MECHANISM OF CHANGES IN NERVE CELLS IN ACUTE EXPERIMENTAL EMOTIONAL STRESS

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KEY WORDS: ganglia; neuron; lysosomes.

The results of a study of the ganglia of the autonomic nervous system in emotional stress [1] showed that during the formation of this state the most marked changes developed not in the sympathetic ganglia, but in structures of the ganglion nodosum of the vagus nerve. In that ganglion the volume of the bodies of nerve cells and their nuclei, the concentration of structural proteins of the cytoplasm and nuclei, and the concentration of water-soluble proteins in homogenates from this formation all decreased significantly.

The next step was to determine what mechanisms lie at the basis of these changes and what causes the nerve cells to lose their protein components in acute emotional stress. The investigation described below was devoted to the study of these problems.

EXPERIMENTAL METHOD*

Acute emotional stress was induced in rabbits by alternate electrical stimulation of the ventromedial hypothalamic nuclei and application of electric shocks to the limb of the immobilized animals in accordance with a specially drawn up stochastic scheme. The duration of individual stimulation of the ventromedial hypothalamic nuclei and of the electric shocks varied from 1 to 3 min. The ventromedial nuclei were stimulated by square pulses with a frequency of 50 Hz, pulse duration 1 msec, and voltage 2-8 V. Electric shocks were applied at a frequency of 50 Hz, pulse duration 3-5 msec, voltage 5-10 V. The experiment lasted 2.5-3 h.

Material taken from five rabbits which died in the course of the experiments was studied. Four rabbits kept in the animal house served as the control.

The ganglion nodosum of the vagus nerve, the superior cervical and stellate ganglia, and also ganglia of the sympathetic chain at the level T4-6 were investigated. After preliminary fixation in situ with 2.5% glutaraldehyde solution in phosphate buffer (pH 7.5) these cell formations of the autonomic nervous system were removed, cut into pieces in the cold in a drop of fixative, and postfixed with 1% osmium tetroxide solution. The tissue samples were dehydrated in alcohols of increasing strength and embedded in Araldite. Semithin sections, cut on the LKB-III Ultrotome, were stained with toluidine blue and examined under the microscope. Ultrathin sections were placed on grids with formvar supporting films and stained with lead citrate. Photomicrographs were obtained under the Philips electron microscope (The Netherlands).

EXPERIMENTAL RESULTS

Under the light microscope, ganglia of different origin in semithin sections did not differ sharply from each other. Each was surrounded by a capsule and divided by layers of connective tissue into separate regions, containing bodies and processes of neurons, glial cells, and blood vessels.

The nerve cells of the various ganglia were similar in their ultrastructure (Fig. 1a, 2a, and 3a). The large circular neurons had an oval nucleus, with smooth outline, 10-12 μm in diameter, located in the middle zone of the cytoplasm. Binuclear cells were seen, especially frequently in the superior cervical and stellate

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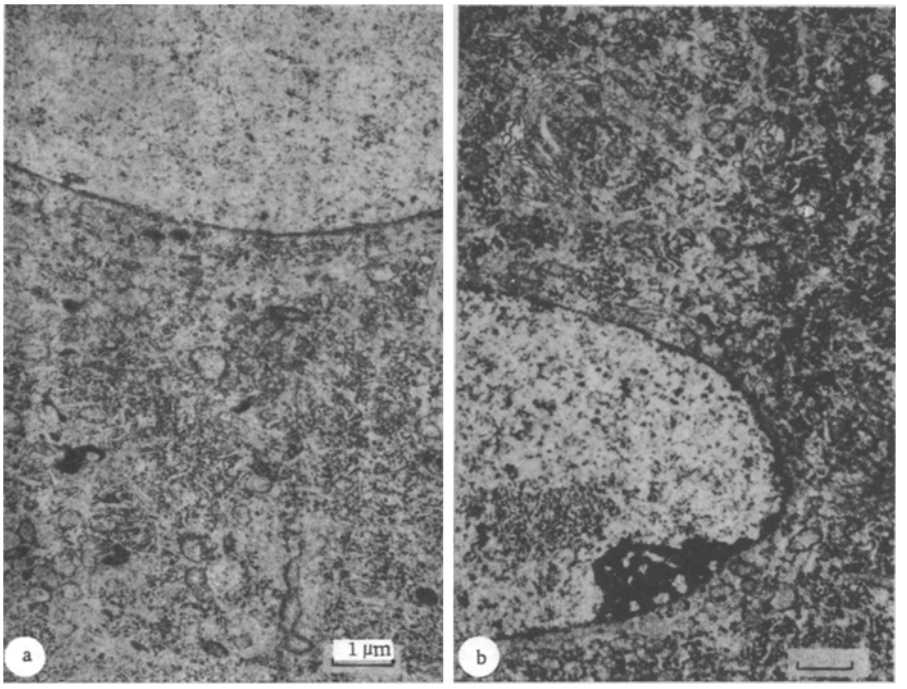

