platelets. This synchronization may be mediated by fibrinogen molecules, which bind to the specific receptors (GPIIb/GPIIIa), forming a kind of loose network. Here the second type of secretion and irreversible platelet aggregation do not take place [1]. Ca^{2+} ions are required for the formation of receptor heterodimer and its binding to fibrinogen [8]. Thus, the requirement, shown in this study, of plasma proteins and Ca^{2+} for recording fluctuations in Spl confirms the hypothesis mentioned above.

This investigation sheds new light on earlier results on the Spl level under normal and pathological conditions. The St of the blood may be considered to be comparatively constant. However, the Spl variability in short periods of time reflects the large measure of chance in the estimation of this index. A study of Spl instability and the factors influencing it is necessary for the investigation of peculiarities of the serotoninergic system under normal and pathological conditions.

REFERENCES

1. N. V. Belitser, M. G. Anishchuk, T. M. Pozdnyakova, *et al., Vestn. Akad. Med. Nauk SSSR,* № 10, 45-56 (1991).
2. A. A. Zozulya, *Zh. Nevropatol. Psikhiatr.*, № 4, 599-603 (1986).
3. N. L. Rabinovich, V. M. Makhotchenko, I. M. Kozinets, *et al., Lab. Delo,* № 12, 729 (1985).
4. A. P. Solomonov and Z. G. Larskii, *Ibid.*, № 2, 80-83 (1973).
5. A. Coppen, C. Swade, and K. Wood, *Brit. J. Psychiat.*, **136**, 235-238 (1980).
6. J. R. Ieni and L. P. Meyerson, *Life Sci.*, **42**, № 3, 311-320 (1988).
7. A. Pletscher, *Experientia*, **23**, 289-291 (1967).
8. E. W. Salzman and J. A. Ware, *Methods for Studying Platelets and Megakaryocytes*, New York (1987), pp. 419-430.
9. S. Stahl, D. Woo, I. Mefford, *et al, Amer. J. Psychiat.*, **140**, 26-30 (1983).

Bioenergetic Parameters of the Brain in Rats with Different Resistance to Hypoxia

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Today we have a body of evidence supporting the notion that the different brain resistance to hypoxia in highly resistant and susceptible animals correlates with peculiarities of the energy-synthesizing function of the brain, which is connected with the oxidation of different substrates [4,7,8,10,11]. In the brain energetic substrates undergo oxidation mostly via the NAD-dependent pathway, which is a limiting link of the respiratory chain during hypoxia [2,5-8,17]. Differences in the degree of its inhibition during oxygen deprivation in resistant and susceptible animals may be a decisive factor in the formation of the initial resistance of the brain to hypoxia.

The goal of our study was to obtain direct confirmation of this assumption by studying the enzyme composition of the respiratory chain in brain mitochondria, as well as some other biochemical param-

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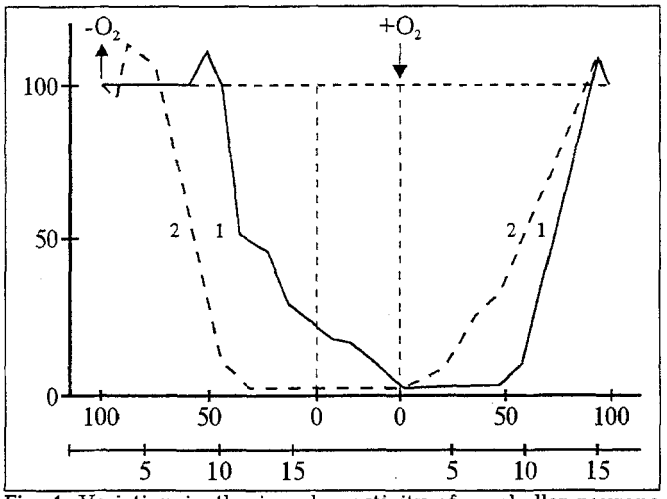


Fig. 1. Variation in the impulse activity of cerebellar neurons from highly resistant (1) and weakly resistant (2) rats during hypoxia of increasing severity ($-O_2$) and ensuing reoxygenation ($+O_2$).

eters in rats with different sensitivity to oxygen deficiency.

