ULTRASTRUCTURE OF NEURONS WITH HYPERCHROMIA AND VACUOLATION FOUND IN NERVE TISSUE AFTER ANOXIA

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Postanoxic changes in cells of the CNS and, in particular, in neurons have been studied sufficiently well [1]. They depend on the type of anoxia, the character of the course of the process, and on other factors, and they can in general be reduced to manifestations of chromatolysis, hyperchromia, and combinations of both. However, whereas ultrastructural changes in the cytoplasm of neurons have been described in fair detail, there is little information on changes taking place in nuclei, especially of hyperchromic neurons. This is easily explained, for it is virtually impossible by traditional electron-microscopic methods to detect the fine structure of dense nuclei of hyperchromic cells. Better prospects for the study of nuclear ultrastructure are provided by the electron-cytochemical method of Bernhard [6, 8].

For a number of years changes in nerve cells of the rat brain in a model of anoxic anoxia have been investigated in detail in the writer's laboratory by methods of light microscopy. The principal results were described in a general monograph [4] in which, in particular, the time course of changes in the neurons was described, the number of neurons undergoing different changes at different periods after the session of anoxia was counted, and so on. Special attention was paid to a group of hyperchromic and contracted neurons, which appeared immediately after the session of anoxia, and which were numerous after 100 days. This group of cells was heterogeneous in composition and whereas the normal structure of some of them was restored in the course of the experiment, others died.

In this paper the results are given of a study of part of this group of cells, namely hyperchromic neurons with signs of vacuolation, revealed in a nerve tissue by Bernhard's method after a single exposure to anoxic anoxia.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 120-160 g. Nerve cells were studied in the sensomotor cortex. The rats were exposed to a single session of anoxic anoxia in a pressure chamber by the method used in the writer's laboratory [4], after which the animals were kept for 100 days under ordinary animal house conditions. The rats were killed by intracardiac injection of a 2.5% solution of glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) under ether anesthesia. Material was embedded in Epon without preliminary fixation in osmic acid. Ultrathin sections were cut on an ultramicrotome (Reichert, Austria), stained for ribonucleoproteins [2], and examined in the EM-420 electron mciroscope (Philips, The Netherlands).

EXPERIMENTAL RESULTS

Two groups of neurons with signs of hyperchromia and vacuolation were found in the sensomotor cortex of animals exposed to anoxic anoxia.

Cells of the first group were medium-sized and small pyramidal neurons and were found in all layers of the cortex. The distinguishing feature of these cells was gross swelling of the cisterns of the endoplasmic reticulum (Fig. 1). Nuclei of these cells were characterized by the almost total absence of condensed chromatin, quite untypical of hyperchromic neurons. The bulk of the nuclei was filled with interchromatin fibrils (ICF) and floccular material, with lower electron density than the ribosomes in the cytoplasm. Discrete perichromatin

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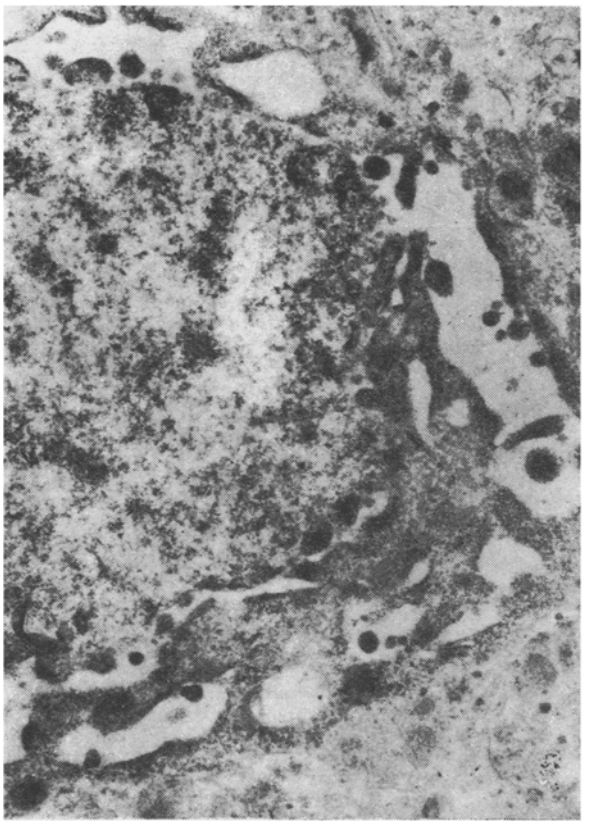

