Protein Synthesis in Neurons and Satellite Glial Cells of Rats After Global Cerebral Ischemia Caused by Cardiac Arrest

M. Sh. Avrushchenko and T. L. Marshak

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 3, pp. 257-260, March, 1997
Original article submitted November 20, 1995

Protein synthesis rates are increased in light and dark neurons and satellite glia cells in various brain areas in rats with completely restituted neurological status after cardiac arrest. However, the increase is provided by different cell types. Only free light neurons are reactive in the hippocampal sector CA1, while both free light neurons as light neurons with glia are reactive in sector CA4; intense protein synthesis is observed in all types of the cerebral cortex neurons and in cortical neurons and in the satellite glial cells of the lateral cerebellar hemispheres.

Key Words: *ischemia; protein synthesis; light and dark neurons; glia; autoradiography*

A decrease in the protein synthesis is one of cell responses to the brain homeostasis disturbances during the postischemic period [13,14]. Previously, we showed that substantial phasic changes in the transcriptional activity of neuronal chromatin in various brain structures are developed by animals without apparent neurological disorders during the postresuscitation period [1]. We also found that these changes correlate with those in the level of protein synthesis in the brain of resuscitated animals [4]. However, it is unclear which cells of the nerve tissue are involved in the postresuscitation changes at the level of protein synthesis. Morphofunctional changes occurring in neurons of various layers of the sensorimotor cortex (SMC) and in the cerebellar Purkinje cells indicate increased protein production [1,3]. In the neuronal populations examined, the degree of changes in the light neurons differed from that in the dark ones. Neuronal dimorphism, i.e., the presence of light and dark neurons in a single population, has been associated with different functional states of these cells [11,12]. Evaluation of the responses of heterogeneous neuronal populations to ischemia is important for elucidation of the mechanisms underlying the postresuscitation brain disorders. Since the glia plays an important role in the nerve tissue function [10,15] and both neuroglial relationships and the state of glia are altered during the postresuscitation period [2,5], it was necessary to estimate protein synthesis rates in the satellite glia (SG) located near light and dark neurons in a heterogeneous neuronal populations.

In the present study we assessed the intensity of protein synthesis in light and dark neurons and the SG in various brain structures on day 4 after cardiac arrest. During this period, the nucleoli increase in size, and the transcriptional activity of chromatin rises [1] in brain neurons of resuscitated animals without apparent neurological disorders.

MATERIALS AND METHODS

A 10-min cardiac arrest was produced in rats by compressing the vascular bundle of the heart [7]. Resuscitation was achieved by indirect massage of the heart in combination with artificial pulmonary ventilation with air. The neurological status of resusci-

Laboratory for General Pathology of Terminal States, Institute of General Reanimation, Russian Academy of Medical Sciences, Moscow; Laboratory of Cytology, Institute of Developmental Biology, Russian Academy of Sciences, Moscow

0007-4888/97/0003-0221$18.00 ©1997 Plenum Publishing Corporation

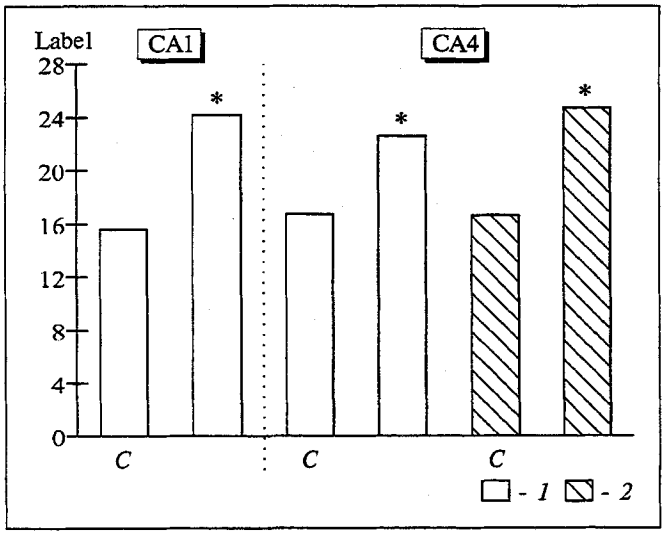

Fig. 1. Labeling intensity of pyramidal cells in hippocampal sectors CA1 and CA4 in resuscitated rats. Here and in Figs. 2 and 3: *1*) free light neurons; *2*) light neurons with glia; *C*) control, **p*<0.05 compared with the control.

