8. I. V. Yamnas, J. F. Burk, D. P. Orgill, et al., Science, **215**, 174 (1982).

CHANGES IN STRUCTURE AND FUNCTION OF CHROMATIN OF CEREBRAL CORTICAL NEURONS IN THE EARLY POSTRESUSCITATION PERIOD IN RATS

M. Sh. Avrushchenko, O. V. Bul'chuk, A. V. Grigor'eva, and V. N. Yarygin

UDC 616.831-008.922.1-008.64-036.17]
-092.9-07:616.8-091.81-036.882

Key words: chromatin; transcription; structure; neurons; clinical death.

Changes in the CNS after severe hypoxia, arising during systemic circulatory arrest, constitute one of the main pathogenetic mechanisms of postresuscitation sickness [9]. Previous investigations have showed that to understand the mechanisms of formation of postresuscitation brain pathology it is essential to study the state of the different components of the protein-synthesizing system of nerve cells. It has been found [2], for instance, that after clinical death, of varied etiology and duration, the nucleus and cytoplasm of the neurons enlarge and their dry weight increases. In the postresuscitation period enlargement of the nucleolus of nerve cells has been observed [8], and the intensity of this process correlates with the duration of previous ischemia and the degree of recovery of the animals' neurological status.

The aim of this investigation was to evaluate the structural and functional state of the transcription apparatus of neurons located in different functional layers of the cerebral cortex of rats in the early stages after systemic circulatory arrest.

EXPERIMENTAL METHOD

The sensomotor cortex of six noninbred male albino rats weighing 160-180 g was studied after a 10-min period of systemic circulatory arrest caused by compression of the vascular bundle of the heart [7], and in three intact rats (control). The medium-sized pyramidal cells of layer III, stellate neurons of layer IV, and large pyramidal cells of layer V of the cortex were investigated 1 and 24 h after resuscitation. To assess the state of transcription in the neurons a histoautoradiographic method was used to demonstrate activity of endogenous RNA-polymerases in fixed cells [13]. The state of transcription was assessed by the intensity of labeling of the nucleolus and the extranucleolar zone, by counting the number of grains of reduced silver separately in pale and dark neurons in each layer of the cortex (staining with methylene blue). By the term dark cells were understood normal, morphologically unchanged neurons with darker staining of their nucleus and cytoplasm [1]. The intensity of labeling of 50 cells was counted in each layer for one animal. The results were subjected to statistical analysis by the Kolmogorov-Smirnov λ test and the φ test [6].

To assess the structural state of the chromatin the method of differential staining of lysine- and arginine-rich histones with ammoniacal silver was used [12]. Although this method was suggested for staining histones, the pattern revealed by ammoniacal silver is essentially determined by the specific nature of interaction of histones with other components of DNP in nuclei of one type or another and, consequently, it reflects differences in the structure of chromatin. It is important to note that according to the existing data [4], there is no unambiguous correlation between characteristics revealed by ammoniacal silver and the level of template activity of the chromatin, determined by Moore's method. Thus the methods which we used characterize different aspects of the state of the neuronal transcription apparatus.

Institute of General Resuscitation, Academy of Medical Sciences of the USSR. N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. A. Negovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 4, pp. 402-405, April, 1990. Original article submitted February 12, 1989.

