytoplasm. No definite conclusion can be drawn from these data regarding the amount of RNA transported from nucleus into cytoplasm of the neurons of the experimental and control mice. However, it should be noted that the maximum of cytoplasmic labeling of neurons of mice receiving guanethidine (the 12-h point in Fig. 1D) is statistically significantly higher than the maximum of the first peak of intensity of labeling above the cytpolasm of the control neurons (6-h point in Fig. 1C). A rather higher intensity of labeling of the nuclei also was observed in the cells of the experimental mice 3 h after injection of the isotope ($P \leq 0.05$). Taken as a whole, these results suggest that rather more RNA incorporating [^{3}H]uridine was synthesized in the neurons of the experimental mice. Meanwhile, the higher intensity of nuclear labeling of neurons from populations with a reduced number of cells can be explained by the lower rate of evacuation and, consequently, by retention of the labeled RNA in the nuclei. The possibility likewise cannot be ruled out that differences in the absolute values of the level of nuclear and cytoplasmic labeling of neurons from the control and experimental populations at the same time points depend on differences in the size of the pool of labeled precursor at the moment of synthesis or in the efficiency of self-absorption of β particles by the material of the cells compared.

The results of analysis of the distribution of neurons from the superior cervical sympathetic ganglia of the control mice and mice receiving guanethidine, at different ages, by the size of their perikarya are given in Fig. 2. Clearly the distribution of the sympathetic neurons with respect to this criterion was virtually the same for the control and experimental animals aged 12 months (Fig. 2C, F), whereas for the experimental mice aged 1 and 6 months (Fig. 2D, E) there was a higher proportion of relatively small neurons compared with the corresponding age control (Fig. 2A, B). This last fact is evidence that the processes of individual development of the sympathetic neurons of the control mice and mice receiving guanethidine in fact differ in certain details. Unlike neurons from mice receiving injections of antibodies against nerve growth factor [6], neurons surviving in the sympathetic ganglia after a course of guanethidine injections do not increase the size of their perikarya in the infantile and juvenile periods. Meanwhile, after both methods of partial desympathization, neurons remaining viable in the sympathetic ganglia of adult and presenile mice [1] exhibit special features in the state of their protein synthesizing apparatus.

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