MATERIALS AND METHODS

The experiments were performed on outbred white male rats weighing 200-250 g. The animals were divided into weakly and highly resistant (WR, HR) groups, according to their resistance to hypobaric hypoxia [12]. In the course of the study of the mitochondrial cytochrome content and the activity of

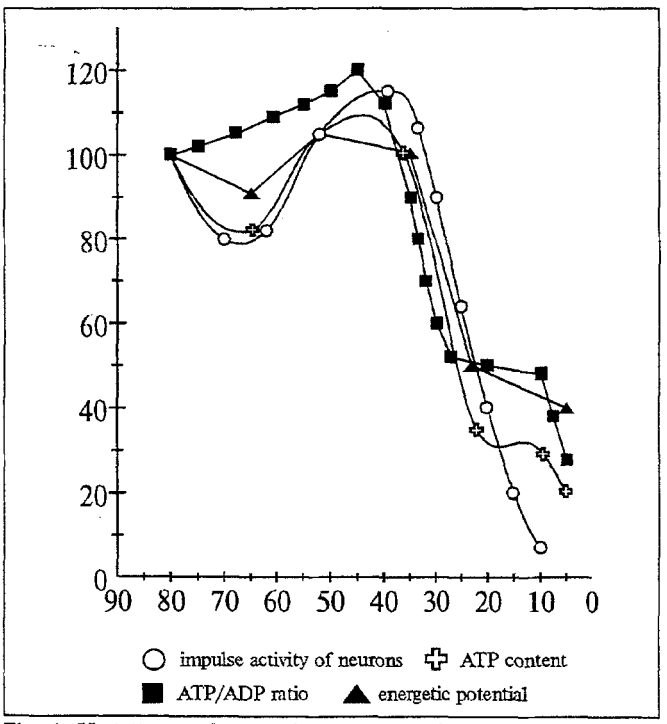


Fig. 2. Variation in the impulse activity and energetic parameters in cerebral cortex slices from rats during hypoxia of increasing severity.

TABLE 1. Variation of the Parameters of Energy Metabolism in Brain Preparations from WR and HR Rats in the Course of Successive Decrease of pO_2 ($M \pm m$).

Parameter	Group of rats	pO_2, %									
		100	90	80	70	60	50	40	30	20	10	5
ATP, μM/g of wet weight (WW)	HR	0.93±0.035	0.92±0.055	0.76±0.054	0.90±0.048	0.97±0.056	0.93±0.046	0.78±0.047	0.46±0.028	0.43±0.038	0.37±0.030	0.34±0.020
	WR	0.93±0.041	0.90±0.045	0.75±0.034	0.88±0.041	1.01±0.052	0.97±0.054	0.76±0.046	0.40±0.035	0.37±0.026	0.31±0.020	0.22±0.018*
ADP, μM/g WW	HR	0.22±0.031	0.23±0.024	0.23±0.021	0.23±0.024	0.24±0.022	0.23±0.022	0.21±0.020	0.18±0.010	0.18±0.007	0.17±0.005	0.34±0.034
	WR	0.30±0.033	0.26±0.024	0.17±0.006*	0.15±0.008*	0.14±0.020*	0.13±0.008*	0.19±0.019	0.26±0.030*	0.26±0.012*	0.20±0.014	0.18±0.026*
AMP, μM/g WW	HR	0.31±0.039	0.36±0.034	0.37±0.034	0.36±0.033	0.34±0.022	0.36±0.033	0.42±0.031	0.48±0.032	0.49±0.032	0.47±0.037	0.47±0.052
	WR	0.20±0.022	0.15±0.020*	0.07±0.006*	0.15±0.018*	0.22±0.022*	0.28±0.023	0.32±0.028	0.35±0.030*	0.37±0.034*	0.41±0.041	0.42±0.052
Energy potential	HR	0.72±0.024	0.69±0.041	0.66±0.039	0.68±0.040	0.71±0.020	0.70±0.021	0.63±0.031	0.50±0.026	0.49±0.013	0.46±0.033	0.44±0.018
	WR	0.76±0.021	0.77±0.050	0.83±0.040*	0.82±0.041*	0.79±0.050	0.75±0.044	0.67±0.042	0.52±0.020	0.50±0.021	0.45±0.031	0.38±0.018