 0007-4888/90/0004-0530$12.50

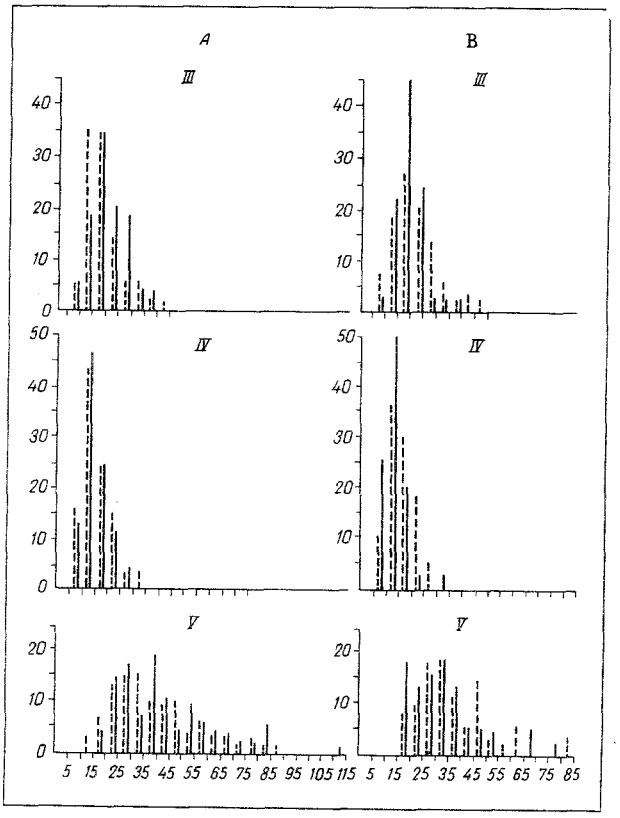

Fig. 1. Distribution of pale and dark neurons of layers III, IV, and V of the sensomotor cortex by intensity of labeling of nucleolus 1 day after resuscitation of rats. A) Pale cells, B) dark cells. Broken line denotes control (intact rats), continuous line — experiment. Abscissa, number of grains of reduced silver; ordinate, number of cells (in %).

EXPERIMENTAL RESULTS

The investigation showed that in the early postresuscitation period significant changes take place in the intensity of labeling of the nucleolus of pale and dark neurons in different layers of the cortex. For instance, analysis of histograms of distribution of pale cells in layer III with respect to this parameter showed that as early as 1 h after resuscitation the proportion of weakly labeled neurons fell whereas the proportion of strongly labeled neurons rose. These changes were more marked 24 h after resuscitation ($p_\varphi < 0.05$). Opposite changes took place in the dark neurons ($p_\varphi < 0.05$); (Fig. 1).

In layer V of the cortex, 1 h after resuscitation, opposite changes in the intensity of labeling of the nucleolus were discovered in the pale and dark neurons, similar to those in layer III, and they persisted until 24 h after resuscitation ($p_\varphi < 0.05$) (Fig. 1). As a result of the changes thus observed, by the end of the 1st day of the postresuscitation period the level of nucleolar labeling of the pale neurons in cortical layers III and IV was raised, whereas that of the dark neurons was lowered by comparison with their levels in intact animals.

In cortical layer IV, 1 h after resuscitation, similar changes were observed in the intensity of labeling of the nucleolus: the number of weakly labeled cells increased, the number with an average density of labeling was reduced ($p_\varphi < 0.05$). However, 24 h after clinical death, the intensity of labeling of the nucleolus reached the control level in the pale neurons, whereas in the dark cells it was unchanged, i.e., it remained below normal (in the experiment there were more weakly labeled cells and fewer with an average labeling density than in the control, ($p_\varphi < 0.005$), see Fig. 1).

Analysis of the distribution of the neurons by intensity of labeling of the extranucleolar chromatin showed that 1 h after resuscitation complementary changes took place in the pale and dark cells of all cortical layers studied (except dark neurons of layer IV): the proportion of cells with an average and high density of labeling was increased, whereas in the proportion of weakly labeled cells was reduced ($p_\lambda < 0.05, p_\varphi < 0.01, p_\lambda < 0.05, p_\varphi < 0.002, p_\lambda < 0.01, p_\varphi < 0.002$, for cortical layers III, IV, and V,

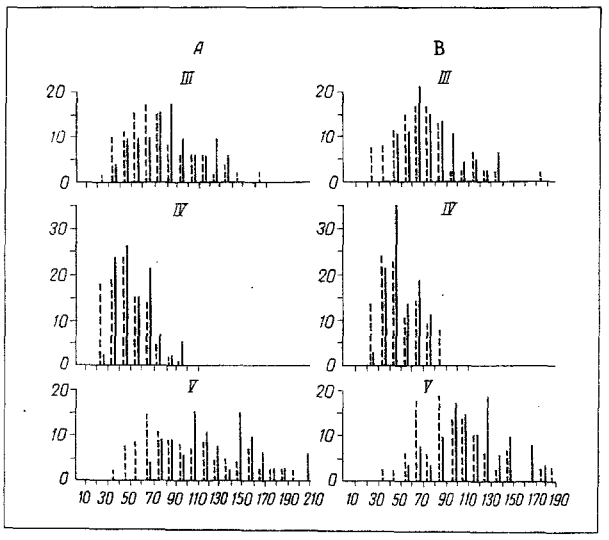

Fig. 2. Distribution of pale and dark neurons of sensomotor cortical layers III, IV, and V by intensity of extranucleolar labeling 1 h after resuscitation of animals. Legend as to Fig. 1.

respectively; Fig. 2). As a result the level of labeling of the extranucleolar chromatin increased relative to the control. Different changes in the character of distribution of the neurons by intensity of extranucleolar labeling were found in the dark neurons of layer IV: 1 h after resuscitation the proportion of neurons with an average density of labeling was increased and the proportion of stronger labeled cells reduced ($p_\varphi < 0.02$), but this did not lead to any change in the level of labeling as a whole. The changes discovered continued in the pale and dark neurons of layers III and IV until 24 h after resuscitation. In layer V by this time the proportion of strongly labeled neurons among both pale and dark types was reduced and the proportion of weakly labeled cells increased ($p_\varphi < 0.001$), leading to a significant decrease in the mean intensity of nucleolar labeling compared with 1 h after resuscitation ($p_t < 0.05$) and to its return to the control level.

