QUANTITATIVE DISTRIBUTION OF NUCLEOLAR NUCLEIC ACIDS IN RAT PURKINJE CELLS DURING VESTIBULAR STIMULATION AND IMMOBILIZATION

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Cytophotometric investigations have revealed quantities of DNA intermediate between the 2c and 4c levels in some of the Purkinje cells of certain animals [1-3]. It has been shown that the excess of DNA is localized in the nucleolus [5]. This supported the hypothesis of amplification of ribosomal DNA in Purkinje cells. Asymmetry of the distribution of nucleolar nucleic acids in Purkinje cells was demonstrated later by UV cytophotometry [4]. Comparison of the quantities of nucleic acids in the perinucleolar chromatin of Purkinje cells modal with respect to this feature, and in cells in which the nucleolar nucleic acids went outside the limits of the normal distribution, revealed an excess of nucleic acids in the latter [4]. It was interesting to study the distribution of nucleolar nucleic acids depending on the functional load on the Purkinje cells and also depending on other nonspecific factors acting on them, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Male Wistar rats (age about 1 month, weight 40-50 g) were rotated horizontally at a speed of 60 rpm for 1 h. The animals were placed in constraint cages, fixed to a disk connected to an electric motor. After rotation for 20, 30, 40, 50, and 60 min three rats at a time were decapitated and the nodulus of the cerebellar vermis was isolated. After fixation in a mixture of formalin, alcohol, and acetic acid (3:1:0.3) for 1 h the pieces of tissue were embedded in paraffin wax. Sections about 5 μ thick were photographed in UV light with a wavelength of 265 nm (MUF-6 instrument, objective 50 × 0.8) before and after extraction of the nucleic acids with 5% $HClO_4$ (90°C, 6 min). The negatives were subjected to photometry on the MF-4 microphotometer. The content of nucleic acids in the nucleoli and cytoplasm was determined as the product of optical density (the difference between the values before and after extraction of RNA) and the area of the corresponding structures, measured planimetrically. At each time of exposure 200-250 cells were studied. The content of nucleic acids in different parts of the Purkinje cells of rats kept for different periods in the same constraint cages as in the experiments, but not rotated, also was measured by the same method.

EXPERIMENTAL RESULTS

Histograms of the distribution of the nucleic acid content in the nucleoli in the control and at different time points of functional stimulation showed a general tendency toward asymmetry (Fig. 1).

Some fluctuation also was observed in the number of cells which were outside the limits of the normal distribution and in the deviation of the fraction of nucleoli with an excess of nucleic acids from the center of the Gaussian curve. For example, after rotation for 10 min these parameters increased. The very small magnitude of the changes and the short duration of functional stimulation of the cells make it unlikely that the changes were significant, still less than they were connected with the cell genome. The histogram of nucleic acid distribution in the nucleoli after rotation for 30 min was narrowed and most of the binucleolar cells had shifted closer to the center of the Gaussian curve. These changes

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TABLE 1. Mean Nucleic Acid Content in Cytoplasm and Nucleoli of Purkinje Cells of Rats after Horizontal Rotation (A) and Immobilization (B) (M ± m)

Duration of exposure, min	RNA content in cytoplasm, conventional units		Nucleic acid content in nucleolus, conventional units	
	A	B	A	B
Control	85±2	85±2	8,2±0,1	8,2±0,1
10	118±2	117±2	9,0±0,2	9,1±0,2
20	90±2	98±2	8,3±0,2	7,7±0,2
30	99±1	113±3	6,4±0,2	7,5±0,3
40	133±2	91±2	8,2±0,2	6,5±0,2
50	92±2	121±2	7,5±0,2	8,3±0,2
60	89±2	—	7,8±0,2	—

may perhaps reflect a transient disturbance of the balance between RNA synthesis and its transport in response to inadequate stimulation of the neurons. The increase in the number of binucleolar cells after rotation for 40 min (corresponding to the peak of RNA accumulation in the cytoplasm of the cells studied; Table 1) was replaced by a decrease after rotation for 50 and 60 min, and this also makes it impossible to connect these changes with amplification of the rRNA of the genes. However, the results of this experiment likewise do not rule out the existence of such a connection. It is not impossible that unbalanced RNA transport leads to loss of a small number of newly formed secondary nucleoli of very low density during photometry. Such a loss could not lead to any mistaken conclusion regarding asymmetry of the histogram of nucleic acid distribution in the nucleoli of the Purkinje cells, for the small number of lost binucleolar cells would lie within the limits of the Gaussian curve at all low densities. The fact that in all six rats tested after rotation for 50 and 60 min the number of binucleolar cells was lower than in each of the three rats tested after rotation for 40 min demands additional verification of the reproducibility of the observed changes so that the possibility that these measurements were simply individual differences can be excluded.