Fig. 1

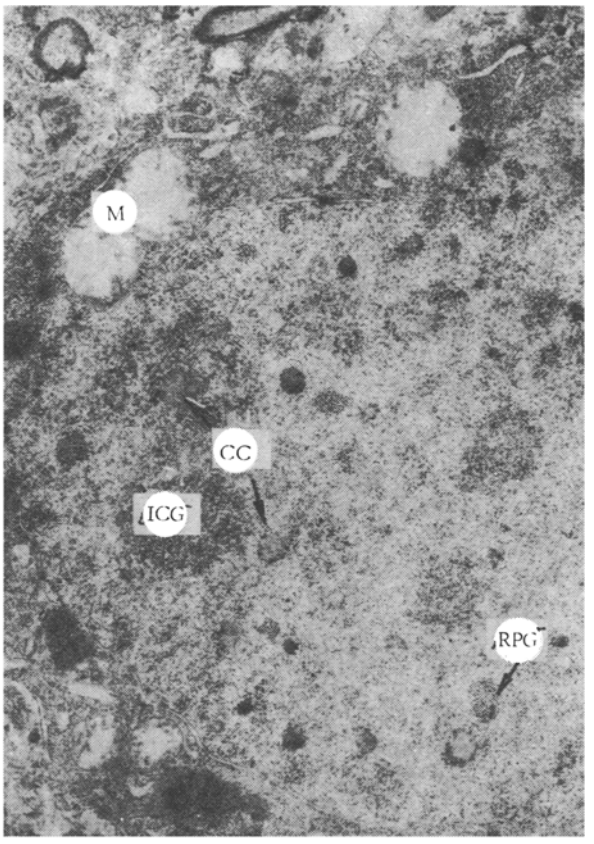

Fig. 2

Fig. 1. Hyperchromic neuron with destructive changes in nucleus and cytoplasm (13,500 ×). Here and in Figs. 2 and 3: sensomotor cortex, anoxic anoxia; stained by Bernhard's method.

Fig. 2. Hyperchromic neuron with swollen mitochondria (M) in cytoplasm (12,500 ×). CC) Conglomerates of condensed chromatin; RPG) concentration of RNP-positive granules.

granules (PCG) could be observed in these nuclei. Concentrations of interchromatin granules (ICG), which are an invariable component of the nuclei of neurons, were not observed here. Virtually no nucleoli likewise were found in the nuclei of this group of cells. Evidence of vacuolation of the cytoplasm varied considerably in different neurons of this group of cells — from small dilatations of the cisterns of the endoplasmic reticulum to fusion of these swollen cisterns into one large vacuole, in which areas of cytoplasm with dissociated and densely packed ribosomes could be seen.

The second group of hyperchromic neurons with evidence of vacuolation of the cytoplasm also consisted of medium-sized and small pyramidal cells, found mainly in layers V and VI of the cortex. Vacuolation of the cytoplasm of these cells was much less marked than in the cells of the first group, and it occurred entirely on account of swollen mitochondria (Fig. 2). The cisterns of the endoplasmic reticulum in the cells of this group were unchanged or slightly dilated. In the cytoplasm, some of the polysomes were dissociated into separate ribosomes. A characteristic feature of the nuclei of the second group of nerve cells was the presence of small round conglomerates of condensed chromatin in them, distributed throughout the plane of section of the nucleus, and not connected with the nuclear membrane, as is usually found in hyperchromic cells (Fig. 2, Fig. 3a). These conglomerates always contained a large number of perichromatin fibrils (PCF) and PCG around their periphery. Another characteristic feature of these cells was the presence of compact concentrations of electron-dense granules in the nuclei, usually in the immediate vicinity of conglomerates of condensed chromatin (Figs. 2 and 3). Individual granules from the concentration were about half the size of the PCG, i.e., they measured approximately 30-35 nm, greater than the mean diameter of ICG. Concentrations of ICG resembled those usually found, characteristic of the nuclei of normochromic neurons, but the fibrils forming separate ICG were not tightly packed to-

