Analysis of Protein Pool of Neuronal Populations of Cerebellar Cortex in Rodents of Different Species

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The protein pool of neuronal population of the cerebellar cortex was studied by interference cytometry in rodents occupying different ecological niches and differing by life style, nutrition habits, and motor activity. In all cell populations protein concentrations in the cytoplasm were higher than in the nucleus in all studied rodents and did not depend on the functional characteristics of neurons. The extreme values of protein content were determined for populations of granular and ganglion cells. High protein concentrations per volume unit of cell structure were detected in functionally different cerebellar neurons of gray rats, characterized by high motor activity and a certain degree of synanthropy, while low values were detected in mole rats, slow-moving underground rodents. Therefore, the specific protein pool of neuronal populations of the cerebellar cortex of rodents can be regarded as adaptation to habitation conditions.

Key Words: *rodents; cerebellum; neuronal populations; interferometry; protein pool*

Adaptation to environmental conditions is an obligatory condition of organism's survival at all levels of organization. Plasticity of motor functions and high adaptation variability of movements set rodents apart from other mammals and explain their high species variability.

Cerebellum plays an important role in the development of the coordination system in rodents [7]. However the contribution of the cerebellum to extension of the adaptation potential of the studied group of mammals received little attention.

We investigated neuronal populations of the cerebellar cortex by measuring the content and concentrations of proteins per volume unit of cell structures as a marker of functional activity of the studied brain compartment in rodents of different species. We characterized the types of neuronal populations and evaluated the contribution of their protein pool in the maintenance of the natural balance between the internal processes in the organism and habitation conditions.

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MATERIALS AND METHODS

Rodents with different degree of dependence on environmental conditions occupying different ecological niches and differing by life style, nutrition, and motor activity were studied. Adult animals were examined (n=59): squirrels (*Sciurus vulgaris*) weighing 406±16 g, gray rats (*Rattus norvegicus*), 272±14 g, mole rats (*Ellobius talpinus*), 40±2 g, field mice (*Microtus arvalis*), 25±2 g, nutrias (*Myocastor coypus*), 3825±241 g. The animals were decapitated under ether narcosis, the cerebellum was fixed in Carnoy fluid, and 5-7-μ sections were made from paraffin blocks. Dry weight of compact substances, 80-90% corresponding to protein content in fixed tissue, was determined in cortical neurons of unstained preparations under a BINAM-L-212 interference microscope at 535 nm. Mean content and concentrations of protein in the cytoplasm and nucleus was evaluated by interference cytometry [3,6]. Associative neurons were analyzed (150 cell of each population): stellate cells of the molecular layer, granular cells of the granular layer, and efferent Purkinje cells (PC). The data were processed statistically using Student's t test.

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RESULTS

The neocortex of mammalian cerebellum is presented by hemispheres functionally connected with cerebral cortex, ensuring coordination between congenital and acquired forms of nervous activity [8, 9,11], which is important in the organism-environment relationships. Protein metabolism plays an important role in the formation of time relationships, the function of neurons in these processes can be evaluated by changes in protein content per volume unit and not by the velocity of their synthesis [4,10]. There are some bio- and histochemical data confirming the peculiar features of the cerebellum [5]. However, quantitative cytochemical characteristics of functionally different neurons of the cerebellum of rodents have never been described.

