Postresuscitation Changes in Neuronal Hippocampal Populations in Rats with Different Learning Ability

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The state of pyramidal cell populations in CA1 and CA4 hippocampal fields was studied in resuscitated and intact rats with different learning ability. Morphometry showed that postresuscitation damage to neurons was more pronounced in good learners compared to poor learners. Interferometry revealed higher protein content in neurons in poor learners compared to successfully trained rats. It was hypothesized that different neuronal resistance to ischemia in rats characterized by different learning ability is determined by some peculiarities in protein metabolism preexisting in intact animals and manifesting in the postresuscitation period.

Key Words: *ischemia; hippocampus; training; morphometry; interferometry*

Previous clinical studies demonstrated different reactivity of the nervous system in patients survived a terminal state. These differences considerably affect the course of postresuscitation period [4]. Animal experiments also showed that mortality, the rate of neurological recovery, and retrieval of memory traces after clinical death correlate with behavioral activity [5]. It was established that recovery of brain functions after resuscitation depends on the severity of rearrangements in neuronal populations, and the major role in the development of these rearrangements is played by changes of protein metabolism in neurons [1]. These data suggest that peculiarities of structural and functional organization of CNS in animals exhibiting different behavioral activity can underlay different tolerance to ischemia-reperfusion. Since learning ability is a typological behavioral index, it is interesting to evaluate the state of neuronal population in intact and resuscitated rats demonstrating different learning ability in a complex food-procuring test. Here we studied morphometric and cytochemical parameters of neurons in the hippocampus, a structure playing an important role in learning [6] and characterized by high sensitivity to ischemia [15].

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MATERIALS AND METHODS

Experiments were carried out on albino rats weighing 160-190 g. The heart was arrested for 10 min [11]. Resuscitation included closed chest massage and artificial ventilation. Food-procuring skill was trained for 1.5 month in intact and resuscitated (10-12 days after postresuscitation) rats in a multialternative maze [13]. The cell density and composition of neuronal populations (fractions of morphologically abnormal cells and normal light and dark cells) in hippocampal fields CA1 and CA4 of intact and resuscitated rats demonstrating low and high learning ability (good and poor learners, GL and PL, respectively, 5 rats per group) were determined by morphometric analysis [1]. In these rats, hippocampal pyramidal neurons (50 CA1 and CA4 neurons from each animal) were studied by interference microscopy. The concentration of dense substances in the nucleus and cytoplasm was determined using a BINAM-L-212 interference microscope. The area of the profile field was measured with MOV-1-15 ocular-micrometer. The dry weight of neuronal nucleus and cytoplasm in fixed preparation (reflects

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protein content in the cell) was calculated using Protein software (Department of Cytochemistry, Brain Research Institute). The results were analyzed statistically using Student's *t* test.

RESULTS

The number of GL rats was the same in intact and resuscitated groups (40 and 50%, respectively, $p>0.05$). Previous studies demonstrated that the number of GL in intact and resuscitated groups was similar after 15-min cardiac arrest [10]. Hence, learning ability is an integral index reflecting peculiarities of the highest nervous activity in intact animals, which is preserved in the postresuscitation period.

Morphometric analysis revealed significant differences in the density and composition of the examined neuronal populations in intact rats demonstrating different learning ability. The total density of neuronal population in hippocampal field CA1 was higher in GL compared to PL rats due to a higher number of dark cells without morphological abnormalities (Fig. 1). Significant variations in the population composition of CA4 neurons were found in intact animals with different learning ability: the relative content of normal light cells was lower, while the content of abnormal cells was higher in GL rats compared to PL (by 11.1 and 27.4%, respectively).

In rats survived clinical death, some differences in the severity of morphological changes were found in animals with various learning ability. In comparison with intact rats, resuscitated GL rats had significant abnormalities in the composition of examined neuronal populations attesting to the development of the dystrophic changes in nerve cells. These changes were pronounced in CA4 hippocampal field (the number of abnormal neurons increased by 31.3%, and the number of normal light cells decreased by 7.1%) and minor in SA1 field. In resuscitated PL rats no disturbances in the density and composition of neuronal populations in hippocampal fields CA1 and CA4 were found. These data suggest that hippocampal neurons in GL rats are more sensitive to ischemia-reperfusion procedure than in PL rats. This observation is also corroborated by comparison of neuronal populations in resuscitated rats with different learning capability: the number of abnormal cells was higher and the number of normal light cells was lower in CA1 and CA4 hippocampal fields of PL rats compared to GL animals (Fig. 2).