Note. Asterisk: $p<0.05$ when compared to the corresponding index in HR animals.

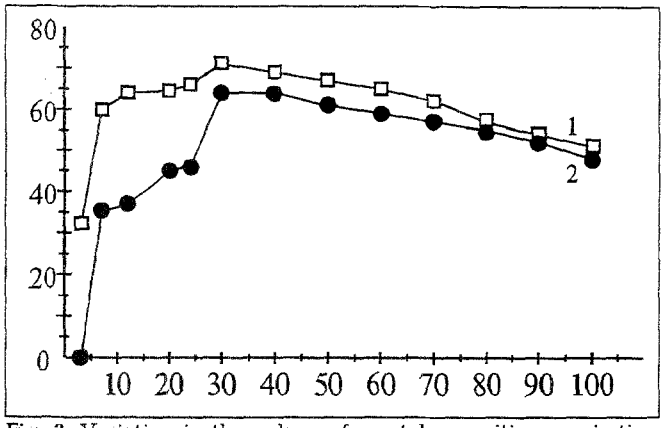


Fig. 3. Variation in the values of amytal-sensitive respiration (V_{ams}, % of initial level) in cerebral cortex slices from highly resistant (*1*) and weakly resistant (*2*) rats during hypoxia of increasing severity.

the respiratory chain enzyme complexes, homogenates or isolated brain mitochondria were used in different experimental series. Homogenates were prepared using a solution containing 0.32 M sucrose, 50 mM Tris-HCl, and 1 mM EDTA, pH 7.4. The brain mitochondria were separated by differential centrifugation [15]. The medium used for separation consisted of 0.32 M sucrose, 5 mM Tris-HCl, 1 mM EDTA, and 0.06% bovine serum albumin, pH 7.4.

The cytochrome content was measured as described elsewhere [9]. Enzyme activity was recorded spectrophotometrically in 0.1 M phosphate buffer, pH 7.4, at 37°C in the homogenates and/or mitochondria having undergone three cycles of freeze-thawing.

NADH-cytochrome C-reductase (rotenone-sensitive) activity was assayed according to [14] in our modification. The incubation mixture contained 12 mM NaN_3, 9.0 mM nicotinamide, 0.15 mM cytochrome C (0.07 mM during the testing of homogenates), and 1.8 mM rotenone. The reaction was initiated by the addition of NADH in different concentrations.

Succinate-cytochrome C-reductase activity was assayed according to [16]; the concentration of cytochrome C was 0.15 mM when testing mitochondria and 0.07 mM in the case of brain homogenates. The reaction was initiated by the addition of succinate in different concentrations.

