individual zones — superior vena cava, atrial septum, oblique vein of the left auricle, and coronary sinus — evidently indicates a regional principle of organization of the SHF pool in auricular tissue.

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INVESTIGATION OF TRANSCRIPTION IN RAT SYMPATHETIC NEURONS AT DIFFERENT STAGES OF POSTNATAL DEVELOPMENT

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UDC 612.89.014.2:577.214:612.66

KEY WORDS: transcription; autoradiography; neuron; postnatal development.

Morphological and functional changes arising in nerve cells in the course of life in connection with events such as differentiation, maturation, variations in functional load, and aging are closely linked with structural changes in the protein-synthesizing apparatus of the cell, leading to changes in the volume and spectrum of proteins synthesized [9, 13]. The concrete mechanisms of these structural changes have received little study, although there is no doubt that some of them at least are responsible for changes at the transcription level.

The aim of the present investigation was an autoradiographic analysis of transcription in the nuclei of sympathetic nerve cells of rats at different stages of postnatal development.

EXPERIMENTAL METHOD

Neurons from the cranial cervical ganglion of rats aged 1, 6, 13, and 25 days and 1, 5, 9, and 30 months (3-5 animals in each age group, 50 cells from each animal) were used as the test object.

To assess the state of transcription in the test neurons an autoradiographic method was used to demonstrate activity of endogenous RNA polymerases in the fixed cells [10]. For this purpose the sections through the cranial cervical ganglion 8 μm thick, cut on a freezing microtome at −20°C, were air-dried and fixed in alcohol—acetone (1:1 v/v) for 5 min at 4°C. The preparations were kept until required at −20°C. To each section 0.02 ml of an incubation mixture of the following composition (in μM) was applied: Tris-HCl buffer (pH 7.9) 100, sucrose 150, ammonium sulfate 80, 2-mercaptoethanol 12, ^{3}H-ATP (specific activity 27 Ci/mmole, from Radiochemical Centre, Amersham, England) 0.02, unlabeled triphosphates 0.6 of each, $MgCl_2$ 8, $MnCl_2$ 2. After application of the mixture the sections were incubated at 37°C for 30 min. The reaction was stopped by washing the preparations thoroughly in distilled water and they were then fixed for 30 min with ethanol—acetic acid (3:1 v/v). Unincorporated triphosphates were removed with 5% TCA (15 min at 4°C), after which the sections were rinsed for 30-60 min in tap water. The sections were dried, coated with type M emulsion, and exposed for 10 days.

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 110-112, July, 1981. Original article submitted September 19, 1980.

0007-4888/81/9207-0987$07.50

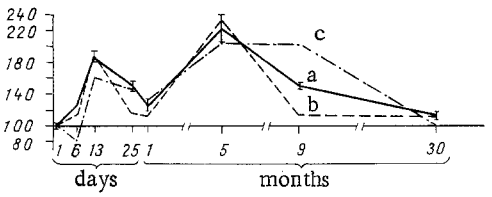

Fig. 1

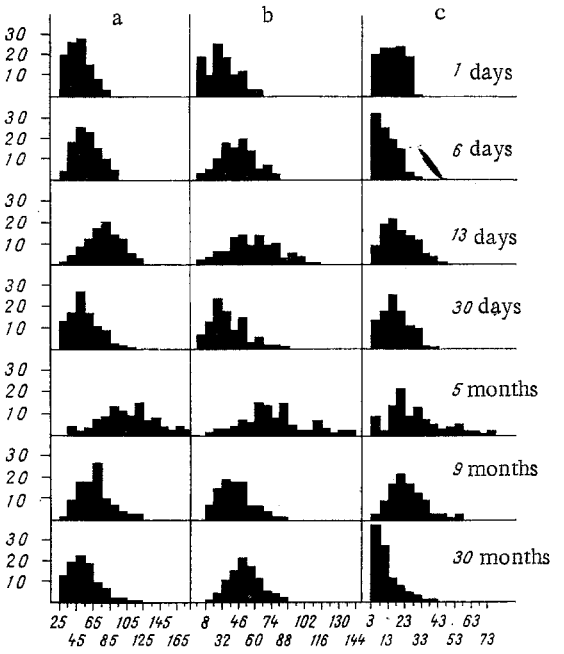

Fig. 2

Fig. 1. Dynamics of changes in mean intensity of nuclear labeling of neurons in cranial cervical ganglion of rat during postnatal development. a) Nucleus, b) nucleoplasm, c) nucleolus. Abscissa, age of animals; ordinate, mean intensity of labeling relative to age of 1 day (in %). Vertical lines show error of mean.

Fig. 2. Distribution of nuclei of cranial cervical ganglion neurons in rats of different ages by intensity of labeling of nuclear structures. a) Nucleus, b) nucleoplasm, c) nucleolus. Abscissa, intensity of labeling (in conventional units — number of grains of reduced silver); ordinate, number of cells (in %).

The state of transcription was assessed from the intensity of labeling of the nuclei, characterized by the number of grains counted visually above the nucleolus and nucleoplasm separately. The total of these values gave the level of nuclear labeling. As the author of the technique emphasizes, initiation of transcription does not take place in the fixed cell and the labeled precursor is incorporated in the course of elongation; the newly synthesized RNA molecules, moreover, remained fixed on the DNA template at the termination points [10]. Comparison of the mean dimensions of one transcribed region of DNA in the genome of certain eukaryotes [6] with the resolving power of autoradiography of histological sections [2] shows that the number of grains of reduced silver detectable in the nucleus by this method is evidently proportional to the number of transcription termination points, so that the number of DNA regions counted at the moment of fixation and, to a certain extent, the relative size of that part of the genome from which information is read, can be assessed indirectly.

