DYNAMICS OF TRANSCRIPTION ACTIVITY OF CEREBELLAR PURKINJE CELL CHROMATIN AFTER SYSTEMIC CIRCULATORY ARREST

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The development of modern resuscitation practice requires particular attention to be concentrated on postresuscitation pathology of the brain [8]. According to clinical observations, an important role in the formation of encephalopathy in patients surviving clinical death is played by damage to the cerebellum [4]. The Purkinje cells of the cerebellum are highly sensitive to hypoxia and they are among the first to be injured during clinical death [8].

It was shown previously that circulatory arrest, even for a short time and followed by complete and rapid compensation of the visible neurological disorders, leads to long-term disturbances of higher nervous activity of resuscitated rats [9]. An important fact is that foci of loss of Purkinje cells or of degeneratively changed neurons were not found in such animals, although considerable changes were observed in the size of the nucleus of the Purkinje cells [2]. Together with the increase in size of the nucleus and cytoplasm of Purkinje cells and of their dry mass, demonstrated by the writers previously [1], this fact indicates the need for a study of the state of the protein-synthesizing system of the Purkinje cells in the postresuscitation period.

The aim of this investigation was to evaluate the transcription activity of nuclear chromatin of Purkinje cells at various times after the onset of circulatory arrest.

EXPERIMENTAL METHOD

Cerebellar Purklnje cells were studied 1 and 24 h and 4 and 7 days after clinical death in 12 noninbred male albino rats weighing 160-180 g, and in three intact animals. Systemic circulatory arrest was induced by ligation of the vascular bundle of the heart for 10 min [6] (the experiments were conducted by T. P. Vasil'eva, on the staff of the Institute of General Resuscitation, Academy of Medical Sciences of the USSR, to whom the authors are grateful). To assess the state of transcription a histo-autoradiographic method was used to detect activity of endogenous RNA-polymerases in the fixed cell [14], as described previously [3]. The level of transcription was assessed by determining the intensity of labeling of the nucleolus and of the extranucleolar zone. Considering the heterogeneity of the Purkinje cell population, pale and dark neurons were studied separately. The term dark cells was taken to mean normal, morphologically unchanged neurons with a darkly stained nucleus and cytoplasm [1]. The intensity of labeling of 50 Purkinie cells was counted for each animal. The results were subjected to statistical analysis by Student's test (p_t), the Kolmogorov–Smirnov test (p_λ), and Fisher's test (p_φ) [5].

EXPERIMENTAL RESULTS

The results showed that changes in template activity of the nucleolar chromatin begin in the early postresuscitation period. Although 1 h after resuscitation the labeling intensity of the nucleolus of the Purkinje cells did not differ from the control, by the end of the 1st day the distribution of the Purkinje cells with respect to this parameter showed a change ($p_\lambda <$

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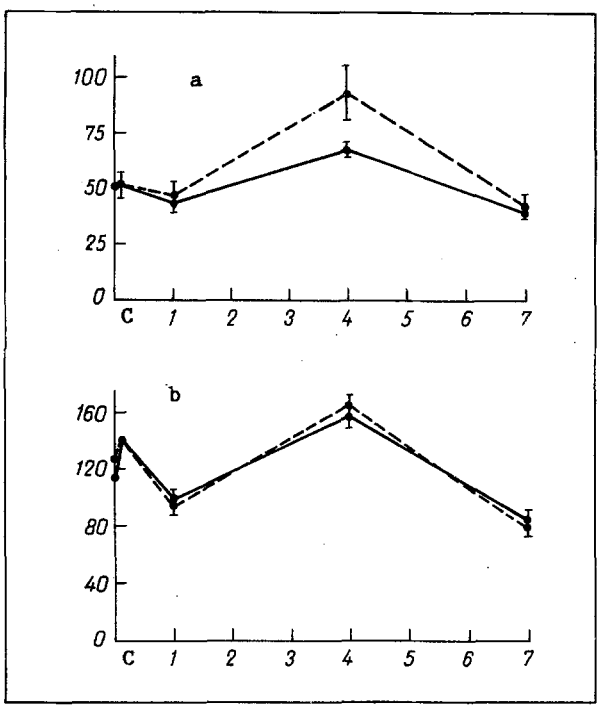

Fig. 1. Changes in transcription activity of nucleolar (a) and extranucleolar (b) chromatin of Purkinje cells of cerebellar cortex in postresuscitation period. Abscissa, time after resuscitation (in days); ordinate, average labeling intensity (in conventional units). C) Control (intact animals). Continuous line — dark neurons, broken line — pale.