Fig. 1. Nerve cell of superior cervical ganglion: a) control; b) experiment: nucleolus lies adjacent to nuclear membrane, enlargement of vacuoles, dilatation of cisternae of Golgi apparatus.

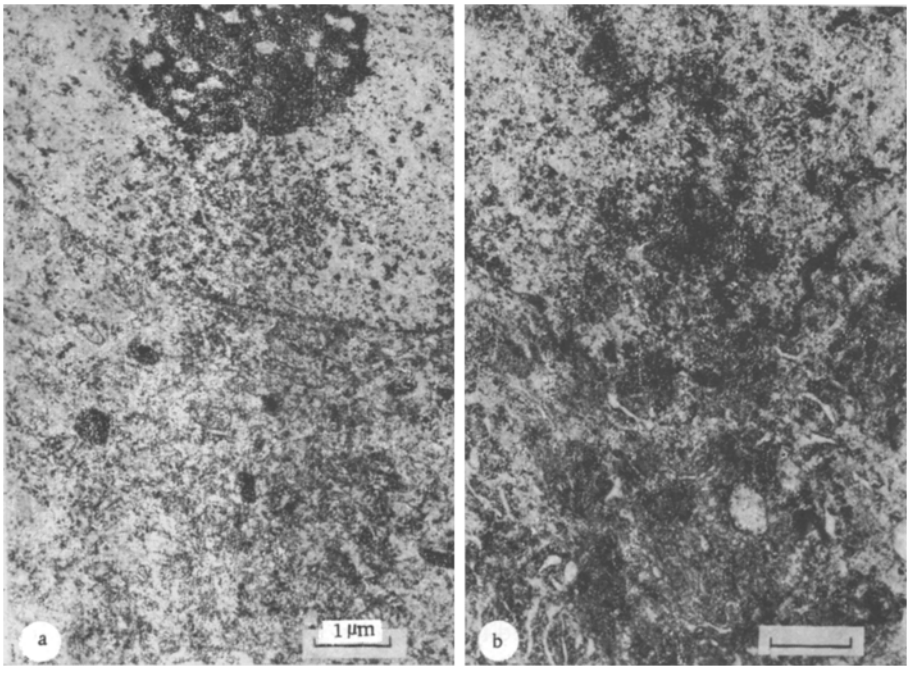

Fig. 2. Nerve cell of stellate ganglion: a) control; b) experiment: hyperplasia of Golgi apparatus, swelling of mitochondria, festooning of nuclear membrane.

ganglia. The double nuclear membrane, with occasional ribosomes attached to its outer layer, surrounded the translucent nucleus. Chromatin was uniformly distributed throughout the area of the nucleus. The nucleus contained one large nucleolus or, less frequently, several nucleoli. The nucleolus consisted of a concentration of electron-dense granules, among which there were pale vacuoles, inclusions of karyoplasm. In the cytoplasm,