Aggregated histograms of distribution of nucleolar nucleic acids in Purkinje cells, generalizing the results of the experiment with immobilization of the animals without rotation (Fig. 2), differed from the histograms of animals subjected to vestibular stimulation. After 10 min of their stay in the constraining cages the number of binucleolar cells was very slightly less than in the control. The number of cells lying outside the limits of the normal distribution also was not increased. Only at one time point (a stay of 20 min in the cage) was correlation found between changes in the nucleic acid content in the nucleoli and cytoplasm (Fig. 2; Table 1) and changes in the degree of asymmetry of the histogram.

At two time points (30 and 40 min) the increase and decrease in the nucleic acid content in the nucleoli were accompanied by similar changes in the number of binucleolar cells and asymmetry of the histograms (in rats exposed to this type of rotation correlation was found at all time points of functional stimulation for the cytoplasm and at most points for the nucleolus; Fig. 1 and Table 1). It is also important to note that the aggregated histograms of the main experiment reproduce the distribution of nucleolar nucleic acids in each individual animal well, at a time when changes in the aggregated histograms of the animals from the control experiments are in fact the sum of what are sometimes even opposite changes in individual animals.

The data described above become more informative when they are compared with changes in the distribution of cytoplasmic RNA (cRNA) in these same cells after exposure to the same factors. Histograms of cRNA distribution during vestibular stimulation (Fig. 3) show the same tendency toward asymmetry and the shifts during 1 h of stimulation reproduce well the shifts in the distribution of the nucleolar nucleic acids. Histograms of the immobilized animals also reveal heterogeneity of the Purkinje cells for the RNA content in their cytoplasm. The smallness of the increase or decrease in asymmetry of the histograms for both procedures studied prevents discussion of the results, but the parallel nature of the changes in the nucleoli and cytoplasm during vestibular stimulation is evidence of the significance of

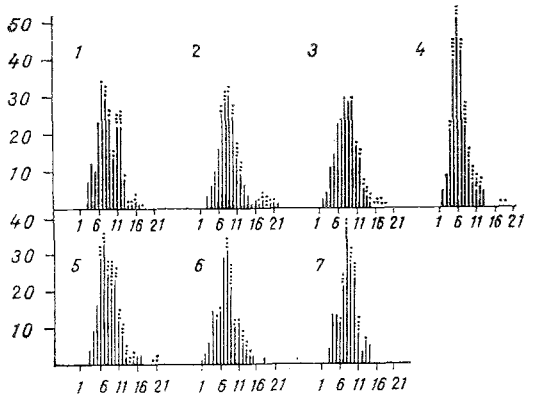

Fig. 1. Distribution of nucleic acid content in nucleoli of Purkinje cells of rats during vestibular stimulation of cerebellum. Abscissa, nucleic acid content (in conventional units); ordinate, number of cells: 1) control; 2) rotation for 10 min; 3) 20 min; 4) 30 min; 5) 40 min; 6) 50 min; 7) 60 min. Straight line denotes mononucleolar cells; each dot represents one binucleolar cell; 200-250 cells from three animals at each time point.

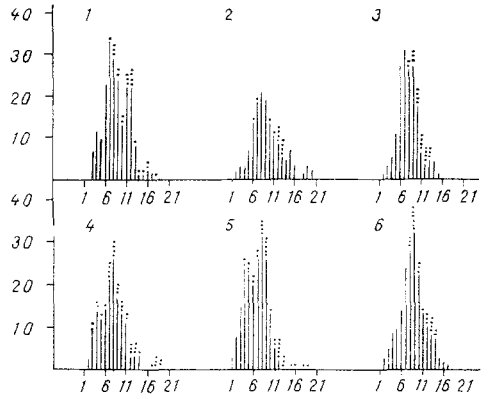

Fig. 2

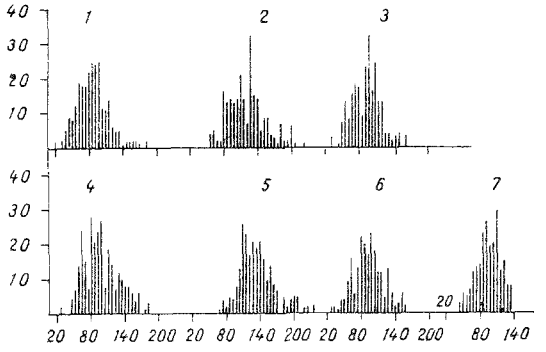

Fig. 3

Fig. 2. Distribution of nucleic acid contents in nucleoli of Purkinje cells after immobilization of rats. Ordinate, number of cells: 1) control, 2) immobilization for 10 min, 3) 20 min, 4) 30 min, 5) 40 min, 6) 50 min; 170-230 cells from two animals at each time point. Remainder of legend the same as in Fig. 1.

Fig. 3. Distribution of RNA content in Purkinje cells after vestibular stimulation of cerebellum in rats. Abscissa, RNA content (in conventional units). Remainder of legend as to Fig. 1.

these changes. The Purkinje cells of the rat cerebellum are heterogeneous for the nucleic acid content in their nucleolus and cytoplasm, and it can be concluded from the character of their heterogeneity that there are two distinct subpopulations of cells.

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