0.05): the fraction of weakly labeled cells was increased whereas that of the moderately and strongly labeled cells increased ($p_\varphi < 0.05$), which led to some reduction in the average level of labeling of the nucleolus in both pale and dark Purkinje cells (Fig. 1a).

A sharp increase in the intensity of labeling of the nucleolus of the Purkinje cells took place 4 days after clinical death compared with levels discovered in the earlier period after resuscitation (by 101.0% in pale neurons, by 53.5% in dark neurons; $p_t < 0.05$). The changes observed are connected with an increase in the fraction of cells with a weak and moderate degree of staining and an increase in the fraction of strongly labeled cells; some cells were found, moreover, which were more strongly labeled than any in intact rats (Fig. 2). These changes led to a significant increase in the average labeling intensity of the nucleolus of the Purkinje cells compared with the control, and the changes were much more marked in the pale cells than in the dark cells (an increase by 76.5 and 29.4% respectively, $p_t < 0.05$) (Fig. 1a). The intensity of labeling of the nucleolus 7 days after circulatory arrest was sharply reduced compared with that on the 4th day after resuscitation, and in both pale (by 56.7%) and dark (by 44.2%) cells ($p_t < 0.05$) had become much lower than in the control (by 23.5 and 27.8% in the pale and dark neurons respectively; $p_t < 0.05$) (Fig. 1a).

Changes in the intensity of labeling of the extranucleolar chromatin were found as early as 1 h after resuscitation, when the distribution of the Purkinje cells by this parameter differed significantly from the control ($p_\lambda < 0.01$): the fraction of weakly labeled cells was reduced whereas that of the moderately strongly and strongly labeled cells was increased ($p_\varphi < 0.05$) (Fig. 3), as a result of which the average level of labeling was increased in both pale and dark cells ($0.05 < p_t < 0.01$) (Fig. 1b). Subsequent changes in the extranucleolar labeling level developed in the postresuscitation period in a manner similar to changes in the intensity of labeling of the nucleolus. Toward the end of the 1st day the labeling intensity was reduced compared with that 1 h after resuscitation (by 34.4 and 31.1% in the pale and dark Purkinje cells respectively; $p_t < 0.05$), falling significantly lower than in the control (by 25.5 and 20.1% in pale and dark neurons respectively; $p_t < 0.005$); on the 4th day after clinical death there was a sharp increase in this parameter compared with its value on the 1st day of the postresuscitation period (by 73.2% in pale and by 60.9% in dark neurons; $p_t < 0.005$), the intensity of labeling in both types of Purkinje cells was 28.5% higher than in the control ($p_t < 0.05$); on the 7th day also, a second decrease was observed (by 53.8% in pale and by 48.5% in dark neurons;

