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CORRELATION BETWEEN DESTRUCTIVE AND REPARATIVE PROCESSES IN RAT CEREBRAL CORTICAL NEURONS AFTER BURN TRAUMA

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KEY WORDS: neurons; rat cerebral cortex; RNA metabolism; electron-microscopic autoradiography.

When the time course of a pathological process is described the reparative phase is generally regarded as one which develops, not at the beginning of the process, but at a certain late, or even the final stage [2]. However, from the theoretical point of view it is evidently correct to consider that repair is triggered by injury and, consequently, it must take place immediately after the beginning of the action of the destructive factor or after a certain latent period necessary for the particular reparative response to be activated. Investigation of these concepts on a model of hepatitis [3] showed that the reparative response, expressed as intensification of RNA synthesis in the hepatocytes located at the periphery of the lobule, develops simultaneously with the spreading of necrosis in hepatocytes at the center of the lobule. Another reparative response leading to much more prolonged and stable compensation and to stimulation of DNA synthesis develops in the preserved cells later, after the lapse of sufficient time for completion of all processes preparatory for DNA synthesis.

Considering the unique character of the biological and pathological processes in the brain, we decided to study the development of reparative and destructive processes in neurons. It was recalled that, unlike in the liver, which can regenerate through division of its cells, neurons have an exclusively intracellular type of regeneration. To investigate the chosen problem the method of electron-microscopic autoradiography was used, for it enables the intensity of RNA synthesis, an important reparative process, to be compared with ultrastructural changes characterizing damage to and repair of the cell.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 180 g were anesthetized with ether and a stage IIIB-IV burn covering 20% of the body surface was inflicted. RNA synthesis was investigated in intact animals (control) and 4, 15, 75, and 144 h after burning (five animals at each time). Altogether 25 animals were used. For electron-autoradiographic investigation of RNA synthesis, the animals were anesthetized with ether and the RNA precursor 5-^{3}H-uridine (specific activity 26 Ci/mmole) was injected into the brain substance (the cerebral cortex in sensomotor area PA^m) through a special needle. The labeled precursor (50 μCi) was dissolved in 0.05 ml of Ringer's solution. Pieces of cortex were removed 3 h after injection of the label and fixed in a 2.5% solution of glutaraldehyde in phosphate buffer, pH 7.4. For the next 24 h the fragments were washed with buffer, with repeated change of the solution, and postfixed in 1% OsO_4 solution. After dehydration in alcohols, the fragments were embedded in an Epon mixture. Light-microscopic autoradiographs were first prepared on semithin sections and the region of cerebral cortex for ultramicrotomy of layer V was chosen on the

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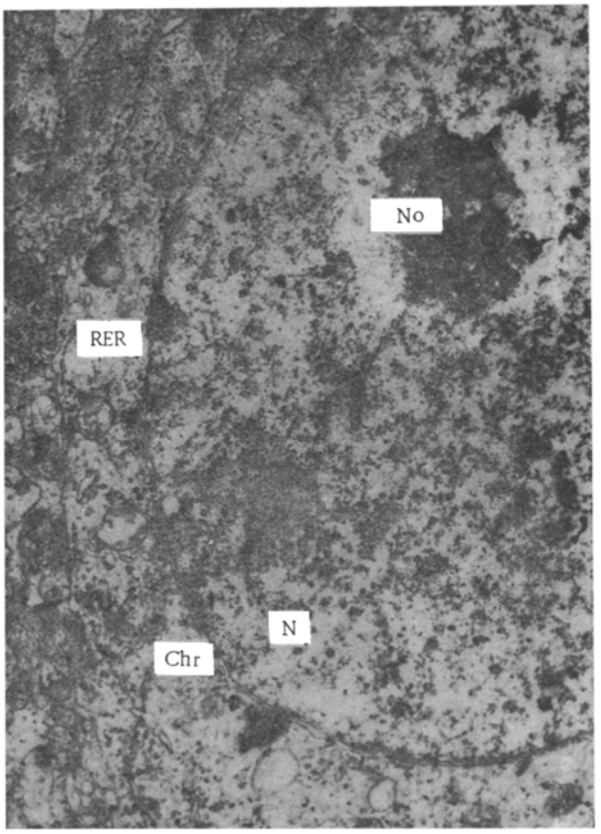

