TIME COURSE OF RNA CONTENT IN CEREBELLAR PURKINJE CELLS IN RATS IN VARIOUS FUNCTIONAL STATES

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Significant quantitative changes in RNA in Purkinje cells were found previously in Purkinje cells of the mouse cerebellum in response to vestibular stimulation [2]. The object of this investigation was to study Purkinje cells in the course of vestibular stimulation and in animals immobilized for different periods. The RNA content was studied in the cytoplasm, nucleus, and nucleoli of the Purkinje cells. The animals were spun under milder conditions than in previous cytochemical investigations of vestibular function [2, 11].

EXPERIMENTAL METHOD

Wistar rats weighing 40-50 g were subjected to horizontal rotation at a speed of 60 rpm for 1 h. The animals were kept in constraining cages, secured to a disk connected to an electric motor. The rats were decapitated three at a time 10, 20, 30, 40, 50, and 60 min after the beginning of rotation, and the nodulus vermis cerebelli, where vestibular fibers are known to be concentrated [9], 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 microscope, 50 × 0.8 objective) before and after extraction of the nucleic acids with 5% $HClO_4$ (90°C, 6 min) [7]. Photometry of these negatives were carried out on an MF-4 microphotometer. The nucleic acid content in the nuclei, nucleoli, and cytoplasm was determined as the product of optical density (difference in values before and after extraction of RNA) and the area of the corresponding structures, determined planimetrically. At each time of stimulation 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 cages as the experimental rats, but not subjected to spinning, was measured by the same method.

EXPERIMENTAL RESULTS

Immobilization of rats may affect the RNA content in the nucleus and cytoplasm of the Purkinje cells (Fig. 1). On comparison with the parameters for intact rats taken from the animal house, changes were not found in all the immobilized animals, but in some animals they were significant. It must be pointed out that a study of several samples of cells from intact animals [2] revealed stability of the RNA content. In the present investigation slight fluctuations in the RNA content were found in immobilized rats. The amplitude of the changes were small — not more than ±15%. However, the RNA content in the cytoplasm of the Purkinje cells after immobilization for 20 min differed significantly in the two rats studied from values obtained after immobilization for 30 min, the latter differed significantly from those obtained after immobilization for 40 min, and these, in turn, differed from the results obtained after immobilization for 50 min. After a small increase, the RNA content in the nucleus fell to a lower level 10 min after the rats had been placed in the cages and during the next 10 min than in intact animals, after which the normal RNA content was restored. It must be emphasized that this method of investigation (UV cytophotometry) enabled both RNA and DNA to be determined in the nucleus. However, the DNA content in the neurons remains

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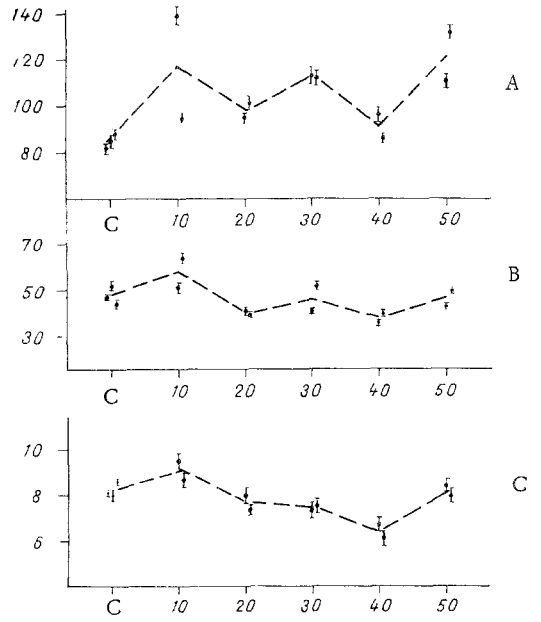

Fig. 1. Nucleic acid content in cytoplasm (A), nuclei (B), and nucleoli (C) of cerebellar Purkinje cells during immobilization of rats. Abscissa, time (in min); ordinate, nucleic acid content (in conventional units). Each point is arithmetic mean of results of UV cytophotometry of 80-130 cells from one rat; short vertical lines denote errors of means. C) Control.

