Protein Content in Rat Brain Neurons Predisposed and Resistant to Emotional Stress

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Protein content was measured by interferometry in the cerebral neurons of August rats predisposed to emotional stress and Wistar rats resistant to it. Protein content was 16-18% lower in the neurons of the third and fourth layers of the sensorimotor cortex and 51% higher in the caudate nucleus neurons (cerebral subcortical nodes) of August rats than in Wistar rats. This indicates an inversion in protein distribution in the cortex and subcortex of August rats which are characterized by typical protein content in different types of neurons and apparently by peculiar cerebral structure and function.

Key Words: *August and Wistar rats; brain; protein; interferometry*

Animals with genetically determined alterations in the nervous system are a convenient model for studies of brain structure and function. Comparison of the brain of August rats predisposed to emotional stress (ES) and of Wistar rats resistant to ES [10] is promising in this field. This problem has been extensively discussed [4,6,8-10,12]. In August and Wistar rats, cardiovascular system [6], lymphoid and connective tissue [9], production and accumulation of heat shock protein 70 in the myocardium [5] differently react to stress. August and Wistar rats differ by the cerebral content of endogenous peptides (substance P, δ-sleep peptide, and β-endorphine) [8,12] and by biogenic amine metabolism [4,6]. August rats slower work out and fix conditioned reflexes than Wistar rats [7], which may be regarded as the evidence of their weaker cortical function. The neurochemical characteristics of the brain cortex were never studied properly in August rats; the main attention was given to the brain stem [4,6,8,12].

Our aim was to measure by interferometry the protein content, an indicator of neuronal functional activity in different neuron types of August and Wistar rats and to examine the neurons of the structural and functional system including the sensorimotor cortex and the caudate nucleus.

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MATERIALS AND METHODS

August and Wistar rats weighing 230±20 g (adult intact males) were examined. The brain of rats decapitated under ether narcosis was fixed in Carnois' fluid, and 7-μ sections were cut from brain fragments embedded in paraffin. Dry weight corresponding to protein content in fixed tissue [2] was measured in a BINAM (L212) interference microscope [3]. The areas of profile fields of neuronal corpus, cytoplasm, and nucleus were measured with MOV-1-15 ocular micrometer. The following characteristics were obtained: protein content in the body of the cell, cytoplasm, and nucleus; protein content per unit of a cellular structure area (UCSA), which was calculated for the cytoplasm and the nucleus by dividing the value of dry weight of solid substances by the area. Pyramidal neurons of the third layer (associative cells), the fifth layer large pyramids (projection efferent cells of the hemispheric sensorimotor cortex), and caudate nucleus associative multipolar neurons (cerebral subcortical cells) were examined. The results were processed using Protein software developed at the Cytochemistry Department of the Institute of Brain Research.

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RESULTS

Smaller cells with lower protein content were revealed in August rats in comparison with Wistar rats (Table 1). The greatest differences (30-40%) were observed in the neurons of the third layer of the sensorimotor cortex (by the area and protein content — cell weight) and in the caudate nucleus

TABLE 1. Protein Content in Cerebral Neurons of August Rats Predisposed and Wistar Rats Resistant to ES

Structures	Wistar		August		Difference from Wistar rats, %
	M±m	difference from caudate nucleus, %	*M±m*	difference from caudate nucleus, %	
Linear parameters of neurons					
Neuron surface area, μ^2					
caudate nucleus	105.47±0.83		73.71±1.01		-30**
cortex, layer III	155.36±1.60	+47	116.85±1.06	+59	-25**
cortex, layer V	361.69±4.69	+243	385.16±4.35	+423	+6**
Cytoplasm area, μ^2					
caudate nucleus	37.54±0.26		29.42±0.43		-22**
cortex, layer III	60.65±0.78	+62	49.61±0.58	+69	-18**
cortex, layer V	196.38±3.39	+423	240.78±3.45	+718	+23**
Nucleus area, μ^2					
caudate nucleus	67.93±0.68		44.29±0.72		-35**
cortex, layer III	94.70±1.09	+39	67.24±0.72	+52	-31**
cortex, layer V	165.32±1.98	+143	144.38±1.50	+226	-13**
Dry weight of solid substances, πg (πg/area of neuron or its components)					
Neuron body					
caudate nucleus	107.14±1.37		102.38±2.48		-4
cortex, layer III	167.76±2.46	+57	107.81±1.64	+5	-36**
cortex, layer V	518.84±11.36	+384	509.75±9.33	+398	-2
Neuron cytoplasm					
caudate nucleus	56.13±0.65		52.15±1.27		-7*
cortex, layer III	97.04±1.54	+73	65.51±1.19	+26	-32**
cortex, layer V	362.50±8.78	+546	396.45±7.68	+660	+9*
Neuron nuclei					
caudate nucleus	51.00±0.85		50.23±1.43		-2
cortex, layer III	70.71±1.31	+39	42.30±0.68	-16	-40**
cortex, layer V	156.35±3.61	+207	113.29±2.26	+126	-28**
Dry weight of solid substances per UCSA, $\pi g/\mu^2$					
Neuron cytoplasm					
caudate nucleus	1.49±0.01		1.76±0.03		+18**
cortex, layer III	1.60±0.01	+7	1.31±0.01	-26	-18**
cortex, layer V	1.83±0.02	+23	1.65±0.02	-6	-10**
Neuron nuclei					
caudate nucleus	0.75±0.01		1.13±0.02		+51**
cortex, layer III	0.75±0.01	0	0.63±0.01	-44	-16**
cortex, layer V	0.94±0.01	+25	0.78±0.01	-31	-17**