tated animals was evaluated using a scoring scale [8]. The protein synthesis rate was estimated on day 4 of the postresuscitation period in 5 experimental rats and 5 intact (control) rats. For this purpose, ^{3}H-leucine (specific activity 1370 TBq/mol) was injected intraperitoneally in a dose of 7 μCi/g body weight 30 min before decapitation under ether anesthesia. Pieces of the SMC, hippocampus, and lateral cerebellum were fixed in Carnoy's fluid, processed using the standard procedures, and embedded in paraffin. After deparaffinization, 5-6-μ sections were coated with type M emulsion and exposed for 30 days. After development, the preparations were stained with cresyl violet by the method of Nissl. Heterogeneous

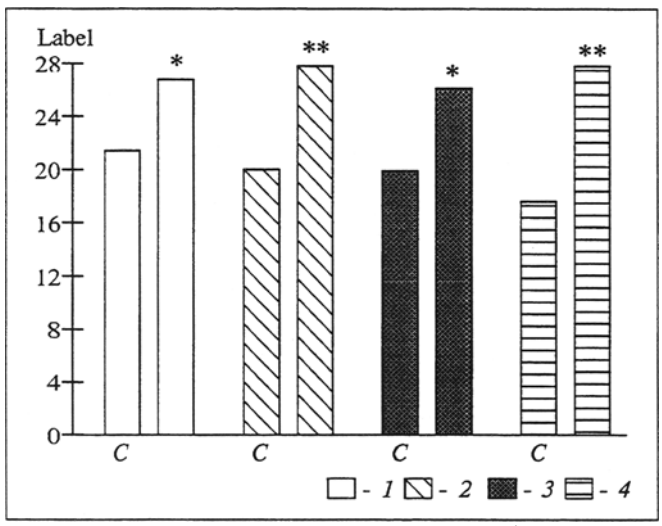

Fig. 2. Labeling intensity of pyramidal cells in cortical layer V in resuscitated rats. Here and in Fig. 3: *3*) free dark neurons; *4*) dark neurons with glia. ***p*<0.025 compared with the control.

populations of pyramidal cells in layer V of the SMC, Purkinje's cells in the lateral cerebellum, pyramidal neurons in hippocampal sectors CA1 and CA4, and the SG located near neurons of the different types were studied. Light and dark neurons as well as free neurons and neurons of both types with SG were counted separately. Labeling intensity was evaluated microscopically (magnification 1250) with the use of a square grid and expressed in arbitrary units. Silver grains were counted in three squares for each nerve cell, and the mean value (label concentration) was calculated per square. Light and dark neurons as well as free neurons and those with SG were counted separately. Fifty or sixty cells were examined in each neuronal population.

The significance of differences was estimated by Student's *t* test.

RESULTS

The apparent (external) neurological status was restored in all experimental rats. In the intact (control) rats, light and dark neurons of the studied populations did not differ in labeling intensity. Four days after resuscitation, labeling intensity in experimental rats was higher than in the control only in free light pyramidal neurons of the hippocampal sector CA1 (by 35.3%) and in both free light neurones and light neurons with SG of the pyramidal cell population in sector CA4 (by 48.8% and 55.1%, respectively; Fig. 1). In the SMC layer V neurons of experimental rats, the labeling intensity was significantly higher not only in free light neurons and light neurons with SG (by 31.2% and 39.0%, respectively), but also in free dark neurons and dark neurons with SG (by 25.2% and 58.0%, respectively; Fig. 2). Thus, protein synthesis was increased in all studied neuronal populations. There were no changes in labeling intensity in the SG adjacent to light and dark neurons.

In the Purkinje's cells of the lateral cerebellum, labeling intensity in experimental rats was much higher than in the control in all types of neurons: by 76.3% in free light neurons, by 84.0% in light neurons with SG, by 77.9% in free dark neurons, and by 78.1% in dark neurons with SG. Moreover, labeling intensity in experimental rats was significantly higher in the SG, both at light (by 55.4%) and dark neurons (by 69.9%) (Fig. 3).