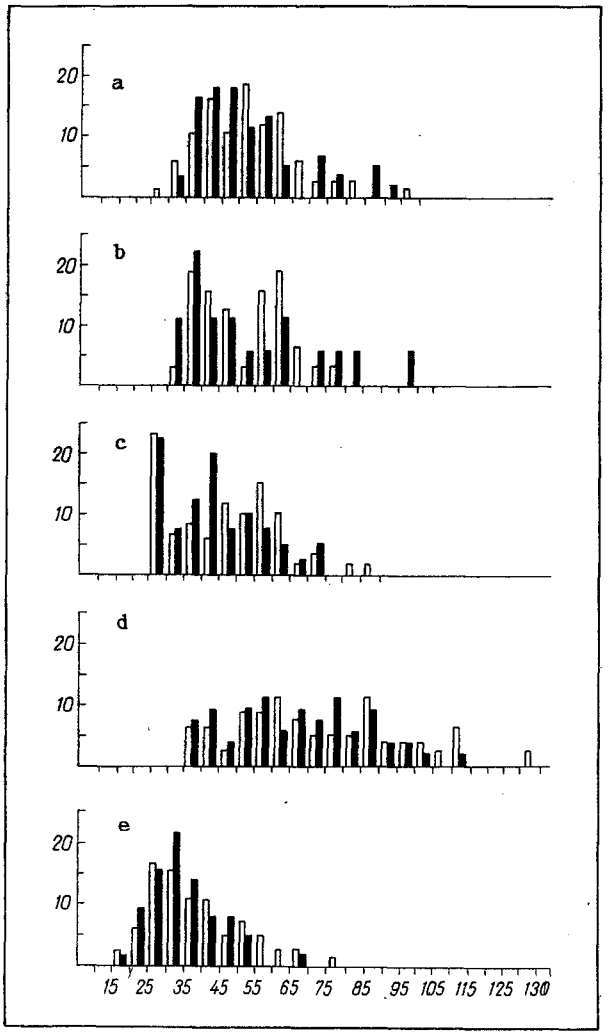

Fig. 2. Distribution of Purkinje cells by labeling intensity of nucleolus at different times after resuscitation. Abscissa, number of grains of reduced silver; ordinate, number of cells (in per cent). Unshaded columns — pale neurons; black columns — dark neurons. a) Control; b) 1 h after resuscitation; c) 24 h after resuscitation; d) 4 days after resuscitation; e) 7 days after resuscitation.

$p_t < 0.05$), i.e., this parameter became much lower than in the control (by 40.6% in the pale and by 33.9% in the dark Purkinje cells; $p_t < 0.025$) (Fig. 1b).

Hence, in the postresuscitation period after systemic circulatory arrest significant changes take place in the template activity of the nuclear chromatin of Purkinje cells. They begin immediately after resuscitation and are phasic in character. Changes in transcription activity of the nucleolar and extranucleolar chromatin are identical in direction and have a similar time course during the postresuscitation process.

Pale and dark nerve cells, which initially do not differ in their level of template activity of the chromatin, undergo changes of different degrees in its transcription activity, the pale cells being more reactive than the dark cells. The essential fact is that pale and dark neurons differ also in the intensity of changes in other parameters in the postresuscitation period (intensity of decline, change from normal cells into morphologically changed cells, enlargement of the cells and an increase in their dry mass) [1], so that pale and dark cells can be regarded as two different subpopulations, reacting differently to the same force.

The increase in transcription activity of chromatin of the Purkinje cells on the 4th day after circulatory arrest for 10 min is in agreement with data obtained by the writers previously for neurons of various layers of the sensomotor cortex of rats resuscitated after clinical death of the same etiology and duration [3]. These results evidently point to activation of protein synthesis