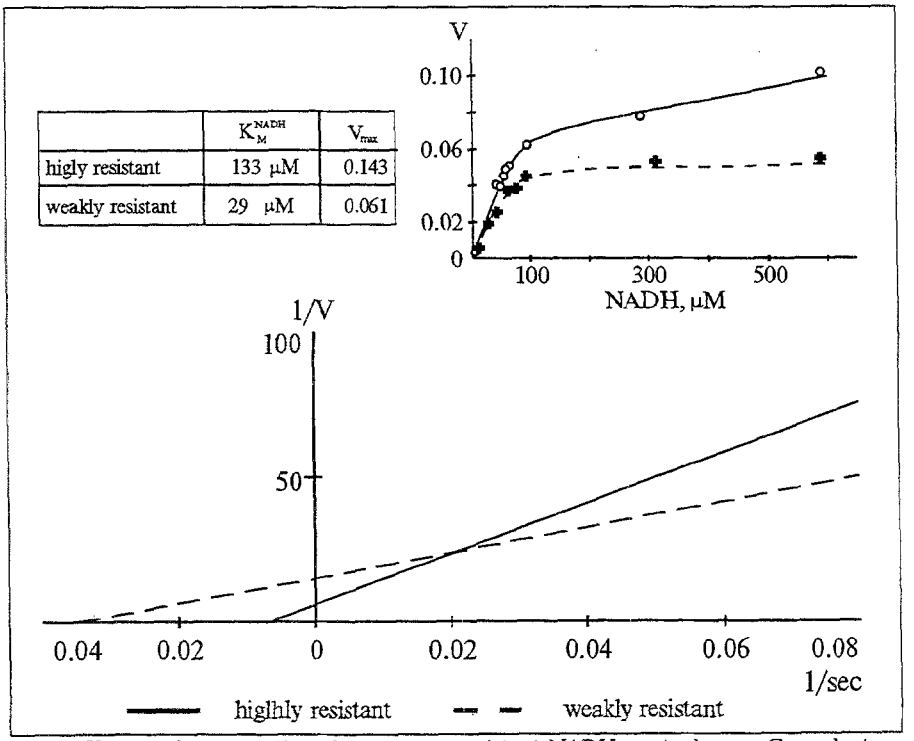
Cytochrome C-oxidase activity was assayed according to [18]. The protein content was recorded using the biuret method [13].

Tissue preparatons (slices) of cerebral cortex and cerebellar cortex from HR and WR animals were prepared by routine methods using a microtome. The preparations were preincubated for 60 min at 36°C in Eagle's medium saturated with carbogen (95% O_2 + 5% CO_2). These conditions of the oxygen regimen were taken as "normoxygenic" (pO_2=100%). Hypoxia of various severity was modeled by the substitution of nitrogen for carbogen. The oxygen content in the medium and the respiration rate in the brain slices were recorded by polarography using a Clark electrode. The spontaneous electrical activity of neurons in the surviving slices of cortex and cerebellum was registered extracellularly using a glass electrode [3].

The adenine nucleotide content in the brain slices was assayed by the luciferin-luciferase method [1].

RESULTS

The study of neuronal impulse activity in the cerebellar slices under the conditions of experimental hypoxia revealed the existence of neurons with differing responses to the oxygen deficiency, i.e., sensitive and resistant neurons (Fig. 1). These neurons differ in the latency of the primary response to hypoxia (3 min and 8.5 min, respectively), in the time of appearance of the phase of impulse activity inhibition (5.5 min and 10.5 min), and the pO_2 value inhibiting impulse activity by 50%. During reoxygenation, the recovery of neuronal electrical activity



	K_M^{NADH}	V_{max}
higly resistant	133 μM	0.143
weakly resistant	29 μM	0.061

Fig. 4. Kinetic characteristics of rotenone-resistant NADH-cytochrome C-reductase in brain mitochondria of highly resistant and weakly resistant rats.

TABLE 2. Kinetic Characteristics of Enzyme Cascades of the Respiratory Chain in Rat Brain Homogenates ($M \pm \sigma$, $n = 5-6$)

Parameter	Highly resistant rats		Weakly resistant rats	
	K_M	V_{max}	K_M	V_{max}
Rotenone-sensitive NCR	123,8±32,8	4,23±1,10	77,9±19,5	2,58±0,54
SCR	1,32±0,37	3,44±0,82	1,14±0,25	3,28±0,69
CO	30,4±4,2	34,78±0,06	20,8±9,1	33,85±7,25