Thus, the rate of protein synthesis was increased in all studied parts of the brain in rats with completely restored neurological status on day 4 after resuscitation. This findings agree with the data on increased protein synthesis rates in the brain of animals with restored neurological status at a particular stage of the postresuscitation period [4]. However,

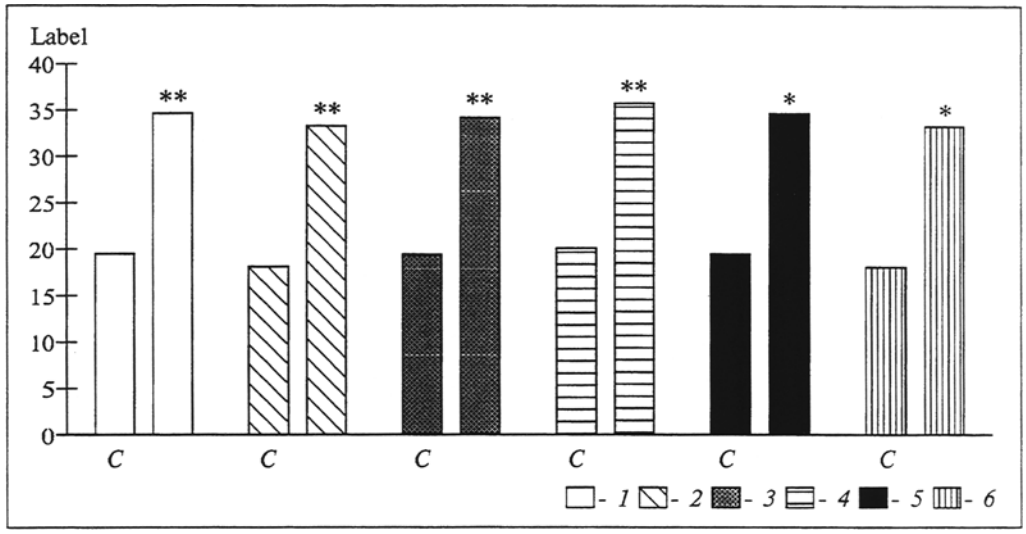

Fig. 3. Labeling intensity of Purkinje's cells and satellite glia in the lateral cerebellum of resuscitated rats. Glia at light (*5*) and dark (*6*) neurons.

the studied neuronal populations, which initially did not differ in the level of protein synthesis, responded in different ways to ischemia. In the pyramidal neurons of hippocampal sector CA1, where no marked degenerative changes or cell loss were observed in restored animals [5], the protein synthesis rate was increased only in free light cells. In sector CA4, where pronounced neuronal changes were observed in the same animals [5], the protein synthesis rate was elevated in both free light cells and the light cells with SG. In layer V of the SMC, where substantial changes occurred in the density and composition of the neuronal population (cell loss, increased numbers of cells with signs of degeneration, and abnormal neuroglial relationships), the rate of protein synthesis was altered in all types of neurons, but not in the SG. Only in the Purkinje's cells of the lateral cerebellum, which is the brain area most vulnerable to clinical death of various etiology and duration [2], changes were noted in all types of neurons as well as in the SG. Therefore, there is a gradation in the involvement of neurons and glia in the postresuscitation process, similar to the gradation revealed by morphometric analysis of heterogeneous neuronal populations [2]. The equal overall protein synthesis rate in the brain tissue (as indicated by biochemical analysis) was achieved at different costs in the studied neuronal populations.

Thus, we have identified the cells responsible for an increase in the protein synthesis rate in the examined brain areas. Activation of protein synthesis in heterogeneous neuronal populations does not necessarily involve all cells. Taken together with our previous data (morphometric analysis of neurons [2], determination of the dry mass of neurons [3], and evaluation of alterations in the transcriptional activity of chromatin [1]), these findings point to a higher responsiveness of light neurons and greater changes in protein synthesis in these cells compared with dark neurons. Through elevation of protein synthesis by neurons, which is probably a manifestation of intracellular regeneration, the density and composition of a neuronal population can be normalized. On the other hand, strong activation of protein synthesis may lead to depletion of neuronal resources, as evidenced by a drastic decrease in chromatin transcriptional activity in neuronal nuclei of various cortical layers and cerebellar Purkinje's cells on day 7 of the postresuscitation period [1]. These changes may be responsible for subsequent impairment of homeostasis in neuronal populations, where degenerative changes and cell loss as well as abnormalities in neuroglial relationships occur on days 4-7 after resuscitation [2]. During this period behavioral and conditioned reflex disturbances were observed in animals without apparent neurological disorders [6,9].

Our results indicate that postresuscitation disturbances in the structure and function of the brain are related to compensatory/adaptive changes in heterogeneous neuronal populations, being determined by the reactivity of neuronal protein-synthesizing system. This phenomenon is important for understanding the mechanisms underlying pathological and compensatory processes in the brain of resuscitated animals after severe ischemia caused by cardiac arrest.

