ELECTRON-MICROSCOPIC STUDY OF CHANGES IN CEREBELLAR PYRIFORM NEURONS IN MICE WITH PROTEIN-CALORIC DEFICIENCY

D. I. Medvedev, P. D. Bonartsev,
I. I. Babichenko, A. I. Kravtsova,
and I. Z. Eremina

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The study of cerebellar cells in animals with protein-caloric deficiency is very important because most neurologic disturbances in nonspecific alimentary dystrophies such as kwashiorkor and marasmus affect the motor sphere [7]. In experimental studies of the structure of the cerebellum in animals on a protein-deficient diet destructive changes were found in axon terminals [14] and synapses [13], development of glial populations was retarded [8], and the total number of neurons was smaller than in the control [9]. However, data on specific changes in the pyriform neurons of the cerebellum (Purkinje cells) are inadequate [10]. The particular features of the study of pyriform neurons are attributable primarily to the heterogeneity of the morphology of these cells, for we know that what have been called dark pyriform neurons [3, 6, 11] are constantly observed in the cerebellum besides the ordinary pale cells. According to some workers, these dark cells reflect a special state of function of the cell [4, 12].

In the investigation described below changes in the relative numbers and ultramicroscopic structure of the various types of pyriform neurons in the cerebellar cortex of mice during prolonged administration of a protein-caloric deficient diet were studied.

EXPERIMENTAL METHOD

Young CBA mice were used. From the 10th to the 40th days of life inclusive the animals were kept on an artificial balanced diet (six control mice) and a diet containing 50% of the components of the diet of the control animals (six experimental mice) [2]. Under pentobarbital anesthesia the animals were perfused with Karnovsky's fixative (pH 7.3-7.4). Sagittal sections through the vermis of the cerebellum were postfixed in OsO_4 and embedded in Araldite. Pyriform neurons in the cerebellar vermis were studied in semi-thin sections stained with methylene blue. The number of dark, pale, and intermediate cells was counted. The number of cells thus obtained was expressed as a percentage of the total number of pyriform neurons counted in one section. The data were subjected to statistical analysis by the χ^2 test. In ultrathin sections cut from trimmed blocks, stained by Reynolds' method, the ultrastructure of the different types of pyriform neurons was investigated in the Tesla BS 500 electron microscope.

EXPERIMENTAL RESULTS

After a study of the semi-thin sections through the cerebellar vermis in the light microscope the pyriform neurons were divided into three types depending on the character of staining with methylene blue: pale, dark, and intermediate.

The pale cells were distinguished by their delicate blue color, comparable in intensity with the surrounding neuropil. The dark cells were sharply distinguished by their intense blue color, similar in intensity to the staining of the nuclear chromatin of the granule cells. Cells of the intermediate type had a nucleus or cytoplasm which was more darkly stained than the surrounding neuropil.