Note. NCR K_M is K_M of NADH-cytochrome C-reductase for NADH. SCR K_M is K_M of succinate-cytochrome C-reductase for succinate. CO K_M is K_M of cytochrome C-oxidase for reduced cytochrome C, mM. V_{max} value of cytochrome C, μM/min per g wet tissue weight.

also proceeded nonuniformly, that is, it occurred earlier in the sensitive than in the resistant neurons (Fig. 1). On average, 69% of neurons in the cerebellar slices of WR rats were found

to be sensitive, while, on the contrary, 63% of neurons in the slices from HR animals were resistant to hypoxia. It may be concluded that the prevailing response of the brain cells to the oxygen deficiency correlated with the individual sensitivity of the rats to oxygen deprivation.

The dynamics of the spontaneous electrical activity in the brain of WR and HR animals during hypoxia of increasing severity was in agreement with the similar alterations of energetic parameters, such as adenine nucleotide content, the ATP/ADP ratio, and the energetic potential (Table 1, Fig. 2). Apparently, in the range of low pO_2 values, the alterations of energy metabolism (loss of macroergic phosphates and reduction of the energetic potential) in the brain of WR animals appeared earlier and were more pronounced than in HR rats.

The similar pattern of phase changes in the energetic parameters and the neuronal background activity (Fig. 2) implies the alternation of the mechanisms of energy metabolism regulation and control in the brain cells for different pO_2 levels. It was found that the activity of the NAD-dependent oxidation pathway plays a crucial role in the establishment of the neuronal reaction to oxygen deficiency. Evaluation of its contribution to the total oxygen consumption by the slice, assayed using amytal, an inhibitor of the NAD-dependent link of the respiratory chain, showed that under conditions of increasing hypoxia a primary compensatory activation of this pathway took place. Activation was more significant in the brain of HR animals. Within the range of low pO_2 indexes the activation gave way to inhibition of NAD-dependent oxidation (Fig. 3). Hence, changes in the activity of NAD-dependent oxidation in the low pO_2 range correlated directly with brain resistance to hypoxia, thus implying a specific role of this pathway in the formation of individual brain sensitivity to oxygen deprivation.

These results are in agreement with the kinetic characteristics of enzyme cascades of the respiratory chain in the rat brain. In both isolated mitochondria and brain homogenates of HR animals the maximal activity of NADH-cytochrome C-reductase significantly exceeded that in the preparations from WR rats (Table 2, Fig. 4). NADH K_M of this enzyme complex from HR rats was much higher than the one in the case of WR animals. High values of K_M and V_{max} in the brain of HR rats enabled NADH-cytochrome C-reductase to function efficiently in the presence of high concentrations of NADH in the cell.

The kinetic characteristics of cytochrome C-reductase and succinate-cytochrome C-reductase in the brain of WR and HR rats did not differ significantly (Table 2). The mitochondrial cytochrome content in the two groups of animals was also equal (Table 3).

The results obtained confirm that the individual sensitivity of the brain to hypoxia can be explained by different levels of activity of rotenone-sensitive NADH-

TABLE 3. Content of Mitochondrial Cytochromes in Brain Homogenate (nM/g Wet Weight) and Isolated Mitochondrial Fraction (nM/mg Protein) from Rats with Different Hypoxia Responsiveness ($M \pm \sigma$)

		Homogenate		Mitochondria	
		HR	WR	HR	WR
Cytochrome	A	7.49±2.86 (16)	6.96±1.26 (15)	0.142±0.02 (5)	0.128±0.056 (7)
	B	4.31±0.91 (15)	4.28±1.22 (14)	0.045±0.019 (5)	0.043±0.024 (7)
	$C \pm C_1$	6.53±1.73 (15)	6.67±1.18 (14)	0.135±0.045 (5)	0.13±0.05 (7)

cytochrome C-reductase and its response to oxygen deficiency in WR and HR rats; this fully corresponds to the data on the role of the NADH-oxidase pathway of energy-rich substrate oxidation in the formation of timely compensatory mechanisms during hypoxia and the regulation of individual brain resistance to it.