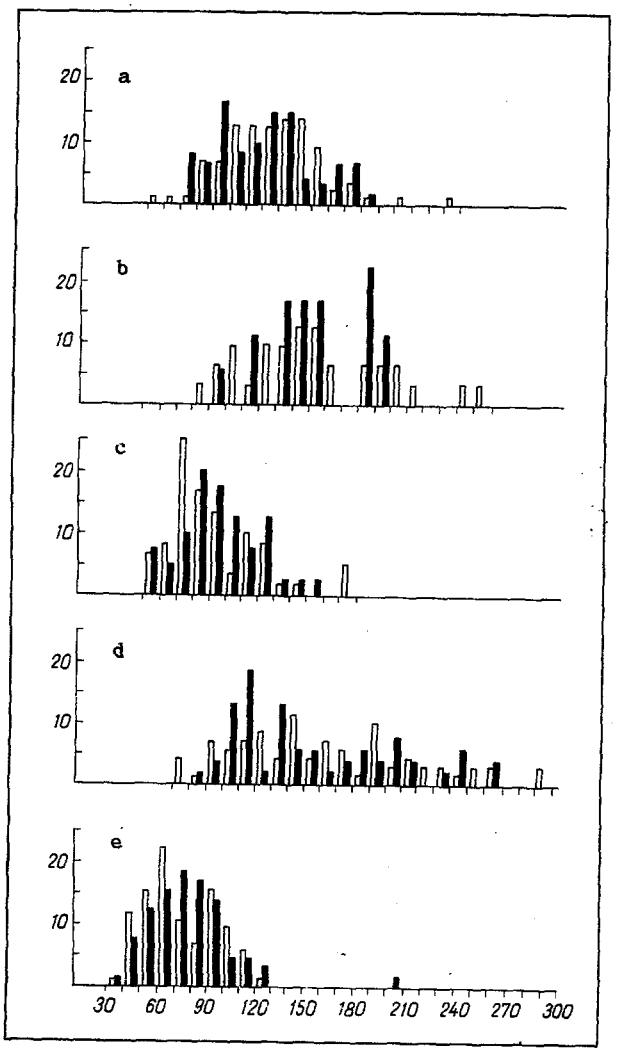

Fig. 3. Distribution of Purkinje cells by labeling intensity of extranucleolar chromatin at different times after resuscitation. Legend as to Fig. 2.

at this stage of the postresuscitation period during compensatory-restorative changes in the brain neurons after severe ischemia.

The increase in template activity of the Purkinje cell protein found in this investigation coincides in time with the significant increase in size of the nucleolus of these neurons discovered by the writers previously [2]. This fact indicates the presence of correlation between changes in size of the nucleolus, found at the light-optical level, and the transcription activity of its chromatin.

The sharp decline in template activity of both nucleolar and extranucleolar chromatin on the 7th day after resuscitation may evidently lead to a decrease in protein synthesis at this stage of the postresuscitation period. It is important to note that it is at this time that disturbances of behavior and condition-reflex activity are observed in resuscitated rats [9]. For the higher nervous activity of animals and, in particular, processes of learning and memory formation, to be maintained in rats, normal protein synthesis in the brain is essential [7, 10]. Disturbance of nucleic acid and protein metabolism is characteristic of many neurodegenerative diseases, accompanied by neurological disorders [13]. In Alzheimer's disease, for instance, there is a sharp decline in transcription activity of the chromatin and the level of protein synthesis falls to 30% of normal [12]. Allowing for the important role of the cerebellum in learning and behavior of animals [11, 15] the results suggest that the substantial changes which we found in functional activity of the genome of the cerebellar Purkinje cells of resuscitated animals and the particularly sharp decline in template activity of the chromatin on the 7th day after resuscitation may be among the causes of formation of disturbances of higher nervous activity arising in the postresuscitation period even in animals with outwardly complete recovery.

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ROLE OF OPIATE MECHANISMS OF THE HIPPOCAMPUS AND SUBSTANTIA NIGRA IN BEHAVIORAL AND SEIZURE DISTURBANCES DURING PICROTOXIN-INDUCED KINDLING

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Kindling is a method of forming epileptic activity (EA) by means of repeated, initially subliminal, epileptogenic procedures [1-5, 13]. Our previous investigations [1, 2, 4, 5] showed that the development of the seizure syndrome during kindling induced by repeated injections of metrazol or picrotoxin, is based on the formation of an epileptic pathological system with determinant structure, in the formations of the hippocampus. It has also been shown that during the formation of EA during kindling, endogenous opioid peptides, playing the role of stabilizers and effectors of activity of the epileptic pathological system, and also of a specific antiepileptic system of the brain in different stages of the pathological process, accumulate in the animals' CNS [3, 6]. The important role of the opiatergic mechanisms of the reticular part of the substantia nigra (RSN) in the appearance and disappearance of EA in the course of kindling induced by electrical stimulation of the amygdala [8], and also by repeated injections of picrotoxin [6], has recently been established. However, the role of the different subtypes of opiate receptors in the mechanisms of kindling is not yet clear. Great importance has recently been attached to the mu- and kappa-opiate systems of the brain in the formation and disappearance of EA during kindling [10]. The aim of the present investigation

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