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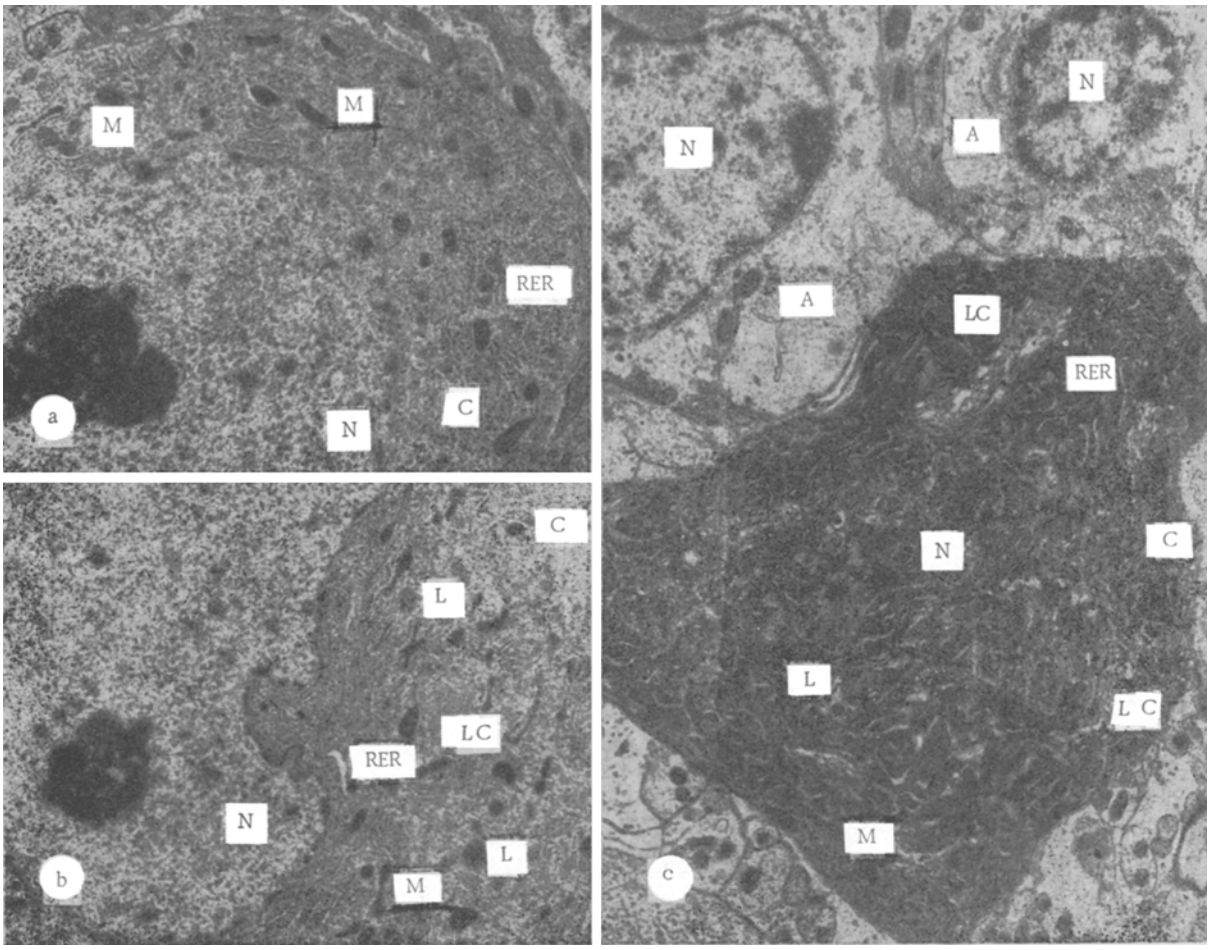

Fig. 1. Main types of pyriform neurons in cerebellar cortex. a) Free arrangement of principal cell organelles in cytoplasm of pale pyriform neuron (10,000 ×); b) concentration of cisterns of rough endoplasmic reticulum and polysomes in region of invagination of nuclear membrane of an intermediate pyriform neuron (10,000 ×); c) increase in electron density of hyaloplasm and destructive changes in cell organelles in cytoplasm of dark pyriform neuron (15,000 ×). N) Nucleus, C) cytoplasm, RER) rough endoplasmic reticulum, LC) lamellar complex, M) mitochondria, L) lysosomes, A) astrocytes.

The results of a study of the relative numbers of pale, intermediate, and dark pyriform neurons in the cerebellar cortex of the experimental and control animals are given in Table 1.

The greatest differences were found in the relative numbers of dark cells. In the experimental animals their number was increased by more than 2.5 times, whereas the number of intermediate cells was significantly lower than in the control.

The electron-microscopic study of dark, pale, and intermediate pyriform neurons revealed a number of significant differences in their ultramicroscopic structure, and on that basis these cells could be divided into various morphophysiological types.

The pale pyriform neurons had an oval nucleus with small aggregations of chromatin around the periphery and with a large nucleolus in the central zone of the nucleus. The perikaryon of these cells had electron-translucent hyaloplasm with well-developed principal cell organelles (Fig. 1a). The nuclei of neurons of intermediate type had much higher electron density of their karyoplasm and an even distribution of chromatin throughout the nucleus and among the nucleoli distributed around the periphery. The nuclear membrane of these cells formed marked invaginations, around which the cisterns of the rough endoplasmic reticulum and polysomes were concentrated in the perikaryon. The cytoplasm of these cells differed in the increased electron density of its hyaloplasm and an increased number of lysosomes and lipofuscin granules (Fig. 1b).