Both cytochemistry and morphometry revealed significant differences between animals with different learning ability. The concentration of dense substances and dry weight of neuronal nuclei in both examined populations were higher in intact PL rats. The concentration of dense substances in the cytoplasm of CA1 neurons and dry weight of the cytoplasm in CA1 and CA4 neurons were also higher in these rats (Table 1). These data indicate that intact PL rats are characterized by high protein content in nerve cells compared to GL rats. Similar differences were revealed in the study of protein metabolism in neurons of the sensorimotor cortex in rats with different motor activity: passive rats were characterized by higher protein content [8]. Since motor activity is a prerequisite of efficient learning, all these experimental findings suggest that the differences in neuronal protein content between GL and PL rats is a characteristic feature of animals with different learning ability. Information load can aggravate these differences. Previous studies showed that maze training induced opposite shifts in neuronal protein metabolism in animals with different learning ability, in particular, it increased the concentration of dense substances and dry weight of cortical neurons in PL rats and reduced these parameters in GL rats [9].

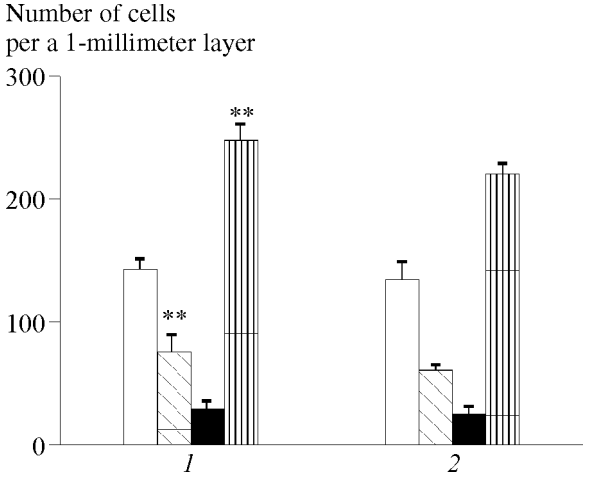

Fig. 1. Distribution density of hippocampal CA1 neurons in intact GL (*1*) and PL (*2*) rats. Here and in Fig. 2: open bars: light neurons; oblique hatching: dark neurons; dark bars: morphologically abnormal neurons; vertical hatching: general population density. *$p<0.01$, **$p<0.05$ compared to PL rats.

Postresuscitation period is accompanied by significant changes in the size of neurons, concentration of dense substance, and dry weights of their nucleus and cytoplasm. However, these changes in the examined neuronal populations are differently expressed in rats with different learning ability (Table 1). In resuscitated rats the size of neuronal nuclei in hippocampal field CA1 decreased (especially in GL rats) and the concentration of dense substances increased (observed only in GL rats). The concentration of dense substances in the nuclei of CA4 neurons (only in GL rats) also decreased. The size and dry weight of these nuclei underwent pronounced and opposite changes in resuscitated rats with different learning ability (increased in