This work was supported by the Russian Foundation for Basic Research (Grant No. 93-04-07).

REFERENCES

1. M. Sh. Avrushchenko, in: *Terminal States and Postresuscitation Disorders* [in Russian], Moscow (1992), pp. 49-59.
2. M. Sh. Avrushchenko, *Anest. Reanimatol.*, No. 5, 41-44 (1994).
3. M. Sh. Avrushchenko and T. L. Marshak, *Byull. Eksp. Biol. Med.*, **95**, No. 3, 105-108 (1983).

4. M. Sh. Avrushchenko, T. L. Marshak, V. I. Fateeva, *et al.*, *Anest. Reanimatol.*, No. 2, 43-46 (1993).
5. M. Sh. Avrushchenko, T. M. Solomatina, A. M. Gurvich, and I. V. Viktorov, *Byull. Eksp. Biol. Med.*, **114**, No. 8, 176-179 (1992).
6. A. M. Gurvich, E. A. Mutuskina, and Yu. V. Zarzhetskii, *Anest. Reanimatol.*, No. 5, 6-9 (1994).
7. V. G. Korpachev, S. T. Lysenkov, and L. Z. Tel', *Pat. Fiziol.*, No. 3, 78-80 (1982).
8. S. P. Lysenkov, V. G. Korpachev, and L. Z. Tel', in: *Emergencies: Clinical Features, Pathogenesis, and Management* [in Russian], Novosibirsk (1982), pp. 8-13.
9. N. I. Nezlina, E. A. Mutuskina, and S. V. Tolova, *Zh. Vyssh. Nervn. Deyat.*, **37**, No. 4, 741-743 (1987).
10. A. I. Roitbak, *Glia and Its Role in Neural Activity* [in Russian], St. Petersburg (1993).
11. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, *Arkh. Pat.*, **38**, No. 5, 48-54 (1976).
12. V. P. Tumanov, *Arkh. Pat.*, **45**, No. 1, 3-12 (1983).
13. H. Hara, T. Sukamoto, and K. Kogure, *Prog. Neurobiol.*, **40**, 645-670 (1993).
14. K. Kogure and J. Kawagoe, in: *Maturation Phenomenon in Cerebral Ischemia*, Berlin (1992), pp. 15-22.
15. L. Z. Pevzner, *Exp. Neurol.*, **65**, 237-241 (1979).

Changes in the Phospholipid Composition of Cat Hepatocyte Plasma Membrane in Hemorrhagic Shock

G. F. Leskova and V. I. Udovichenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 3, pp. 261-263, March, 1997
Original article submitted November 18, 1995

Progressive elimination of phosphatidylcholine from the hepatocyte plasma membranes is observed during the development of hemorrhagic shock in cats. The phosphatidylinositol content decreases 30 min after the start of bleeding and then gradually increases. The phosphatidylethanolamine content is increased on the 30th and 60th min of bleeding, while the content of phosphatidylserine increases 1 h after the start of bleeding. At the peak of hemorrhagic shock the major shifts are observed in the phosphatidylcholine and phosphatidylinositol contents.

Key Words: *phospholipids; plasma membranes; hepatocytes; hemorrhagic shock*

Changes in the structure and function of cell membranes are one of the major determinants of the pathogenesis of shock at the cellular and subcellular levels [10]. Therefore, it is important to evaluate the role of structural modifications occurring in the plasma membrane phospholipids, since they strongly determine functional activity of the cell [1,5,6,8,9]. Bearing in mind that the liver is a target organ of hemorrhagic shock, we analyzed changes in the phospholipid composition of the hepatocyte plasma membrane in hemorrhagic shock.

Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

Experiments were performed on cats (n=34, body weight 3.0±0.5 kg) under Nembutal anesthesia (40 mg/kg intraperitoneally). Hemorrhagic shock was produced by the method [16]. The animals were injected with 2000 units/kg heparin 30 min prior to bleeding. Blood pressure was reduced to 40 mm Hg within 30 min and maintained at this level for 1 h. Intact cats given the same dose of heparin served as the control. Liver was incised after perfusion with cold 1 mM $NaHCO_3$ 30 min, 1, and 1.5 h (experimental cats) and 1, 1.5, and 2 h (control cats) after the start of bleeding. Hepatocytes were isolated as described [4], and total lipid extraction was performed by the method [13]. Phospholipids were frac-

0007-4888/97/0003-0224$18.00 ©1997 Plenum Publishing Corporation