Note. For each neuron population representing a cell type, 150 neurons were examined in three August and three Wistar rats. *$p<0.01$, **$p<0.001$.

neurons (differing by the area). In August rats, only cell nuclei of large pyramids of the fifth layer had a 13% smaller area and a lower (by 28%) protein content, while the cytoplasm area of these neurons and the content of proteins in the cytoplasm were 23 and 9% higher, respectively. Estimation of protein content per UCSA showed significant differences in all examined neurons (Table 1). In August rats, the protein content per UCSA was lower in the cortical neurons and in the third and fifth layers, while in the caudate nucleus neurons it was higher than in Wistar rats. In cortical neurons (both in the cytoplasm and nuclei) the content of proteins was 10-18% lower, while in the caudate nucleus neurons 18-51% higher in August than in Wistar rats.

In Wistar rats, the area and protein content of neurons (cell weight and protein content per UCSA) gradually increased in the cytoplasm and nuclei of neurons in the direction from the caudate nucleus to the third layer pyramids and the fifth layer pyramids (Table 1). In August rats, only neuronal area decreased in the same direction; cell weight increased nonuniformely, and in some cases even decreased, for example, in the third layer neurons. The protein content per UCSA decreased in this cell series. This is due to a higher concentration of proteins in subcortical neurons in August rats in comparison with cortical neurons. The cytoplasm and nuclei of the caudate nucleus neurons contained 1.76 and 1.13 pg protein, respectively (pg/μ^2). The protein content was lower in the cortical neurons: by 26 and 44% in the third layer and by 6 and 31% in the fifth layer in the cytoplasm and nuclei, respectively.

The level of protein metabolism in the brain adequately reflects the functional activity of the brain. Proteins are produced mainly in the neuron body [13,14]. Functional activity of a nerve cell can be evaluated by the protein content per UCSA. The shift of protein content per UCSA in the same direction in the cytoplasm and nucleus (proteins are produced mainly in the neuronal bodies) indicates a stable alteration of proteins in neurons of August rats. August rats differ from Wistar rats by the protein content and by the biogenic amine metabolism, which was described for individual nuclei of the brain stem [4,6]; therefore, it is probable that protein metabolism in these animals is different.

August rats acquire conditioned reflexes (alimentary, time, and spatial and time differentiation) slower than Wistar rats and gray laboratory rats [7]. A lower conditioned reflex activity and decreased protein content in the cortical neurons of August rats are probably related. Nerve tissue proteins are represented mainly by nucleoprotein complexes. The higher the conditioned reflex function, the higher the potential capacity and force of excitation processes [11] and RNA concentration in the brain. The correlation between these parameters is stronger in the cortex than in the subcortex [1]. The decreased content of proteins per UCSA in the cortical neurons of August rats suggests a lowered ability of these animals to develop conditioned reflexes, which may be due to weaker function of the associative neurons (third layer) than of the projection efferent neurons (fifth layer). Inversed ratio of cortical/subcortical proteins in August rats (decreased content of proteins per UCSA in the cortical neurons, most pronounced in the third layer, and their increased content in the caudate nucleus neurons) may also contribute to the attenuation of the cortical neuron function.

Thus, August rats predisposed to ES are characterized by typical protein levels in different types of neurons, which suggest specific structural and functional organization of the brain in this rat strain in comparison with Wistar rats resistant to ES.

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REFERENCES

1. V. K. Bolondinskii and V. K. Fedorov, *Zh. Vyssh. Nervn. Deyat.*, **24,** No. 3, 513-520 (1974).
2. V. Ya. Brodskii, *Cell Trophics* [in Russian], Moscow (1966).
3. L. M. Gershtein, *Tsitologiya,* **17,** No. 4, 437-441 (1975).
4. A. V. Gorbunova, R. M. Salieva, N. N. Lobanova, *et al., Ros. Fiziol. Zh.,* **81,** No. 5, 14-22 (1995).
5. A. V. Zamotrinskii, I. Yu. Malyshev, and N. P. Larionov, *Byull. Eksp. Biol. Med.,* **120,** No. 7, 30-32 (1995).
6. T. M. Ivanova, Yu. G. Skotselyas, V. I. Bolyakin, *et al., Zh. Vyssh. Nervn. Deyat.,* **29,** No. 5, 1052-1059 (1979).
7. T. A. Mering, *Ibid.,* **38,** No. 3, 550-552 (1988).
8. R. M. Salieva, E. V. Koplik, Z. A. Kamenev, *et al., Byull. Eksp. Biol. Med.,* **108,** No. 10, 464-466 (1989).
9. V. V. Serov, I. V. Tomilina, and K. V. Sudakov, *Ibid.,* **119,** No. 6, 571-573 (1995).
10. K. V. Sudakov, *Systemic Mechanisms of Emotional Stress* [in Russian], Moscow (1981).
11. V. A. Troshikhin, L. N. Kozlova, Zh. A. Kruchenko, *et al., Formation and Development of the Main Types of Higher Nervous Activity in the Ontogenesis* [in Russian], Kiev (1971).
12. E. A. Yumatov, O. I. Kirillova, M. Poppai, *et al., Zh. Vyssh. Nervn. Deyat.,* **37,** No. 2, 371-372 (1987).
13. W. Link, U. Konietzko, G. Kausselmann, *et al., Proc. Natl. Acad. Sci. USA,* **92,** No. 12, 5734-5738 (1995).
14. E. R. Torre and O. Steward, *J. Neurosci.,* **12,** No. 3, 762-772 (1992).