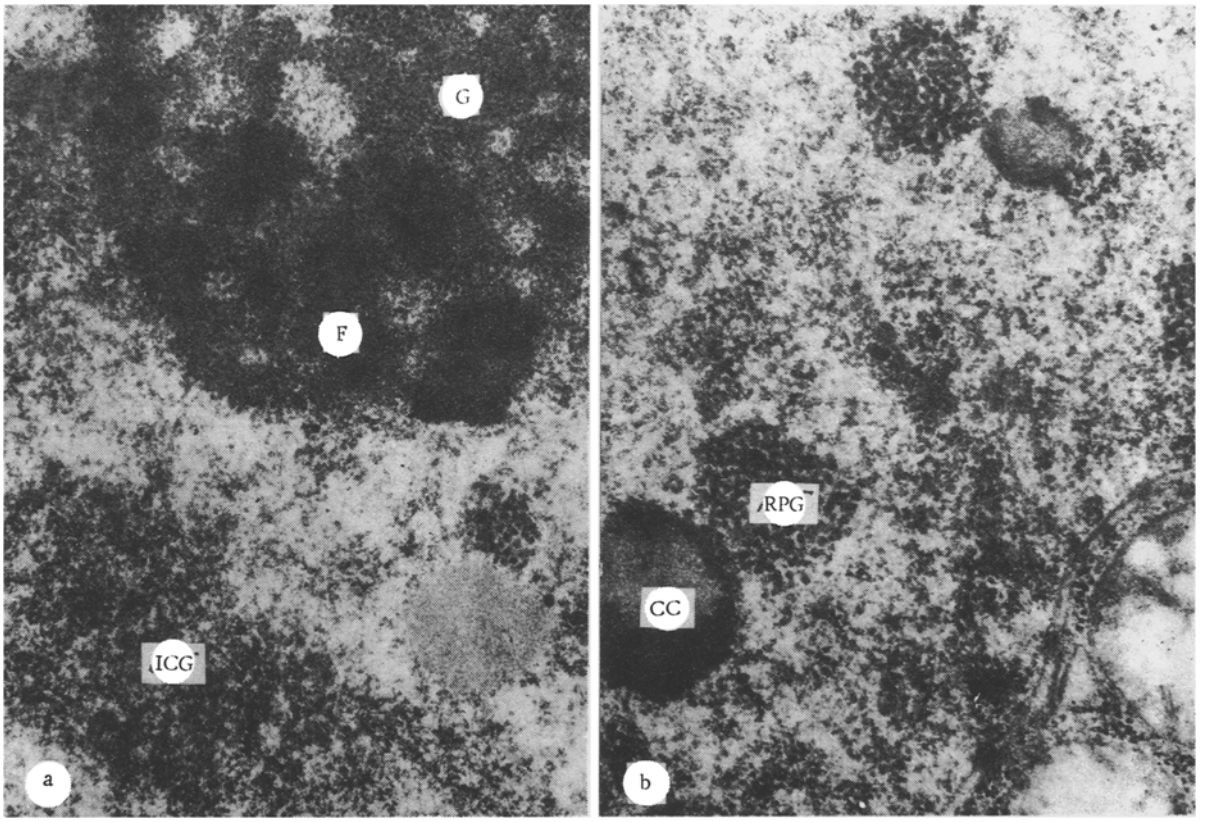

Fig. 3. Fragments of hyperchromic neurons with swollen mitochondria (46,000 ×). a) Conglomerates of condensed chromatin and concentrations of electron-dense granules in nuclei; b) concentrations of loosely packed ICG. G, F) Granular and fibrillar zones of nucleolus respectively. Remainder of legend as to Fig. 2.

gether, so that the whole concentration appeared to be loose in structure (Figs. 2 and 3b).