Fig. 1. Pale neuron in layer V of rat sensomotor cortex 15 h after burn trauma. Decrease in size of RER component of rough endoplasmic reticulum (RER) and in number of free polysomes, i.e., chromatolysis (Chr). Chromatolysis is combined with intensive RNA synthesis in nucleus (N) and nucleolus (No) of neuron. 12,000 ×.

basis of the results of their analysis. Serial electron-autoradiographs were prepared with the aid of type M emulsion [3]. After exposure for 1 month the preparations were developed and examined in the JEM-100B microscope. Quantitative analysis of the autoradiographs included determination of the density of grains of silver above the test structures: nucleolus, extranucleolar zone of the nucleus, and the cytoplasm of the neuron. For this purpose the number of grains of silver above the nucleus and nucleolus was counted on the negatives, the areas of cross section of the nucleus and nucleolus were measured after which the labeling densities in the nucleous (d_2) and extranucleolar zone of the nucleus (d_1) were calculated (in relative units) and the ratio between them (r) was determined. The value of r served as indicator of the relative intensity of RNA synthesis in the nucleolus compared with the corresponding parameter for synthetic processes taking place in the extranucleolar zone of the neuron nucleus. The significance of differences between the parameters compared was determined by Student's t test. The labeling density was compared in four groups of cells: pale and dark neurons with no evident dystrophic changes; pale and dark neurons with dystrophic changes. The numerical results were subjected to statistical analysis on the M-220 computer.

EXPERIMENTAL RESULTS

Analysis of the morphological changes in the neurons at different times after burn trauma revealed various structural changes in them, evidence of intensification of two simultaneously developing processes in these cells, namely destructive and reparative, with a mosaic character of distribution.

Intracellular destructive processes after burn trauma were manifested both as ultrastructural changes in the nucleus and cytoplasm and as a disturbance of neuroglio-vascular relationships. As early as 4 h after burn trauma changes in structures of the rough endoplasmic reticulum (RER) were observed in individual neurons: segmental and, sometimes,

TABLE 1. Mean Labeling Density in Extranucleolar Part of Nucleus and Nucleolus of Neurons of Control Animals and 4, 15, 75, and 147 h after Burn Trauma (M ± m)

Parameter	Time after burn trauma, h				
	control	4	15	75	147
Number of cells investigated	225	262	285	120	241
Labeling density in extranucleolar zone of nucleus (d_1)	50,8±4,4	90,0±4,7*	154,3±6,6*	182,0±10,6*	185,2±8,9*
Labeling density in nucleolus (d_2)	188,7±15,2	349,6±24,0*	957,0±31,1*	955,0±50,9*	821,5±35,5*
Ratio of labeling density in nucleolus to labeling density in nucleus ($r=d_2/d_1$)	4.6±0,5	5,0±0,4	7,7±0,3*	7,5±0,9*	5,8±0,3

Legend. *$p < 0.05$ Compared with control.