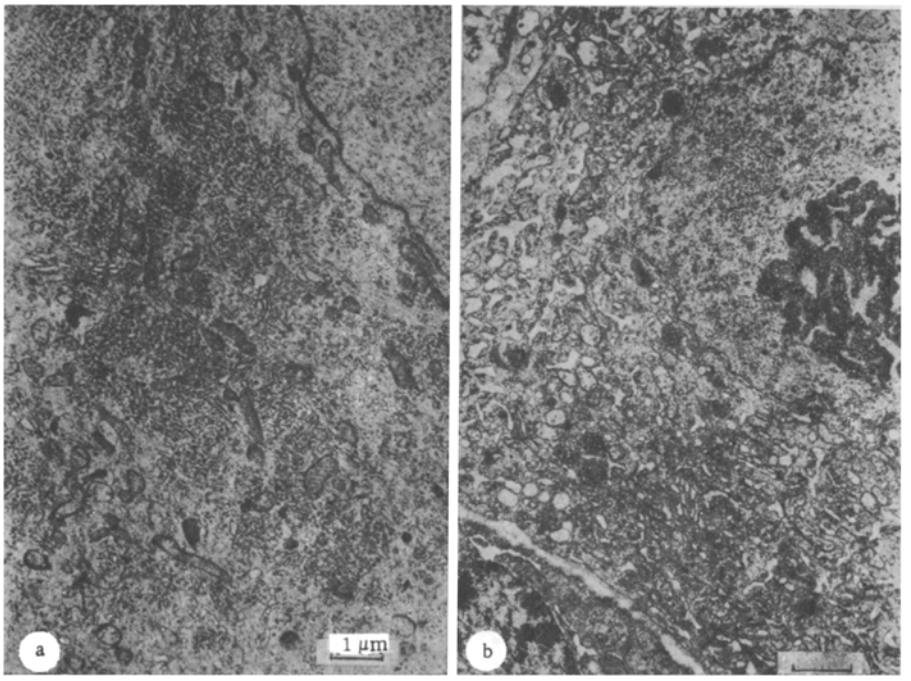

Fig. 3. Nerve cell of ganglion nodosum: a) control; b) experiment: dilatation of cisternae of Golgi apparatus, sharp increase in number and size of lysosomes, reduction in number of ribosomes in cytoplasm, dilatation of cisternae of endoplasmic reticulum.

the rough endoplasmic reticulum consisted of wide cisterns, lying almost parallel to one another, and separated by spaces 0.2-0.5 μm wide. The outer surface of the membranes bounding the cisternae was speckled with irregularly distributed ribosomes, but over large areas of the membranes they were absent. Freely lying groups consisting of five or six ribosomes, surrounding one central ribosome, were seen between the cisternae of the endoplasmic reticulum in the cytoplasmic matrix. In the perinuclear zone the Golgi apparatus was represented by a group of flattened cisternae and numerous vesicles of different kinds. It was usually formed by five to seven large cisternae, separated by very narrow spaces, superposed one above the other, forming "rouleaux." Mitochondria, oval or rather elongated in shape and not more than 1 μm in length, with a moderately electron-dense matrix were irregularly distributed in the cytoplasm of the perikaryon. Branched mitochondria were rarely seen. Single lysosomes appeared as round bodies 0.3-0.5 μm in diameter with fine-grain contents. The single membrane surrounding the lysosome was separated from the matrix by an electron-transparent halo, and in thickness it was similar to the cytoplasmic membrane. Lysosomes were rectangular, curved, or cup-shaped, and contained curved and spirally coiled membranes, granules of different sizes and density, and transparent vacuoles. In the spaces between the concentrations of elements of the endoplasmic reticulum neurofibrils ran in different directions.