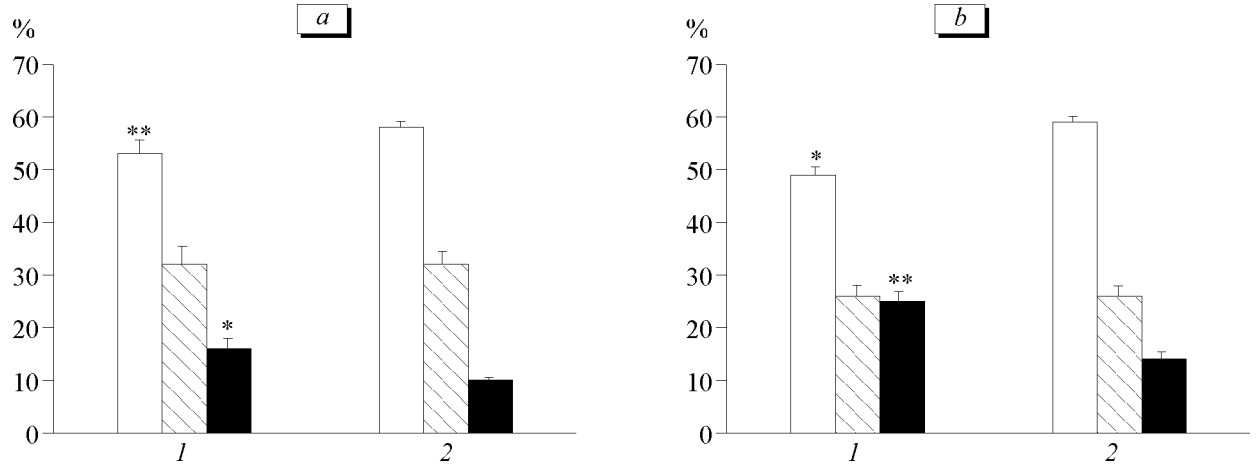

Fig. 2. Population of hippocampal pyramidal CA1 (*a*) and CA4 (*b*) neurons in GL (*1*) and PL (*2*) resuscitated rats.

GL rats and decreased in PL rats). In resuscitated rats, the size and dry weight of the cytoplasm of CA1 hippocampal neurons decreased (only in PL rats), while in CA4 neurons the size of cytoplasm increased (especially in GL rats) and the concentration of dense substances decreased (only in GL rats, Table 1) compared to the corresponding parameters in intact controls. It should be emphasized that that the pronounced postresuscitation changes did not mask the regularity observed in intact rats: increased concentration of dense substances in the nuclei and cytoplasm and higher dry weight of the nuclei of CA1 and CA4 neurons and increased dry weight of cytoplasm in CA4 neurons (Table 1).

Our experiments demonstrated pronounced morphological and cytochemical peculiarities of the examined neuronal populations in animals with different learning ability. Degenerative changes in neurons in the postresuscitation period disturb the composition of neuronal populations (only in GL rats). It should be noted that CA4 pyramidal neurons of the hippocampus were less resistant to postresuscitation damage than CA1 neurons. This is consistent with our previous data on the severity of degenerative changes and sensitivity of protein-synthesizing system in these neuronal populations after clinical death [1]. Different vulnerability of CA1 and CA4 neurons can result from different functional load and specific physiological roles of these cells in integral hippocampal activity [3,6]. It was shown that the concentration of dense substances and dry weight of the corresponding neuronal populations in PL rats are higher than in GL rats. Sharp

TABLE 1. Parameter of Nucleus and Cytoplasm of CA1/CA4 Pyramidal Neurons in the Hippocampus ($M \pm m$, n=5)

Parameter		Intact		Resuscitated	
		GL rats	PL rats	GL rats	PL rats
Area, μ²	nucleus	96.97±1.37	88.21±1.61⁺	87.08±1.40*	84.18±1.21**
		88.68±1.27	101.10±1.43⁺	98.30±1.46*	96.56±1.43**
	cytoplasm	75.02±1.41	76.021±1.870	75.09±1.50	67.64±1.41*⁺
		85.15±1.43	88.70±1.86	95.25±2.61*	94.64±1.78**
Concentration of dense substances, pg/μ³	nucleus	0.76±0.01	0.89±0.02⁺	0.82±0.01**	0.90±0.02⁺
		0.86±0.01	0.99±0.02⁺	0.83±0.01**	0.96±0.02⁺
	cytoplasm	1.50±0.01	1.58±0.02⁺	1.48±0.02	1.54±0.02⁺⁺
		1.66±0.02	1.69±0.02	1.48±0.02*	1.63±0.02⁺
Dry weight, pg	nucleus	73.93±1.65	79.09±1.78⁺⁺	70.89±1.43	75.09±1.78
		76.08±1.50	99.93±2.28⁺	80.58±1.61**	92.90±2.47**⁺
	cytoplasm	112.32±2.30	119.72±3.08	109.83±2.28	104.32±2.51*
		140.97±1.43	149.79±3.68	140.01±3.29	153.63±3.38⁺⁺

