CHROMATIN TEMPLATE ACTIVITY IN PURKINJE AND GRANULE CELLS OF THE RAT CEREBELLAR CORTEX DURING POSTNATAL DIFFERENTIATION

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The first month of postnatal life of rodents is the concluding period so far as differentiation of cerebellar cortical cells is concerned and it is characterized by the formation of axons, dendrites, and synapses [3], by the formation of mediator metabolism [5], by increased synthesis of calcium-binding proteins [7] in Purkinje cells, by the appearance of specific immunologic markers of nerve cells [6], and by other processes suggestive of both quantitative and qualitative changes in protein synthesis and, consequently, in transcription. The study of transcription parameters in the rodent cerebellum during early postnatal development has received the attention of several investigators [4, 9, 10], but most investigations in this field have been have been undertaken on a total preparation of cerebellar chromatin, so that it is impossible to judge the relationship between the changes discovered and any particular morphological or functional group of nerve cells forming the cerebellar tissue. In the present investigation Moore's autoradiographic method [8] was used to study template activity of cerebellar cortical cells; this method is based on demonstration of activity of endogenous RNA-polymerases actually in histological sections, so that not only average values of transcription activity for a concrete type of cell could be analyzed, but this parameter could also be subjected to population analysis.

EXPERIMENTAL METHOD

Purkinje cells and granule cells from the cerebellar cortex of rats aged 7, 14, and 30 days and 1 and 3 months were used as the test objects. From each animal 20 Purkinje cells and 30 granule cells were counted. Each age group consisted of 3 or 4 rats. The label above the nucleolus and in the extranucelolar zone of the nucleus was counted separately for Purkinje cells. Histograms of distribution of the nuclei by intensity of labeling were constructed by averaging data for all the animals of each age group. To carry out the RNA-polymerase reaction the cerebellar hemispheres were cut on a freezing microtome at −20°C into sections 8 μ thick, which were dried in air and fixed in a mixture of alcohol and acetone (1:1) for 5 min at 4°C. The sections were kept at −20°C until required for use. To each section 0.02 ml of a 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-UTP 0.02, unlabeled triphosphates 0.6 of each, $MgCl_2$ 8, $MnCl_2$ 2. The sections were incubated at 37°C for 30 min. The reaction was stopped by thoroughly washing sections in distilled water, after which they were postfixed for 30 min with ethanol and acetic acid (3:1). Unincorporated triphosphates were removed by treatment of the material with 5% TCA (15 min at 4°C), after which the sections were washed for 30-60 min in tap water, dried, covered with type M emulsion, and exposed for 10 days. The level of nuclear template activity was estimated as the number of grains of reduced silver, which was determined visually. The value of this parameter is evidently proportional (with the method used) to the number of transcription termination points. To assess the significance of age differences between the average values of template activity thus determined, Wilcoxon's test was used.

EXPERIMENTAL RESULTS

Analysis of nuclear chromatin template activity of the Purkinje cells of the rat cerebellum during postnatal differentiation showed that its mean level changes unequally for

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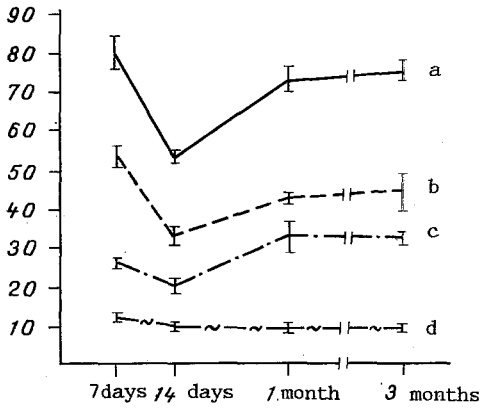

Fig. 1. Age dynamics of mean labeling of nuclear structures of Purkinje cells and granule cells in the rat cerebellar cortex. a) Nucleus; b) nucleoplasm; c) nucleoli (Purkinje cell); d) nucleus (granule cell). Abscissa, age of animals; ordinate, mean labeling level (number of grains of reduced silver).