We detected high content of water-insoluble proteins in astrocytes of the small neuronal population of molecular layer of the cerebellum in nutrias and squirrels (Table 1). Astrocytes of field mice were characterized by the lowest content of proteins: these values in the nucleus and cytoplasm being 41.6 and 38.1% lower than in squirrels, respectively. The linear size of cells in these rodents was also low (Table 1). The maximum concentration of compact substances was detected in astrocyte nuclei of nutrias (16.6% higher than in squirrels) and in the cytoplasm in gray rats. The concentrations of compact substances in cells of gray rats was higher than in squirrels (by 11.4% in the nucleus and by 3.4% in the cytoplasm) and 7.1% lower than in the nuclei of nutrias and 1.98% higher than the cytoplasm of nutrias. The area of neurons of molecular layer was the highest in squirrels, the values in nutrias being only 10.9 and 8.6% lower for the cytoplasm and nucleus, respectively (Table 1). In astrocytes of gray rats, neurons with small structures predominated, their nuclei being the smallest in the studied group of animals (40.7% smaller than in squirrels). The cytoplasm area in gray rats was 30.1% smaller than in squirrels and was intermediate between mole rats (21.6% smaller) and field mice (6.9% larger). The lowest concentration of compact substances was observed in mole rats: 15.2% lower than in gray rats in the cytoplasm and 20.2% lower than in nutrias in the nuclei. Astrocyte cytoplasm area in mole rats was intermediate between the values in nutrias and gray rats and the area of nuclei intermediate between gray rats and field mice (Table 1).

In granular layer granulocytes, the only excitatory neurons of the cerebellar cortex, the maximum concentrations of compact substances were detected in gray rats (15.9 and 28% higher in the cytoplasm and nucleus, respectively, than in squirrels); the values in field mice were close to these (4.4 and 25.3% higher than in squirrels). The maximum linear sizes were those of squirrel granulocytes; the cytoplasm area was 10.3% smaller in gray rats. As for nucleus area, it was the smallest in granular layer neurons and molecular layer cells of gray rats (37.5% less than in squirrels). Squirrel granulocytes were characterized by high levels of compact substances. In gray rats the cytoplasm content of water-insoluble proteins was 5% higher than in squirrels, while in the nucleus their content was minimum (19% lower than in squirrels). In granulocytes of nutrias the concentrations of compact substances in the nucleus and cytoplasm was, respectively, 27.1 and 19.8% lower than in gray rats, in which these values were maximum. Granulocytes of nutrias were characterized by a high nucleus-cytoplasm ratio. A high concentration of compact substances was detected in granulocytes of field mice, but the content of protein in them was medium. The lowest values of protein pool were observed in granulocyte cytoplasm of mole rats: protein content was high and their concentration low in the nucleus (Table 1).

PC, the only efferent cells of the cerebellar cortex, form abundant synaptic bonds and regular transverse rows. All impulses directed to the cerebellum are concentrated in these cells and then through cerebellar nuclei are sent to the respective formations in the spinal cord and brain. Morphologically similar PC can differ by their functions. Autoradiographic studies revealed a correlation between tinctorial properties of neurons and the level of their metabolism. It was proven that the major part of the ganglion cell layer consists of "dark" neurons, which can be regarded as the population reserve. Our interferometry data on the protein pool in ganglion neurons and other neuronal populations extend our knowledge on the protein structure of functionally different neurons of the cerebellar cortex in rodents.

In the ganglion cell layer the maximum concentration of compact substances per cell surface unit was detected in gray rats; squirrels are close to them by concentrations in the nucleus (8.2% lower) and field mice by concentrations in the cytoplasm (12.3% lower). The area of PC nucleus was the largest in squirrels, while in field mice both the cytoplasm and nucleus areas were the smallest. Minimum concentrations of protein substances were detected in the nuclei of ganglion cell layer of nutrias, which had large area, and in mole rat PC cytoplasm which had small area. The areas of neuronal nuclei in squirrels and of cytoplasm in nutrias directly correlated with the content of compact substances in these structures: the content of proteins was maximum in PC nuclei of squirrels and nutria cytoplasm characterized by maximum area. The smallest cells were detected in the ganglion cell layer of field mice; the content of protein in their PC nuclei and cytoplasm was low (37.4 and 46.6% lower than in squirrels). Similar values were found in mole rats (Table 1).