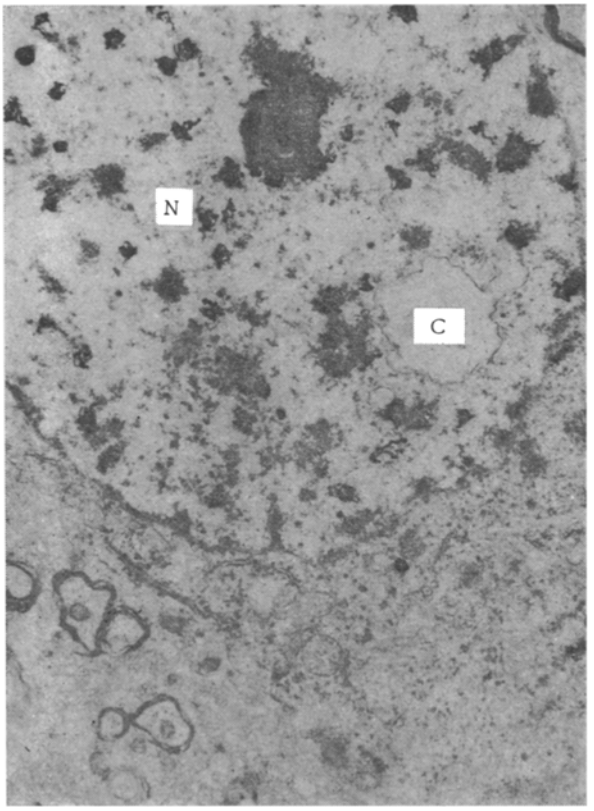

Fig. 2. Pale neuron in layer V of rat sensomotor cortex 15 h after burn trauma. Intensive RNA synthesis in nucleus of neuron together with presence of a large cavity (C) in nucleus (N). 13,000 ×.

total chromatolysis (Fig. 1), vacuolation of the karyoplasm (Fig. 2), and considerable dilatation of the tubules and cavities of RER (Fig. 1). Mitochondria with a translucent matrix, and with fragmentation and disintegration of their cristae were frequently seen (Fig. 3).

Some morphological features of the neurons of the experimental animals, distinguishing them from those of the control animals, can be regarded as a manifestation of intracellular repair processes. These changes include: an increase in the fraction of euchromatin, an increase in the size and number of the nucleoli, and increase in extent of the nuclear membrane and the number of pores in it, and accumulation of granular material near the pores of the nuclear membrane. Reparative processes in the neurons, assessed on the basis of structural changes, could be distinguished at the earliest times of observation (4 h). They reached their peak of development after 15 h, and later remained at the same level until the end of observation.

RNA synthesis, recorded by electron-microscopic autoradiography, is a more precise and more quantitative parameter of repair processes in the zone of the sensomotor cortex chosen

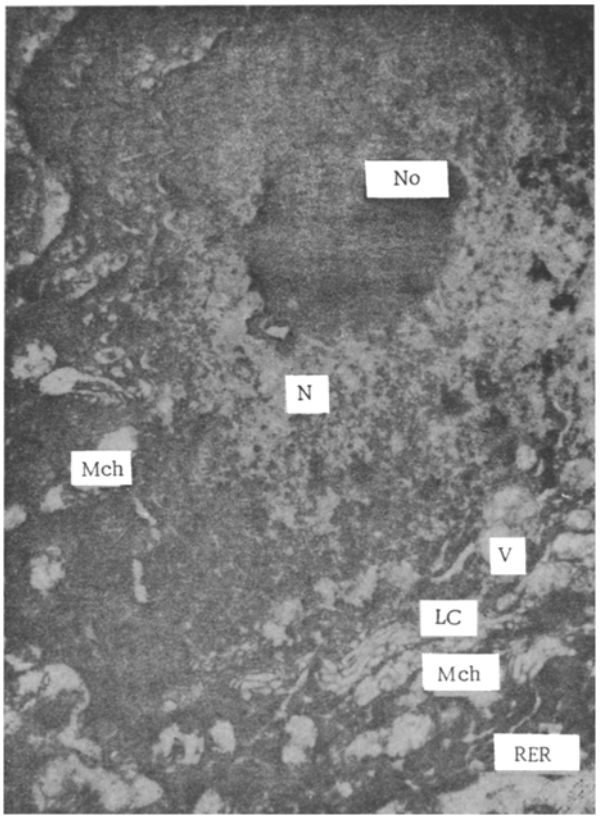

Fig. 3. Dark pyramidal neuron in layer V of rat sensomotor cortex 15 h after burn trauma. Dilation of tubules of RER, swelling and destruction of cristae of mitochondria (Mch). Considerable vacuolation of lamellar complex (LC), with numerous vacuoles (V). Intensive RNA synthesis in N and No of neurons. 12,000 ×.