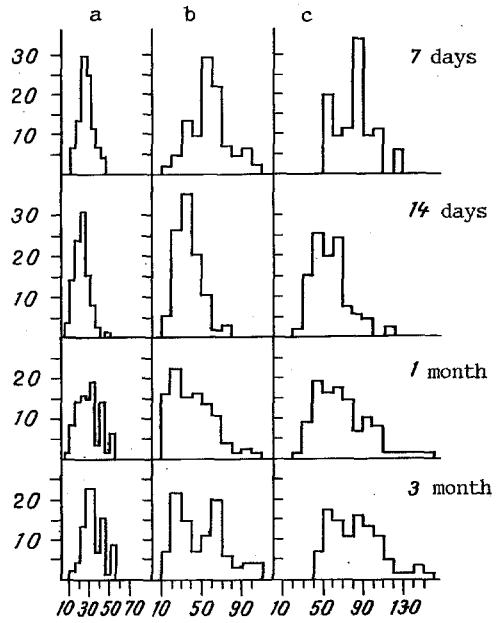

Fig. 2. Distribution of nuclei and nuclear structures of Purkinje cells of rat cerebellar cortex at different ages depending on labeling level. a) Nucleoli; b) nucleoplasm; c) nucleus. Abscissa, labeling level (number of grains of reduced silver); ordinate, number of cells (in percent).

nucleolar and extranucleolar chromatin. For instance, the mean level of nucleoplasmic labeling fell sharply between the 7th and 14th days after birth, and rose again toward the end of the first month whereas the nucleolar labeling level did not change significantly before the 14th day. This parameter changed between the 14th and 30th days in a similar way to the level of nucleoplasmic labeling. After completion of differentiation, between the 1st and 3rd months of postnatal ontogeny, the quantity of label did not change significantly in either the nucleolar or the extranucleolar region of the chromatin (Fig. 1a, b). The dynamics of total nuclear labeling during the period of differentiation of Purkinje cells now being investigated reproduces in principle that observed for the nucleoplasm (Fig. 1a).

Histograms of distribution of Purkinje cells depending on the labeling level showed that differences were not significant when average values were compared. On analysis of histograms for nucleoplasmic labeling (Fig. 2b) the first feature to be noted was the presence of three distinct classes of activity, within which values of template activity of the extranucleolar chromatin of the cerebellar Purkinje cells of rats aged 1 week and 3 months were grouped in something closely resembling a normal distribution. The maximum of each successive class was about twice as high as the maximum of the one before. However, where the percentage of cells belonging to classes 1 and 2 was about equal for the cerebellum of rats aged 3 months, the percentage of cells belonging to class 2, i.e., with average intensity of labeling of the nucleoplasm, in rats aged 1 week was about twice as high as those of class 1. The population of nerve cells studied in rats aged 2 weeks consisted mainly of cells belonging to class 1 with respect to their nucleoplasmic labeling level. At this age there were virtually no intensively labeled cells. The pattern observed in the case of this parameter in rats aged 1 month resembled rather more closely that in rats aged 3 months: in this case there were already a few cells with intensively labeled nucleoplasm, but cells in class 1 were predominant.

The character of distribution of the nerve cells based on the intensity of nucleoplasmic labeling, as shown by the averaged histograms, corresponded sufficiently closely to the pattern obtained by analysis of material from each animal separately.

It is difficult to compare these results with those obtained by other workers because the overwhelming majority of investigations in this field known to us were conducted on the whole cerebellum by biochemical methods. Nevertheless it is interesting to note that, according to data obtained by Morrison et al. [9] by 2-dimensional gel-electrophoresis, a set of actively translated mRNAs, characteristic of the adult rat cerebellum, is formed between the 7th and 14th days after birth. This suggests a connection between the change in template activity discovered during this period in the Purkinje cells of the rat cerebellum and a change in the spectrum of proteins synthesized during postnatal differentiation of these nerve cells [9].

