ELECTRON-AUTORADIOGRAPHIC INVESTIGATION OF RNA SYNTHESIS IN CEREBRAL CORTICAL NEURONS OF RATS AFTER BURNS

E. Ya. Sanovich, V. P. Tumanov, and A. A. Pal'tsyn

UDC 617-001.17-092.9-07:616.831-008.939.633.2-092-18-076.4

KEY WORDS: neuron; nucleolus; RNA synthesis; autoradiography; burns.

The subject of burns has attracted the attention of clinicians in different specialties and experimental workers for over a century, but important problems to do with the pathogenesis of this condition still remain unsolved. Much can be done along these lines through the use of modern methods of morphological investigation, by means of which we can penetrate deeper into the intimate mechanisms of disturbances of CNS functions. Injury to the CNS gives rise to the principal clinical effect of shock. Previous investigations [8-11], using the electron microscope, showed that burns significantly modify protein metabolism in the neuron and cause destruction of the rough endoplasmic reticulum. Since this reticulum contains RNA granules it can be postulated that burns significantly modify RNA metabolism in the neuron, which is an important regulating structure of the CNS, it is essential to discover how repair processes in the neuron are provided for, i.e., how intracellular regenerative processes take place in the neuron [6].

For the experimental study of these problems the present investigation was undertaken, using the modern technique of electron-microscopic autoradiography, by means of which the dynamics of RNA synthesis in the neuron of the CNA can be recorded structurally.

EXPERIMENTAL METHOD

A burn of the IIIB-IV degree, covering 20% of the body surface, was inflicted on noninbred albino rats weighing 180 g under ether anesthesia. RNA synthesis was investigated in intact animals (control) and 1 and 12 h and 3 and 6 days after burning (five animals at each time). Altogether 25 animals were used. For electron-autoradiographic study of RNA synthesis, under ether anesthesia the RNA precursor uridine-5-^{3}H (specific activity 26 Ci/mmole) was injected through a special needle into the brain substance (sensorimotor cortex, area PA^m). The dose of the labeled precursor (50 μCi) was dissolved in 0.05 ml Ringer's solution. Pieces of cortex taken 3 h after injection of uridine-^{3}H were fixed in 2.5% glutaraldehyde solution made up in phosphate buffer, pH 7.4. The pieces were then washed with buffer for 24 h, with repeated changing of the solution, and then postfixed in 1% OsO_4 solution. After dehydration in alcohols the pieces were embedded in Epon. Light-microscopic autoradiographs were first prepared on semithin sections, and depending on the results of their analysis the region for cutting ultrathin sections (the 5th layer) was chosen. Serial electron autoradiographs were prepared with M emulsion by the method described by Sarkisov et al. [7]. After exposure for 1 month the preparations were developed and examined in the IEM-100B microscope. The area of cross section (the product of the maximal and minimal diameters) of the nucleolus and nucleus of the neurons was measured on the negatives and the density of label in the nucleolus and in the nucleus (the ratio of the number of grains of reduced silver located above these zones and their area of cross section) was determined. The numerical experimental results were subjected to statistical analysis on the M-220 computer [3].

EXPERIMENTAL RESULTS

The study of light-microscopic autoradiographs on semithin sections revealed incorporation of label into nuclei of neurons and glial and endothelial cells. A marked decrease in the concentration of the label was found

Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. I. Kuzin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 733-736, December, 1980. Original article submitted June 20, 1980.