Thus, 1 h after clinical death, the template activity of the chromatin was modified in all layers of the cortex tested, and these changes persisted until 24 h after resuscitation. The intensity of nucleolar labeling is known to be an indicator of the level of ribosomal RNA synthesis. It has been shown [14] that the level of *r*RNA synthesis correlates positively with the intensity of ribosome production and, consequently, it indirectly characterizes activity of intracellular protein synthesis. If these results are examined from this point of view it can be concluded that activation of protein synthesis takes place in the early postresuscitation period in pale neurons, but this process is inhibited in dark neurons. The results of this investigation confirm the previous hypothesis [2] that pale and dark neurons play different roles in the maintenance of homeostasis of the population and they are evidence of the greater functional activity of pale than of dark cells.

Changes in the intensity of labeling of extranucleolar chromatin toward the first hour after resuscitation were identical irrespective of the type (pale or dark) of neuron: in all cortical layers the level of labeling rose, and remained high until 24 h after resuscitation. Only in layer V was the intensity of labeling of extranucleolar chromatin back at the control level 24 h after clinical death. It is also important to note that these changes were the same in both pale and dark cells. The increase in the level of extranucleolar labeling characterizes the intensity of synthesis of *m*RNA and, to a lesser degree, of *t*RNA, i.e., it is evidence primarily of intensification of the function of structural genes. Comparison of the data on changes in nucleolar and extranucleolar labeling indicates that these processes are relatively independent. The question whether intensification of the level of transcription of extranucleolar genes is linked with the activation of new groups of genes will be solved to some degree by results obtained by staining nuclei with ammoniacal silver.

Under normal conditions nuclei of pyramidal and stellate cells, stained with ammoniacal silver, have a different and stable structure, characterized by a definite content of large and small globules of uniformly brown color ("clumps" and "grains," respectively), which show up clearly against a yellow background. Considerable complementary changes were found 1 h after resuscitation in all cortical layers: the proportion of nuclei with a high content of brown clumps and with a few grains per nucleus fell significantly. In addition, the size of the grains in the pyramidal cells increased as a result of the indistinctness of its outlines. Changes in the nuclei 24 h after resuscitation were even more marked in character. The process of reduction of the

Fig. 3. Pyramidal cells in sensomotor cortex of intact rats and rats resuscitated from clinical death. a) Control, b) 1st day after resuscitation. Stained with ammoniacal silver.

number of brown clumps per nucleus increased in intensity and, besides, numerous cells in which clumps and grains were not at all detectable appeared among the pyramidal cells of layers III and V (in layer V they accounted for half the total number). The nuclei of these pyramidal cells had a homogeneous pale brown color and contained a large orange nucleolus, surrounded by a yellow border (Fig. 3).

As has been shown previously for sympathetic neurons and neurons of the cerebellar cortex, the unique structure of the nucleus as revealed by ammoniacal silver is linked with the fact that the cell belongs to a particular morphological and functional type, it is formed during postnatal differentiation, and it is distinguished by stability during different functional loads, despite the change in transcription activity of the chromatin under these circumstances [4, 5]. Meanwhile, powerful pathological influences can induce changes in the character of nuclear staining [11]. All these considerations, together with the absence of any mutually equivalent correlation between the type of staining and the quantitative parameters of chromatin template activity [4] indicate that the picture revealed with ammoniacal silver is linked with the qualitative aspect of transcription, namely, with the set of genes that can be expressed in cells of the given type. Consequently, changes in the character of staining of the nuclei with ammoniacal silver may indicate more profound structural changes in chromatin than a quantitative change of template activity. It is these changes in chromatin structure that are found in the nuclei of cerebral cortical neurons of rats subjected to systemic circulatory arrest. In this case 1 h after resuscitation only a redistribution of the cells by content of globular structures in the nucleus within the characteristic norm of range could be detected, whereas 24 h after resuscitation nearly half of the neuron population already contained nuclei never found in intact rats. The changes discovered ought evidently to be accompanied by a change in the spectrum of expressed genes in the cells and, as a result of this, a change in the set of synthesized proteins [11]. Although this hypothesis requires direct experimental confirmation, nevertheless the disturbances of brain metabolism arising during severe hypoxia due to systemic circulatory arrest are so profound [10] that reprogramming of the neuron genome can logically be expected.