Note. *p<0.001, **p<0.05 compared to intact rats; ⁺p<0.001, ⁺⁺p<0.05 compared to GL rats.

inhibition of protein synthesis is the main cause of postischemic brain pathology [14]. It was demonstrated that the loss and degenerative changes in neurons during postresuscitation develop against the background of decreased protein synthesis [1]. Our findings suggest that the higher tolerance of neurons to ischemia-reperfusion procedure in PL rats in comparison with that of GL rats can be related to a larger "safety margin" in PL rats due to enhanced protein content before clinical death. This advantage is preserved even in the postresuscitation period, when protein content decreases.

Differences tolerance to hypoxia can be explained by peculiarities of energy metabolism, specificity of cerebral circulation, and activity of lipid peroxidation in the brain [7,12]. The previously established correlation between the degree and the rate of neurological recovery in animals survived clinical death, on the one side, and the level of protein synthesis in the brain, on the other side [2], as well as our present findings on the interrelation between protein content in neurons and their vulnerability during postresuscitation period attest to an important role of protein synthesis in the development of different tolerance to ischemia.

In conclusion, learning ability is an index of individual and typological properties of an organism, which is intimately related to structural and functional organization of the brain. At the level of neuronal populations, this connection is manifested by the differences in their composition and density, as well as the intensity of protein metabolism in neurons. The revealed peculiarities of morphological and functional organization in animals with different learning ability are characteristic of intact rats, but they are preserved during postresuscitation period and determined different tolerance to ischemia-reperfusion damage indicate that individual and typological peculiarities of the organism should be taken into consideration during prophylaxis and treatment of posthypoxic encephalopathies.

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REFERENCES

1. M. Sh. Avrushchenko and A. V. Volkov, *Vestn. Ros. Akad. Med. Nauk*, No. 10, 26-32 (1997).
2. h. Avrushchenko, T. L. Marshak, V. I. Fateeva, *et al., Anest. Reanimatol.*, No. 2, 43-46 (1993).
3. O. S. Adrianov, *Zh. Vyssh. Nervn. Deyat.*, **45**, No. 3, 441-452 (1995).
4. G. V. Alekseeva, *Anest. Reanimatol.*, No. 6, 15-20 (2000).
5. G. E. Beglarova and N. B. Kabdualieva, in: *Neuronal Resuscitation and Rehabilitation* [in Russian], Omsk (1999), pp. 12-14.
6. O. S. Vinogradova, *Hippocampus and Memory* [in Russian], Moscow (1975).
7. I. V. Gannushkina, A. L. Antelava, and M. V. Baranchikova, *Byull. Eksp. Biol. Med.*, **118**, No. 10, 360-363 (1994).
8. L. M. Gershtein, A. S. Kamysheva, T. L. Chebotareva, *et al., Zh. Vyssh. Nervn. Deyat.*, **41**, No. 2, 300-304 (1991).
9. L. M. Gershtein, V. N. Shpin'kova, and K. A. Nikol'skaya, *Ibid.*, **48**, No. 8, 847-854 (1998).
10. Yu. V. Zarzhetskii, E. A. Mutuskina, and I. E. Trubina, *Byull. Eksp. Biol. Med.*, **129**, Suppl. 2, 21-24 (2000).
11. V. G. Korpachev, V. P. Lysenkov, and L. Z. Tel', *Pat. Fiziol.*, No. 3, 78-80 (1982).
12. M. L. Kuklei, S. L. Stvolinskii, A. A. Boldyrev, and I. V. Gannushkina, *Byull. Eksp. Biol. Med.*, **118**, No. 10, 384-387 (1994).
13. K. A. Nikol'skaya, Sh. K. Segimbaeva, and L. G. Voronin, *Zh. Vyssh. Nervn. Deyat.*, **28**, No. 1, 91-105 (1978).
14. R. W. Neumar, *Ann. Emerg. Med.*, **36**, No. 5, 483-506 (2000).
15. H. Onodera, H. Aoki, and K. Kogure, *Progr. Brain. Res.*, **96**, 271-280 (1993).