TABLE 1. Individual Mean Statistical Parameters for Undernourished and Control Mice

Experimental conditions	Body weight, g	Number of cells, %		
		dark	intermediate	pale
Normal	15,4	3	51	46
	14,3	4	18	78
	15,8	3	57	40
	14,7	10	68	22
	12,5	8	42	48
	19,0	10	29	60
Mean statistical parameter	15,0	7±3	44±7	49±7
Undernourished	8,6	36	40	24
	7,4	29	29	42
	8,9	7	23	70
	7,7	9	42	49
	6,4	54	16	30
	9,8	20	50	30
Mean statistical parameter	7,8	26±5	33±6	41±6
P	<0,01	<0,001	<0,01	<0,1

The nuclei of the dark cells were considerably deformed, reduced in size, and had uneven outlines. The nucleoli and chromatin were poorly distinguishable against the background of the high electron density of the karyoplasm. The ultrastructure of the perikaryon of the dark cells differed substantially from that of the pale and intermediate cells in the marked changes of dystrophic and destructive character. The hyaloplasm of the dark neurons had high electron density, and elements of the lamellar complex and also cisterns of the rough and smooth endoplasmic reticulum were sharply dilated in it (Fig. 1c). The space between the cisterns of the rough endoplasmic reticulum was densely packed with free ribosomes. The mitochondria also had high electron density. Their cristae were often fragmented and individual regions of the mitochondrial matrix were highly translucent. The cytoplasm contained many lysosomes, lipofuscin granules, and vacuoles.

Quantitative analysis of changes in the relative numbers of dark, pale, and intermediate pyriform neurons in the cerebellar cortex of mice developing on a protein-caloric-deficient diet revealed a marked increase in the number of dark cells, mainly on account of the number of intermediate cells.

In the nucleus and in all the principal organelles of the perikaryon of the dark cells (lamellar complex, mitochondria, endoplasmic reticulum) changes of a dystrophic and destructive character were observed. Meanwhile, around the dark cells a marked reaction of the astroglia was frequently discovered, and could indicate the irreversible character of the changes described above.

The fact that after the animals had been kept on a diet deficient in proteins and calories the number of dark cells increased sharply can be taken as evidence of a marked reduction in the compensatory hours of the CNS under these conditions.

At the same time the increase in the number of dark cells is not a specific response of the cerebellum to malnutrition, for similar changes have been observed in the cerebellum and in the cerebral cortex in hypoxia [1], and also in the spinal cord during increased functional loading [5].

The increase in the number of dark cells in the cerebellar cortex in dietary protein deficiency observed in the present experiments may probably be a universal typical response of the CNS irrespective of the character of the harmful factor acting on the animal.

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PATHOGENESIS OF DISTURBANCES OF THE HARD TISSUES OF THE TEETH IN THYROTOXICOSIS

A. I. Volozhin and V. I. Kobeleva

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In thyroid hyperfunction lesions of the hard tissues of the teeth, classified as erosion, become more frequent [1, 3]. However, the mechanism of development of these lesions has not been discovered; moreover, data on the surface ultrastructure of the tooth and its mineral phase in thyrotoxicosis are not available. The investigation described below was undertaken to study these problems.

EXPERIMENTAL METHOD

Experiments were carried out on 150 female albino rats weighing 120-180 g, receiving the ordinary animal house diet. Thyrotoxicosis was produced in 75 animals by daily injection of a solution of the sodium salt of L-thyroxine in a dose of 400 μg of the dry powder/100 g body weight. Control animals received injections of 0.2 ml of physiological saline. The rats were killed at different times after the beginning of the experiment — 5, 10, 15, 20, and 30 days. After sacrifice the incisors were extracted and one batch of them (400 teeth) was dried to constant weight whereas the other (200 teeth) was fixed in 12% neutral formalin.

The dried teeth were ground in an agate mortar to a powder, from which samples weighing 100 mg were prepared, mixed with 100 mg buffer (calcium phosphate), homogenized on an amalgam mixer, and pressed into tablets 1 cm in diameter by means of a press under a pressure of 10.13×10^4 GP. The surface of the tablets was smooth and flawless. The tablets thus prepared were placed in the cuvette compartment of an x-ray fluorescence instrument (VRA-2, from Carl Zeiss, East Germany), then irradiated for 100 sec, after which the intensity of emission for the elements copper, zinc, iron, and cobalt was determined and the background level deducted.

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