0007-4888/80/9012-1743$07.50

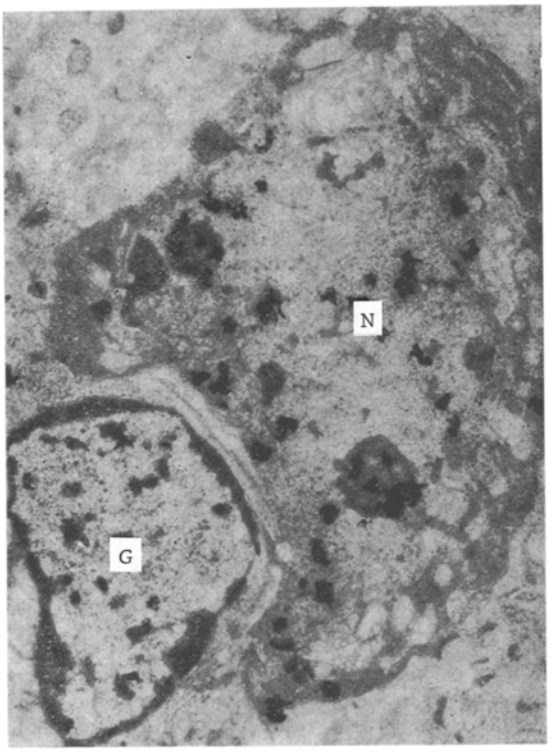

Fig. 1

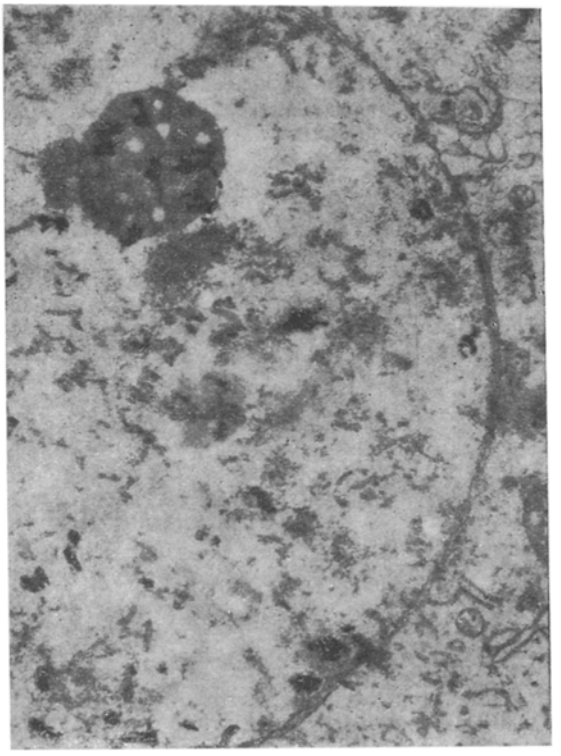

Fig. 2

Fig. 1. Differences in intensity of incorporation of uridine-^{3}H into a neuron (N) and glial cell (G) (oligodendrocyte). 18,000×.

Fig. 2. Distribution of label in neuron after injection of uridine-^{3}H: highest concentration of grains of silver above body of nucleolus. 25,000×.

as the distance from the site of injection increased. This fact led us to decide not to compare absolute values of density of label in the control and experimental animals. Since it was impossible to guarantee that the cells chosen for investigation were the same distance away from the site of injection, the difference between the absolute values of the densities could be attributable not only to differences in the rate of incorporation of the precursor, but also differences in the closeness of the cells compared to the site of injection of the isotope.

The higher rate of incorporation of uridine-^{3}H in neurons than in glial and endothelial cells was very clearly revealed in electron-autoradiographs (Fig. 1). In the neurons the highest concentration of label was found in the nucleolus or, more exactly, in its body – the nucleonema (Fig. 2). Very dense labeling was observed above the extranucleolar part of the nucleus. Only single grains of silver were observed in the cytoplasm.

Many experiments of different kinds have shown [12-16] that the nucleolus is the site of synthesis of ribosomal RNA (rRNA). This rRNA is also contained in the Nissl's bodies. The action of pathogenic factors and, in particular, of burns, on the nervous system causes significant changes in the structure of the apparatus of protein synthesis in the neuron – the Nissl's substance. In light-microscopic sections these changes are manifested as paler staining by Nissl's method – tigrolysis [1, 2, 4, 5]. Restoration of the Nissl's substance can take place by hyperproduction of rRNA by the nucleolus. The state of synthesis of nucleolar RNA can thus be used to judge destructive and regenerative changes in the protein-synthesizing function of the nerve cell. The level of synthesis of nucleolar RNA in each cell studies was determined as the ratio between the density of its label to that of the extranucleolar part of the nucleus.

Increasing or decreasing distance of the test cell from the site of injection of the isotope changed its concentration for the cell as a whole but did not affect the ratio between the density of labeling of nucleolus and nucleus, which depends only on the rate of RNA synthesis in different cell structures. Consequently, by using this approach it was possible to determine the level of synthesis of nucleolar RNA relative to that of nuclear RNA. The values obtained by this analysis are given in Table 1. In all animals with burns RNA synthesis in the nucleoli was more rapid than in the control. A very slight increase in its rate could be seen as early as

TABLE 1. Ratio of Labeling Density of Nucleolus to Labeling Density of Extranucleolar Zone of the Nucleus in Neurons of the 5th Layer of the Rat Cerebral Cortex

Group of animals	Time from burning to injection of isotope	Number of cells tested	$M \pm m$	t	P
1	Control	255	4,933±0,455	—	—
2	1 h	262	5,051±0,424	0,190	>0,05
3	12 h	285	7,713±0,304	5,077	<0,001
4	3 days	120	4,476±0,859	2,616	<0,01
5	6 days	241	5,805±0,328	1,552	>0,05

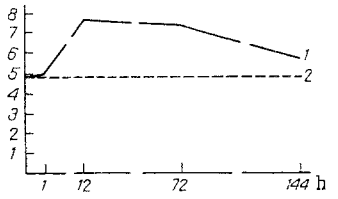

Fig. 3. Rate of rRNA synthesis in cerebral cortical neurons of rats after burns: 1) experiment; 2) control. Abscissa, time between burning and injection of isotope (in h); ordinate, ratio of density of label in nucleolus to density of label in extranucleolar zone of nucleus.

1 h after burning, in the period of shock. Synthesis of nucleolar RNA in the animals 12 h after burning was more than 1.5 times faster than in the control, and it remained at almost the same level until 3 days. On the 6th day the rate of RNA synthesis in the nucleoli of the burned animals fell appreciably but was still higher than the control level.