The discrete character of distribution of the Purkinje cells by levels of template activity, clearly revealed on the histograms for nucleoplasmic chromatin of rats aged 3 months, it noteworthy, for a similar picture was obtained by the author previously for rat sympathetic nerve cells [1], although the presence of different types of cells in the cranial cervical ganglion makes an unequivocal interpretation of this result difficult. Purkinje cells constitute a qualitatively homogeneous population, in which only one morphological and functional type is represented. This suggests that the classes revealed in the present investigation characterize the template activity of Purkinje cells quantitatively. A virtually similar discrete character of distribution with respect to functional activity was demonstrated for adult rat hepatocyte nuclei by Nemirovskii et al. [2], who analyzed the size of the nuclear DNA fraction highly sensitive to depolymerization as the parameter of chromatin activity.

Analysis of histograms of distribution of Purkinje cells by levels of nucleolar labeling also indicates the existence of discrete classes of activity; nuclei with an average and high intensity of labeling, moreover, appear for the first time in rats aged 1 month, when the character of their distribution already corresponds to the adult type (Fig. 2a).

Absolute values of nuclear labeling for granule cells toward the end of differentiation of the cerebellar cortex were much lower than those for Purkinje cells (Fig. 1d). Analysis of the age dynamics of mean template activity of the nuclear chromatin of granule cells revealed some difference in its parameter in its character compared with that for Purkinje cells, as well as absence of statistically significant differences for animals aged 14 days and 1 and 3 months. Between 7 and 14 days this parameter for granule cells fell by a small amount, as did the corresponding parameter for Purkinje cells, due to the virtual disappearance of intensively labeled nuclei (Fig. 1d).

Comparison of the age dynamics of template activity of the cerebellar cortical cells with changes in the corresponding parameter for sympathetic nerve cells, which the writer estimated previously by the same (Moore's) method [1], leads to the conclusion that in both cases, at certain stage of postnatal differentiation, the mean level of chromatin template activity falls, and in the case of macroneurons this is followed by a rise, although the actual times of these changes differ for the different parts of the nervous system compared, evidently due to differences in the pattern of formation of nerve cell function in central and peripheral divisions of the nervous system.

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ULTRASTRUCTURE OF THE SINUS AND ATRIOVENTRICULAR NODES IN EXPERIMENTAL MYOCARDIAL INFARCTION

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It has now been established that death from myocardial infarction is often based on disturbances of the cardiac rhythm. Lesions of the conducting system of the heart are a significant factor. However, recognition of this fact alone does not explain the morphological changes taking place in the nodes of the conducting system in myocardial infarction.

The aim of this investigation was an electron-microscopic study of the sinus (SA) and atrioventricular (AV) nodes of the rat heart in experimental myocardial infarction, using colloidal lanthanum as electron-microscopic tracer, and using morphometric methods to evaluate the ultrastructural changes.

EXPERIMENTAL METHOD

Myocardial infarction was induced in 20 noninbred albino rats weighing 180-200 g by ligation of the left coronary artery. The operation was performed under ether anesthesia. Ten rats served as the control. The experimental rats were killed under ether anesthesia 1 day after the beginning of the experiment. The heart was stopped by exposure to cold. The SA node material was taken with the lower part of the superior vena cava and adjacent right atrial myocardium, whereas material of the AV node was taken with the upper part of the ventricular and lower part of the atrial septum [4]. Pieces of tissue were fixed for 2 h in 2.5% glutaraldehyde at 4°C and washed with phosphate buffer (pH 7.4). The material was postfixed in 1% OsO_4 for 2 h. Oriented embedding [8] was used when the fragments were embedded in Araldite.

Ultrathin sections were cut on the Ultrotome-5 (LKB, Sweden), stained on the Ultro Stainer (LKB, Sweden), and examined under the UÉMV-100K electron microscope. Permeability of the sarcolemma was estimated with the aid of colloidal lanthanum, prepared from lanthanum nitrate (from Serva, West Germany) by the method in [9] in the modification [6]. The electron micrographs were analyzed quantitatively by the method in [2] under a magnification of 10,000. The mean number of mitochondria per electron micrograph, the mean number of cristae per mitochondrion, the mean area of 1 mitochrondrion, the mean total area of the mitochondria per electron micrograph, and the mean total number of cristae per electron micrograph were determined. The energy efficiency of the mitochondria (EEM) was calculated

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