The results of this investigation thus show that in the early postresuscitation period significant changes take place in the structural and functional state of the nuclear chromatin of neurons in different functional layers of the cerebral cortex. Considering that the state of the protein-synthesizing apparatus plays an important role in the maintenance of functionally adaptive activity of the neuron [3], it can be tentatively suggested that the changes in transcription activity of chromatin and the profound disturbances of its structure revealed by these experiments may lead to the formation of posthypoxic encephalopathies. Further investigations of neuronal chromatin in the later stages of the postresuscitation period, when the degree of reversibility of changes in the transcription apparatus also in the neurological status and higher nervous activity of the resuscitated animals can be evaluated, will evidently give the answer to this question.

LITERATURE CITED

1. M. Sh. Avrushchenko, Byull. Éksp. Biol. Med., **92**, No. 9, 363 (1981).
2. M. Sh. Avrushchenko and T. L. Marshak, Byull. Éksp. Biol. Med., **95**, No. 3, 105 (1983).
3. V. Ya. Brodskii, Cell Nutrition [in Russian], Moscow (1966).
4. A. V. Grigor'eva and V. N. Yarygin, Tsitologiya, **27**, No. 2, 186 (1985).
5. A. V. Grigor'eva and V. N. Yarygin, Tsitologiya, **28**, No. 8, 837 (1986).
6. E. V. Gubler, Computerized Methods of Analysis and Diagnosis of Pathological Processes [in Russian], Leningrad (1978).
7. V. G. Korpachev, S. P. Lysenkov, and L. Z. Tel', Patol. Fiziol., No. 3, 78 (1982).
8. T. L. Marshak and M. Sh. Avrushchenko, Byull. Éksp. Biol. Med., **102**, No. 10, 477 (1986).
9. V. A. Negovskii, Essays on Resuscitation [in Russian], Moscow (1986).
10. V. A. Negovskii, A. M. Gurvich, and E. S. Zolotokrylina, Postresuscitation Sickness [in Russian], Moscow (1987).
11. G. V. Chernykh, A. V. Grigor'eva, and V. N. Yarygin, Acupuncture in Clinical and Experimental Medicine [in Russian], Ustinov (1987).
12. M. M. Black and H. R. Ansley, Science, **143**, 693 (1964).
13. P. M. Moore, Exp. Cell Res., **111**, No. 2, 318 (1978).
14. J. Tesaric, Y. Kopecu, M. Plachot, and J. Mandelbaum, J. Reprod. Fertil., **78**, No. 2, 463 (1986).

COMPARATIVE CHANGES IN MICROANATOMICAL ORGANIZATION OF LYMPH NODES DRAINING AND LOCATED IN A ZONE OF VENOUS STASIS

Yu. I. Borodin, V. N. Grigor'ev, and T. Zh. Umbetov

UDC 616.14-008.811.6-092.9-07:616.438-091.8

Key words: venous stasis; popliteal lymph nodes; renal lymph nodes

Vascular pathology occupies a leading position in the morbidity structure of the population of developed countries; in the last decade there has been a rapid increase in the incidence of diseases accompanied by disturbance of the drainage function of the veins [2, 4, 7]. Meanwhile, the important role of the lymphatic system in the compensation of circulatory disorders arising in the presence of venous stasis has been proved quite conclusively during these years [2, 4-6], and a role of particular importance in these processes is played by the lymph node, as an instrument for the redistribution of fluid and cells between the blood vascular and lymphatic systems [2, 9, 10].

The state of lymph nodes located in a zone of venous stasis has been investigated in fair detail [2, 4, 6]. However, there have been no investigations of the structural and functional organization of lymph nodes draining a zone of venous stasis, but not located in it. The aim of this investigation was accordingly to compare the fine structure of lymph nodes lying in a zone of venous stasis and involved in the drainage of lymph from it, and also of lymph nodes located outside the zone, but receiving lymph from the region of stasis.

Department of Normal Anatomy, Novosibirsk Medical Institute. Laboratory of Functional Morphology, Institute of Physiology, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 4, pp. 405-408, April, 1990. Original article submitted November 2, 1988.

 0007-4888/90/0004-0534$12.50