The increase in the rate of synthesis of nucleolar RNA can be regarded as an intracellular regenerative reaction, expressed as the more intensive production of new ribosomes and aimed at restoring the rough endoplasmic reticulum of the neurons injured by the burn trauma. On the basis of these results it was possible to determine quantitatively the magnitude and duration of the reaction of hyperplasia of the ribosomes which developed in response to injury. They showed that the reaction develops very quickly (1 h) after injury and reached a high level after 12 h. The rate of rRNA synthesis fell more slowly than it rose, and it had not returned to its normal level even at the end of the 6th day after trauma (Fig. 3).

By means of electron autoradiography it is possible to judge intracellular regenerative processes in the CNS even in the initial period of the pathological process. This method shows that repair processes begin to develop simultaneously with destructive changes in the cell. Transmission electron microscopy did not reveal the development of repair processes in the neuron before the 10th-15th day [4], whereas the results of autoradiography at the ultrastructural level show reliably that intracellular repair processes begin earlier (within a matter of hours).

LITERATURE CITED

1. A. P. Avtsyn and V. A. Shakhlamov, The Ultrastructural Bases of Cell Pathology [in Russian], Moscow (1979).
2. N. N. Bogolepov, Ultrastructure of the Brain in Hypoxia [in Russian], Moscow (1979).
3. A. I. Kurochkina and N. G. Astasheva, in: Cybernetics in Medical and Biological Research [in Russian], Moscow (1971), pp. 16-26.
4. S. Nichkov and G. N. Krivitskaya, Acoustic Stress and Cerebrovisceral Disturbances [in Russian], Moscow (1969).
5. É. N. Popova, S. K. Lapin, and G. N. Krivitskaya, Morphology of Adaptive Changes in Nerve Structures [in Russian], Moscow (1976).

6. D. S. Sarkisov, Regeneration and Its Clinical Importance [in Russian], Moscow (1970).
7. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, Adaptive Reorganization of Biorhythms [in Russian], Moscow (1975).
8. V. P. Tumanov, Arkh. Patol., No. 11, 62 (1973).
9. V. P. Tumanov, "Electron-microscopic investigation of changes in the CNS in burns and during the action of radiation," Author's Abstract of Doctoral Dissertation, Moscow (1974).
10. V. P. Tumanov, Arkh. Patol., No. 3, 41 (1976).
11. V. P. Tumanov and M. D. Malamud, Changes in the CNS in Burns and Radiation and Combined Trauma [in Russian], Kishinev (1977).
12. J. E. Edstrom, J. Biophys. Biochem. Cytol., 8, 47 (1960).
13. R. P. Perry, Exp. Cell Res., 29, 400 (1963).
14. R. P. Perry, Natl. Cancer Inst. Monogr., 14, 73 (1964).
15. R. P. Perry, Natl. Cancer Inst. Monogr., 23, 527 (1966).
16. F. M. Ritossa and S. Spiegelman, Proc. Natl. Acad. Sci. USA, 53, 737 (1965).

STUDY OF THE SUBCELLULAR LOCALIZATION OF $^{45}Ca^{++}$ DURING ABSORPTION BY EPITHELIUM OF THE RAT SMALL INTESTINE

I. A. Morozov, V. B. Spirichev, and Yu. A. Lysikov

UDC 616.341-018.73-073.916-033.1

KEY WORDS: ultrastructure; calcium; absorption; enterocyte.

Despite great advances made in recent years in the study of the mechanisms of absorption of Ca^{++} in the intestine, many structural and biochemical aspects of this process still remain unexplained [2, 16]. In particular, the problem of concrete pathways for the transfer of Ca^{++} through the layer of intestinal epithelium still remains unsolved.

The most widely held hypotheses are those based on the assumption that Ca^{++} ions pass through the apical membrane into the cytosol of the enterocyte, and are then expelled from it by the Ca pump on the basal side of the cell [2, 7, 16]. A few electron-microscopic investigations have yielded evidence that Ca^{++} is carried through the enterocyte in a sequestered state, in "packets," evidently not mixed with the cytosol [15]. According to other data, Ca^{++} is carried across a tight junction and then along the lateral membrane, along an intercellular canal [3].

A definite defect of the above investigations is that the methods used did not enable the absorbed Ca^{++} ions to be differentiated from the endogenous cation which is constantly present in the cell and its structures. This difficulty can be overcome by using electron microscopy in conjunction with electron autoradiography, and by the use of radioactive $^{45}Ca^{++}$ as the absorbed ion. The essence of the method is to identify the absorbed radioisotope by means of halides of heavy metals, reduction of which by β-radiation at the site of localization of the isotope leads to deposition of an electron-dense deposit of the reduced metal in these areas [12].

This method was used in the present investigation to study the localization of $^{45}Ca^{++}$ during its absorption in the small intestine of young, growing rats.

EXPERIMENTAL METHOD

Under ether anesthesia, 0.5 ml of 0.25 mM $^{45}CaCl_2$ (2 mCi/mmole) in 0.9% NaCl was injected into a 5-cm segment of the proximal portion of the small intestine of a young (80-100 g) male Wistar rat, isolated in situ

Institute of Nutrition, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 736-738, December, 1980. Original article submitted March 26, 1980.

 0007-4888/80/9012-1746$07.50