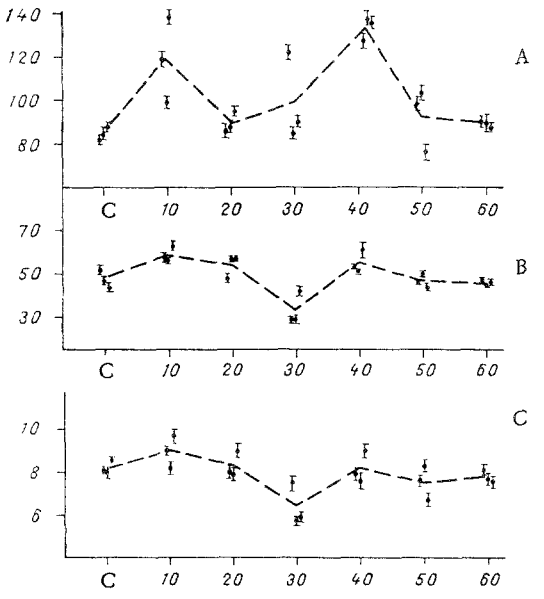

Fig. 2. Nucleic acid content in cytoplasm (A), nuclei (B), and nucleoli (C) of cerebellar Purkinje cells during horizontal rotation of rats. Each point is arithmetic mean of results of UV cytophotometry of 60-100 cells of one rat. Remainder of legend as to Fig. 1.

unchanged [1] both at the times when we studied it, and at other times. The RNA content in the nucleolus of the immobilized animals fell gradually until 40 min and then returned to normal.

Changes in the RNA content in Purkinje cells during vestibular stimulation were more significant (Fig. 2). During observation for 1 h a complete period was found with an interval of about 30 min between maxima and minima. The changes in nucleus and cytoplasm were in phase. Their amplitude was +30% of the mean level, which is characteristic of many circumhoral fluctuations [3]. Fluctuations in the nucleolus were less marked, although their general character was similar to that observed in the nucleus as a whole.

Definite shifts in the RNA content in the cerebellar Purkinje cells thus took place in rats in the two altered states studied. In immobilized animals quantitative changes in RNA may be due to stress: excitation of some nerve cells and inhibition of others, together with hypoxia, etc. We know that repeated stress affects the cyclic GMP content in the rat cerebellum [10]. Hypoxia, although admittedly more severe than that which can be expected in the present experiments, affects the RNA content in neurons [5]. During stimulation of vestibular function of the cerebellum, however, changes in the RNA content in the Purkinje cells were marked than in the stressed which may have accompanied vestibular stimulation. Consequently, the physiological character of the effect was evidently substantiated. The causes of physiologically determined changes in the RNA content in nerve cells have often been discussed [1]. Fluctuations in the RNA level in the nucleolus and in the nucleus as a whole are definitely linked both with its synthesis and with its processing, chiefly with movement of the ribosomes from nucleus into cytoplasm. Changes in the RNA content (especially a decrease) in the cytoplasm can be explained both by RNA transport into the axon, demonstrated in [12], and also, perhaps, by a change in the state of the RNA. In the latter case, for example, because of modified connections between RNA and proteins, changes in the coefficient of RNA absorption may be recorded rather than changes in the number of molecules. Finally, the regular breakdown of a small part of the cytoplasmic RNA cannot be ruled out.

The character of changes in RNA in the stimulated Purkinje cells closely resemble the circumhoral rhythms studied by the use of various markers: protein synthesis, dimensions of the cells and nuclei, activity of many enzymes, etc. [3, 4, 6, 8]. Similar fluctuations with a period of about 1 h were found previously in Purkinje cell nuclei during vestibular stimulation of the cerebellum in mice. This rhythm was not observed in the cytoplasm. The present writer has found that changes in RNA in the nucleus and cytoplasm of Purkinje cells fluctuate in phase.

LITERATURE CITED

1. V. Ya. Brodskii, Cell Nutrition [in Russian], Moscow (1966).
2. V. Ya. Brodskii and L. A. Manunyan, Tsitologiya, 11, 1528 (1967).
3. V. Ya. Brodskii (W. Y. Brodsky), J. Theor. Biol., 55, 167 (1975).
4. V. Ya. Brodskii, Tsitologiya, 18, 397 (1976).
5. N. N. Demin and L. Z. Pevzner, Tsitologiya, 11, 1536 (1969).
6. N. V. Nechaeva, "Biological rhythms in the secretory function of the salivary gland (quantitative cytological investigations)," Doctoral Dissertation, Moscow (1977).
7. A. F. Kuznetsova and V. Ya. Brodskii, Tsitologiya, 10, 392 (1968).
8. K. N. Yarygin, N. V. Nechaeva, V. I. Fateeva, et al., Byull. Eksp. Biol. Med., No. 12, 711 (1979).
9. A. Brodal, O. Pompeiano, and F. Walberg, The Vestibular Nuclei and Their Connections, Anatomy and Correlations, London (1962).
10. M. G. Corda, G. Biggio, and G. L. Gessa, Brain Res., 188, 287 (1980).
11. J. Jarlstedt, Acta Physiol. Scand., 67, 271 (1966).
12. J. Peterson, J. Bray, and L. Austin, J. Neurochem., 15, 741 (1978).