To estimate the significance of age differences between the mean values of intensity of labeling Student's t-test was used, and to compare the histograms, Wilcoxon's test was used.

EXPERIMENTAL RESULTS

The curve given in Fig. 1a demonstrates the presence of definite age dynamics of the mean level of "nuclear" labeling in the neuron population studied. By the 13th day, for instance, it showed a considerable increase, but in animals aged 25-30 days it had fallen again. In animals aged 5 months this index was up again, after 5 months it began to fall, and in old rats it reached a minimum. The statistical significance of the difference between the age points was not below 99% on average.

Analysis of the corresponding histograms, reflecting the character of distribution of cells with different intensities of labeling in the test sample (Fig. 2a) shows that age differences in the mean level of "nuclear" labeling were due to changes in the relative percent-

ages of weakly, moderately, and strongly labeled cells with age. For instance, whereas in day-old animals practically all neurons studied could be conventionally defined as weakly labeled (25-75 conventional units), in the week-old rats a definite percentage of moderately labeled nuclei (75-125 conventional units) had begun to appear, and in animals aged 13 days these nuclei accounted for about half of the total. In rats aged 1 month there was again a sharp rise in the percentage of cells with weakly labeled nuclei. By 5 months the majority of cells in the population of the cranial cervical ganglion were moderately labeled and, in addition, a considerable number of strongly labeled cells appeared (125-185 conventional units); the last group practically disappeared by the age of 9 months and were absent in old rats, in which the majority of cells were weakly labeled.

Differential analysis of the intensity of nucleolar and nucleoplasmic labeling showed that the character of the age dynamics of this parameter differed in the two cases. The course of the curve for nucleoplasmic labeling (Fig. 1b) was similar at all age points except the last (old) to that described above for nuclear labeling, or in other words, the former determined the latter. On the right-hand side of the curve it will be noted that the value of this parameter did not differ statistically significantly ($P > 0.05$) for rats aged 9 and 30 months.

Analysis of the histograms for nucleoplasmic labeling shows that the age shifts in mean values for the ganglion as a whole were due in this case also to changes in the ratio of cells with different intensities of labeling. The character of the changes as a whole was similar to that described above for "nuclear" labeling, the only difference being that in this case strongly labeled cells were beginning to appear at the age of 13 days.

The dynamics of the mean values of nucleolar labeling was similar in character to that for nucleoplasmic within the interval from 6 days to 5 months. In the period from 1 to 6 days the mean intensity of nucleolar labeling fell a little ($P < 0.05$), and between 5 and 9 months it did not change significantly ($P > 0.05$), but later, in rats aged 30 months, it was reduced by almost half. Examination of the corresponding histograms (Fig. 2c) shows that in this case the age changes in mean values described above also reflect changes in the relative percentages of weakly, moderately, and strongly labeled cells in the neuron population of the cranial cervical ganglion during ontogeny. It must be specially mentioned that in old rats the majority of cells had a very low intensity of nucleolar labeling. For most cells this index did not exceed 10 conventional units (within a range of 3 to 70 conventional units).

It is also important to note that although there were no significant differences in the mean intensity of nucleolar labeling, the histograms for rats aged 5 and 9 months differred highly significantly ($P < 0.01$).

It can thus be concluded from the data described above that the number of transcription regions in the genome of neurons in the cranial cervical ganglion of rats changes throughout the animal's life in such a way that these changes do not affect all cells of the ganglion simultaneously (which would cause the whole histogram to shift), but only a certain group of cells.

On the whole the age changes in the number of transcription regions in the nuclei of the neurons studied can be reduced to variations in this parameter in the course of cell maturation, leading ultimately to an increase in its value in adult animals compared with young, followed by a decrease in mature, and more especially in old rats; the dynamics of this decrease differs, moreover, for nucleolar and nucleoplasmic DNA.

If the changes observed are assessed in the context of nerve cell function, it can evidently be legitimately suggested that the significant rise in the number of transcription regions of DNA between the 6th and 13th day is connected with differential activation of specific genes in the course of cell differentiation of the neurons studied. This is confirmed by data in the literature on the phenomenology of morphological and physiological changes taking place during this period in the corresponding nerve cells, reflecting the formation of their specific function [5, 9, 13]. As regards the sharp fall in this parameter toward the end of the first month of life, it can conjecturally be linked with inactivation of the nonspecific part of the genome during differentiation, which as we know from the literature, may be delayed compared with the state of activation of specific genes [3]. The rise in the number of transcription regions in the corresponding cells until the 5th month is evidently due to an increase in the functional potentials of the cells in connection with

growth, and not to differentiation. It is difficult as yet to give an unambiguous interpretation of the fall in this parameter in mature and old animals.

Investigation of transcription in postnatal ontogeny has been undertaken by many workers, on different objects and by different methods. Their reports by no means always contain an indication of the concrete mechanisms responsible for the change in transcription level. Nevertheless, it can be concluded from analysis of age differences in transcription in nerve cells, in agreement with the results of the present investigation, that the rise in its level is associated with differentiation in the period from 1 day to 1 month [4, 7, 8]. The decrease in template activity of DNA in cells of various organs during aging is also in harmony with this conclusion [1, 11, 12, 14].

The results of the present investigation link these changes in transcription with a definite mechanism, namely a change in the number of transcription units of DNA, and in this context they are in good agreement with the observations of Cutler, who showed by the molecular hybridization method that there is an increase in the spectrum of transcribed genes in brain cells of mice aged 4 months compared with newborn mice, after which this parameter falls gradually until old age, when it reaches the level found in young animals [7].

On the whole a comparison of the results of the present experiments with data in the literature suggests that there are definite rules that govern changes in transcription in nerve cells during postnatal ontogeny.

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