In rabbits which died during emotional stress the most marked changes were found in structures of nerve cells of the ganglion nodosum of the vagus nerve and the stellate sympathetic ganglion (Figs. 2b and 3b). In these ganglia, more especially the ganglion nodosum, the outlines of the nuclear membrane were changed and appeared festooned in sections. The nuclear pores and the perinuclear spaces were widened in these nuclei. The number of free ribosomes was appreciably reduced in the perinuclear zone. The nucleolus was enlarged and often shifted toward the periphery of the nucleus; the vacuoles in the nucleolus were enlarged. In sections the nucleolus appeared "untwisted" (Fig. 3b). In the cytoplasm the cisternae of the rough endoplasmic reticulum were dilated and its membranes had lost the ribosomes bound with them. Adjacent mitochondria were collected into groups and swollen, the regular arrangement of the cristae was disorganized, and their matrix was translucent and had undergone vacuolation. Very large, elongated mitochondria, measuring 3-4 μm in length, could be seen in the peripheral zones of the cytoplasm. The most prominent feature, found as a rule only in neurons of the ganglion nodosum, was a sharp increase in their number of lysosomes compared with normal. The lysosomes also were increased in size (Fig. 3b). In the Golgi apparatus the outer cisternae were dilated, their number increased, and the vesicular apparatus hypertrophied. "Framed" vesicles were more frequent than in the control. Electron-transparent regions whose appearance was attributed to the outflow of

hydrolytic enzymes from the lysosomes could be seen in the cytoplasm. In some cells autophagosomes could be seen, inside which some of the organelles, especially mitochondria, and the outlines of the endoplasmic reticulum were undergoing lysis.

In nerve cells of the superior cervical ganglion changes in acute emotional stress were expressed as some increase in the lumen of the cisternae of the Golgi apparatus, displacement of the nucleolus toward the periphery of the nucleus, and enlargement of the vacuoles in the nucleolus (Fig. 1b). Changes in the nerve cells in the ganglia of the sympathetic chain could be reduced to swelling of the mitochondria, accompanied by translucency of their matrix, and by some widening of the cisternae of rough reticulum and also of the Golgi apparatus. The nuclei played virtually no part in the activation of the neurons.

It can be concluded from the results of this investigation that the predominant changes in acute emotional stress arise and develop in the Golgi apparatus of nerve cells of the ganglion nodosum of the vagus nerve. These changes lead to the formation de novo of a large number of lysosomes – endogenous sources of proteolytic enzymes. There is reason to suppose that in acute emotional stress nerve cells function at the expense of energy formed by lysis of proteins. The results thus suggest that changes in protein metabolism of the nerve cell of the ganglion nodosum in acute emotional stress are linked with the increased production of enzymes, localized in the lysosomes, by the cell.

LITERATURE CITED

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EFFECT OF LONG-TERM INTERNAL IRRADIATION ON NEURONS OF THE HYPOTHALAMIC ARCUATE AND SUPRACHIASMATIC NUCLEI AND MEDIAN EMINENCE IN RATS

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The decisive factor in radiation-induced disturbance of activity of the neuroendocrine system is injury to its central stage – the hypothalamus and pituitary [1]. Through the releasing hormones the hypothalamus maintains the optimal level of hormones of the peripheral endocrine glands. It has been shown that of the hypothalamic formations it is the arcuate and suprachiasmatic nuclei (AN and SCN respectively) which are the source of formation of most releasing hormones [2, 4, 5], which are carried along the axons of secretory neurons into the median eminence and are discharged into the capillary blood stream in the primary portal plexus. It is therefore interesting to study the fine structure of neurons of AN and SCN, and also of the median eminence, in rats during long-term internal irradiation, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 72 noninbred male rats aged 3 months, which were given a single intravenous injection of the radionuclide preparation ^{75}Se-selenomethionine in a dose of 1.22×10^4 Bq/g body weight. The animals were killed 2 weeks and 1, 3, 6, 9, 12, and 18 months after injection of the radionuclide. AN and SCN of the hypothalamus and the median eminence were taken for electron-microscopic study. The number of granules of secretion and the number of empty vesicles in nerve terminals of the median eminence ending on portal capillaries and also the number of granules of secretion in nerve fibers located not more than 2-3 μm away from the capillary were counted on electron micrographs.

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