TABLE 1. Protein Fund in Neurons of Cerebellar Cortex in Rodents (*M*±*m*)

Parameter, cortical layer	Squirrel	Nutria	Gray rat	Mole rat	Field mouse
Cytoplasm area, μ^2					
molecular	33.58±0.53	30.70±0.45**	23.48±0.42*	29.97±0.32**	21.96±0.25*
ganglion-cell	241.90±3.11	269.80±3.78**	191.33±3.16*	127.66±1.66*	121.14±1.64*
granular	16.88±0.19	14.26±0.15*	15.14±0.18**	14.55±0.20*	14.45±0.19*
Nuclear area, μ^2					
molecular	30.91±0.43	27.53±0.43**	18.31±0.54*	23.36±0.38*	18.50±0.27*
ganglion-cell	86.70±1.25	78.20±1.13**	77.80±1.61**	65.44±0.91*	55.14±0.92*
granular	14.76±0.26	14.07±0.19	9.22±0.25*	11.78±0.27*	9.68±0.21*
Content of compact substances, pg					
in cytoplasm					
molecular	49.30±1.08	45.47±1.00	35.23±0.81*	38.69±0.91**	30.49±0.65*
ganglion-cell	325.05±7.04	364.14±9.79**	312.72±8.98	167.27±4.17*	173.70±4.25*
granular	26.47±0.52	20.90±0.49*	27.80±0.71	20.80±0.47*	23.69±0.72
in nuclei					
molecular	21.60±0.44	22.99±0.67	14.07±0.50*	15.78±0.61**	12.62±0.25*
ganglion-cell	67.94±1.83	56.96±1.66**	67.51±2.68	49.77±1.24*	42.49±1.14*
granular	11.20±0.32	9.96±0.26	9.07±0.40**	9.96±0.37	9.04±0.36**
Concentration of compact substances, pg/μ^3					
in cytoplasm					
molecular	1.46±0.02	1.48±0.02	1.51±0.02	1.28±0.02**	1.39±0.02
ganglion-cell	1.34±0.02	1.35±0.03	1.61±0.03*	1.31±0.02	1.43±0.02**
granular	1.57±0.02	1.46±0.03	1.82±0.03**	1.43±0.02**	1.64±0.04
in nuclei					
molecular	0.70±0.01	0.84±0.02**	0.78±0.02**	0.67±0.02	0.68±0.01
ganglion-cell	0.78±0.01	0.73±0.01	0.85±0.02	0.76±0.01	0.76±0.01
granular	0.75±0.01	0.70±0.01	0.96±0.02**	0.84±0.02	0.94±0.03**

Note. *$p<0.001$, **$p<0.01$ *vs.* squirrel.

Hence, the following differences were revealed in neurons of the first genetic group [2] in the cerebellar cortex of rodents: associative granulocytes, as the only excitatory neurons of the cerebellar cortex, were characterized by high concentration and low content of water-insoluble proteins, while efferent PC had low concentrations and high content of proteins. Interstitial neurons of the molecular layer belonging to the second genetic group occupied an intermediate position. Our data agree with the results of bio- and histochemical studies [4] and confirm the functional characteristics of neuronal populations of the cerebellar cortex.

The content and concentration of proteins in cellular components of all types of neurons did not depend on the structure and function of cells and were higher in the cytoplasm than in the nucleus. The highest and lowest concentrations of proteins in the cytoplasm of all types of neurons of cerebellar cortex were observed in animals living in holes: highly active gray rats (maximum) and less active mole rats (minimum), which can be regarded as an indicator of high adaptation of these animals to environmental factors.

The characteristic features of protein pool in cerebellar neuronal populations in rodents are explained by a high level of protein metabolism typical of nerve cells [1,4,10], which is required for compensation of their loss during vital activity. Normally, protein destruction is compensated by their parallel synthesis, the quality of protein in working cells being not only restored, but even increased. However protein synthesis takes place only in cell body, while the protein pool is utilized both in the cell body and axon, where it is transported with different intensity, depending on the functional state of the organism.

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