for investigation. Examination of the light-microscopic and electron-microscopic autoradiographs showed that incorporation of labeled uridine into the brain cells was recorded mainly in the nucleus, and in a particularly high concentration, in the nucleolus. With the period of circulation of the labeled precursor used (3 h) the quantity of label in the cytoplasm was exceedingly small. Neurons were distinguished by the highest density of labeling compared with other brain cells. The results of determination of the labeling density in them are summarized in Table 1. Clearly RNA synthesis in both nucleus and nucleolus of the neurons rose sharply during the first few hours after burn trauma (4 h), i.e., repair processes were recorded simultaneously with the appearance of the first destructive changes. RNA synthesis in the experimental animals remained at a much higher level than in the control until the end of the observations.

Since chromatolysis is a manifestation of dystrophy in nerve cells, special attention was paid to RNA synthesis, as a process aimed at restoring the components of the rough endoplasmic reticulum. For this purpose a parameter "r" was introduced — the ratio of labeling density in the nucleolus to that in the nucleus (Table 1).

Other investigators [4-8] have shown that the nucleolus is the site of synthesis of ribosomal RNA (rRNA). RNA synthesis was increased in the experimental animals both in the extranucleolar zone of the nucleus and in the nucleolus, and the increase in the value of r observed pointed to a particularly high intensity of rRNA synthesis, detectable even against the background of the general RNA hyperproduction in the neuron. rRNA synthesis reached its peak value 15 and 75 h after burn trauma, after which it fell until 147 h, as was reflected also in the morphologically apparent decrease in the intensity of chromatolysis.

A very important aspect of the study of compensatory and adaptive processes is determination of the actual cells in which preparative and destructive processes develop: are they the same or different? To answer this question with respect to neurons, we compared

the labeling density in cells with dystrophic changes and in cells with no such changes. As has already been pointed out [1], dark neurons have a higher level of RNA synthesis than pale neurons. Meanwhile dark neurons with dystrophic changes had a higher intensity of RNA synthesis, although not statistically significantly, than dark neurons with no dystrophic changes. Similar data also were obtained when pale neurons were compared. This is shown by examination of the autoradiographs (Fig. 1-3), which revealed maximal labeling in the nucleus and nucleolus of damaged neurons. In other words, nerve cells with marked dystrophic changes were characterized by a high level of RNA synthesis.

As was observed previously for hepatocytes [3], a reparative process such as RNA synthesis is recorded in neurons simultaneously with destructive changes. However, unlike in hepatocytes, it does not develop in different cells, but in the same cells, due to the intracellular type of regeneration which is a feature of neurons.

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ACTION OF CORTICAL EXTRACTS FROM THE LEFT AND RIGHT CEREBRAL HEMISPHERES ON RESTORATION OF CONDITIONED REFLEX ACTIVITY IN RATS AFTER UNILATERAL EXTIRPATION OF THE FRONTAL CORTEX

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Proof has recently been obtained of neurochemical asymmetry of the brain of man and animals [9] and of considerable differences in the changes taking place in neurotransmitter concentrations after injury to the left or right cerebral hemispheres [12, 13]. Unilateral injury to the cerebellum [1], vestibular nuclei [6], or cerebral cortex [2] of animals and the creation of unilateral generators of pathologically enhanced excitation (GPEE) in the brain [5, 6] or spinal cord [8] have been shown to lead to the appearance of peptide regulators in the brain tissue with a lateralized influence on muscle tone. The disparity between the biochemical consequences of injury to the hemispheres suggests that, in principle, they can be selectively corrected and, consequently, partial recovery of disturbed functions may be possible with the aid of endogenous factors specific for each hemisphere.

To test this hypothesis, the investigation described below was undertaken to study the effect of low-molecular-weight components of cortical extracts of the left and right hemi-

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