Most of the area of the nucleus was filled with ICF, the density of which was considerable, and this created an increased electron density of the whole nucleus. Small concentrations of PCG, not directly connected with conglomerates of condensed chromatin, also were found in the nuclei of this group of cells. Nucleoli were located in the center of the nucleus, and under low power, alternating fibrillary and granular zones were very clearly distinguishable in them (Fig. 3b). Near the nucleoli no condensed chromatin was observed.

The two groups of hyperchromic neurons with vacuolation of their cytoplasm described in this paper correspond to what has been called ischemically homogenized disease of nerve cell [1]. Analysis of the ultrastructure of the cytoplasm and nucleus, on the basis of electron-cytochemical data obtained by Bernhard's method, enabled the state of the plastic processes in these cells to be characterized. For instance, nerve cells of the first group are neurons which have undergone irreversibly degenerative changes, and have died. This is shown by the grossly swollen cisterns of their endoplasmic reticulum, the absence of the "standard" set of ribonucleoprotein (RNP) particles in the nuclei, and the presence of floccular material in them. The latter is characteristic of nuclei of neurons in which autolysis is taking place [3, 5]. According to Bogolepov [1], these cells will subsequently undergo neuronophagy.

Another picture is observed in the second group of neurons which, according to the results of light-optical investigation, are very similar to cells of the first group with minimal changes. Neurons of the second group are cells which have either recovered their synthetic activity or have become adapted to functioning after the trauma they have undergone. The presence of polysomes in the cytoplasm and the presence of all types of RNP particles in the nucleus are evidence of the existence of synthetic processes by the DNA-RNA-protein synthesizing system in these neurons. Admittedly, synthesis of RNA and protein itself must be depressed in these cells and also modified somewhat compared with normochromic neurons. This is shown by the appearance and unusual character of condensation of the chromatin in the

form of small conglomerates, distributed throughout the nucleus. The increase in the quantity of condensed chromatin is proportional to the decrease in transcription activity of the nucleus [7]. Concentrations of ICG with indistinct separate granules, an increased number of PCG, collected into "bunches" and unconnected with condensed chromatin, partial segregation of the nucleus, and also the appearance of RNP-positively stained granules, which are never observed in the nuclei of normochromic neurons, also point to changes in RNA and protein synthesis. Similar granules were discovered by Swanson and co-workers [9] in nuclei of neurons attacked by herpes simplex virus.

Thus two groups of nerve cells with signs of hyperchromia and vacuolation are found in the sensomotor cortex of rats exposed to anoxic anoxia: cells with irreversible degenerative changes and cells with no signs of irreversible degeneration, but with changes in their DNA-RNA-protein synthesizing apparatus.

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STEREOHISTOLOGIC ANALYSIS OF THE MYOCARDIUM OF HOMOIOTHERMIC ANIMALS DURING COOLING

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The cardiovascular system, which plays an important role in adaptation of the organism to cold stress [1], is in a state of functional strain when exposed to this factor, and this frequently leads to the development of irreversible pathological changes in the myocardium [9, 13-16]. To understand the mechanisms of formation of compensatory-adaptive reactions in the myocardium during hypothermia, quantitative morphologic investigations are very important [6, 10]. However, the time course of quantitative structural changes in the muscular and connective tissue of the myocardium during exposure to low temperatures has not been fully investigated [5].

The aim of this investigation was a stereologic study of parenchymatous-stromal interrelations in the myocardium of rats subjected to long-term hypothermia.

EXPERIMENTAL METHOD

Experiments were carried out on 26 male Wistar rats aged 4 months and weighing initially 200-230 g. The animals were subjected to continuous hypothermia (except when feeding) in a

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