REFERENCES

1. F. I. Ataullakhanov and A. V. Pichugin, *Biofizika*, **26**, № 1, 86-88 (1981).
2. R. V. Balasyavichys, Mechanisms of Ischemic Alteration of Heart Mitochondria Functional Activity, Ph.D. Thesis (Biology), Kaunas (1988).
3. I.G. Vlasova, L.D. Luk'yanova, and N.A. Agadzhanyan, *Fiziol. Zh. SSSR*, **72**, № 3, 330-336 (1986).
4. A.M. Dudchenko, Mitochondrial Enzyme Activity and Content of Energy Metabolism Components in the Cerebral Cortex of Rats with Different Hypoxia Sensitivity. Ph.D. Thesis (Medicine), Moscow (1976).
5. L.D. Luk'yanova, B.S. Balmukhanov, and A.T. Ugolev, *Oxygen-Dependent Processes in the Cell and the Cell Function* [in Russian], Moscow (1982), p.301.
6. L. D. Luk'yanova, *Pharmacological Correction of Hypoxic States. Moscow* [in Russian], (1989), p. 11-44.
7. L. D. Luk'yanova. *Itogi Nauki i Tekhniki*, **27**, 5-26 (1991).
8. L. D. Luk'yanova, V. E. Romanova, and G. N. Chernobaeva, *Byull. Eksp. Biol.*, **112**, № 7, 49-51 (1991).
9. E. N. Mokhova and I. V. Zhiganova, Mitochondria: Energy Accumulation and Enzymatic Process Regulation. Moscow (1977), p. 139.
10. V. E. Romanova, Cellular Models for Selection of Bioactive Compounds according to Criteria of Respiration Kinetic Parameters and Coupled Processes of Oxidative Metabolism. Ph.D. Thesis (Biology), Moscow (1984).
11. G. N. Chernobaeva, Specific Features of Oxidative Metabolism Regulation in the Brain of Animals with Different Individual Sensitivity to Oxygen Deficiency. Ph.D. Thesis (Biology), Moscow (1985).
12. G. N. Chernobaeva and L.D. Luk'yanova, *Pharmacological Correction of Hypoxic States* [in Russian], Moscow (1989), pp. 160-164.
13. H. U. Bergmeyer, *Meth. Enzymat. Analys.*, **111**, 86-88 (1983).
14. C. L. Hoppel and C. Cooper, *Arch. Biochem.*, **135**, 173-183 (1969).
15. H. McIlwain and R. Rodnight, *Practical Neurochemistry. London* (1962), pp. 189-210.
16. G. L. Sottocasa, B. O. Kuylenstierna, E. Lars, and A. Bergstrand, *J. Cell Biol.*, **32**, 415-438 (1967).
17. A. H. Toleykis, *J. Molec. Cell. Cardiol.*, **12**, Suppl. 1, 169 (1980).
18. D. C. Wharton and A. Tzagoloff, *Meth. Enzymol.*, **10**, 245-250 (1967).

Antioxidant Activity of Anticonvulsive Drugs

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Recent studies have aimed at elucidating the molecular mechanisms of action of preparations used in anticonvulsive therapy [6,15]. This is explained by the inadequacies of current treatments for epilepsy and various convulsive syndromes [13]. It was established in the last decade that disturbed regulation of lipid peroxidation (LPO) is an important component in the pathogenesis of epilepsy [2,5,7-11,14]; one of the proofs of this thesis is the demonstrated marked anticonvulsive effect of antioxidants belonging to various classes of chemical compounds [1,3,4,7,9,10,12]. Therefore, at least for some of the known anticonvulsive agents, a relationship between the molecular mechanisms of anticonvulsive action and the system of LPO regulation may be assumed.

In order to test this hypothesis, we studied the effect of anticonvulsive preparations belonging to dif-

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