Approaches to Prediction of the Adaptive State of the Brain Energetic System under Conditions of Hypoxia

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We showed that mitochondrial creatine kinase activity can be predicted from the ATP level and that changes in ATP content can be evaluated by activity of mitochondrial creatine kinase under experimental conditions.

Key Words: *creatine kinase; adenosine triphosphate; ischemia; adaptation; regression model*

Oxygen deficiency leads to rapid development of metabolic disturbances in the brain primarily involving the energy metabolism [3,6,7,11]. Organism's response to acute oxygen deficiency is characterized by activation of immediate regulatory mechanisms; this activation is most efficient under conditions of hypoxic preconditioning [4,10,12].

An approach based on the use of empirical dependencies between the experimental parameters under different hypoxic conditions can be useful for solving various practical problems related to formulation of the criteria of organism's resistance to hypoxia. This approach provides the possibility of choosing regression models for predicting the energetic state of the brain by a calculation method.

Creatine kinase reaction is an effective mechanism of emergency correction of ATP content in response to considerable decrease in oxygen consumption rate.

The aim of this study was evaluation and prediction of the energetic state of the brain by ATP content and activity of mitochondrial creatine kinase (mCK) at different terms of circulatory disturbances and during adaptation to ischemia and approximation of the empirical dependency of ATP levels and activity of mCK in the brain tissue under different experimental conditions.

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MATERIALS AND METHODS

Experiments were performed on outbred albino male rats (n=144) weighing 180-200 g. The animals were maintained under vivarium conditions. The control and experimental group consisted of 44 and 100 rats, respectively. Series of experiments are shown in Table 1. Brain ischemia was modeled by double bilateral ligation of the common carotid arteries under Nembutal anesthesia (30 mg/kg body weight intraperitoneally). The brain tissue was examined 30 min and 18 h after surgery. Hypoxic preconditioning was performed in a flow pressure chamber (ascent to an altitude of 7000 m, atmospheric pressure 310 mm Hg, 1 h for 1 and 4 days). The positive effect of single and 4-fold preconditioning was verified on the model of acute ischemia with 30-min and 18-h exposure. Activity of mCK was measured potentiometrically with some modifications [1]. The concentration of ATP was measured by column chromatography on Ecteola-cellulose (Cl-form) [9].

The data were processed statistically using BIOSTAT software according to biomedical statistics recommendations [2].

We used a regression model approximating the dependence between mCK activity and ATP content in the brain tissue; coefficients were determined by the method of least squares, Newton's method, or method of conjugate gradients. The function was described by an equation:

$$y=1.3991\times x-0.2038 \quad \textbf{(1)};$$

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$$y(x,t)=(0.73772-0.16538\times\cos\frac{154.6875}{t})\times x+$$

$$(0.499876+0.516566\times\cos\frac{142.5}{t}) \quad \textbf{(2)},$$

where x is the concentration of ATP (μmol/liter), y is mCK activity (μg-eq. H^+/mg protein/min), and t is the time of ischemia (min).

RESULTS

Thirty minutes after bilateral ligation of the common carotid arteries, the content of ATP and activity of mCK considerably decreased (by 33 and 47%, respectively) compared to the corresponding parameters in intact animals (Table 1). Eighteen hours after bilateral ligation of the common carotid arteries, the ATP content slightly increased (by ~8%) and mCK activity increased by 33% compared to those observed after 30-min hypoxic exposure. Both parameters remained below the corresponding values in intact animals.

The use of methods of mathematical analysis allowed us to create a regression model approximating the relationship between mCK activity and ATP content in the brain of intact animals **(1)** and rats exposed to hypoxia **(2)**. Using formulas **(1)** and **(2)** we calculated activity of mCK from experimentally measured ATP content and (after modifications of the formulas) the level of ATP from mCK activity in the group of intact

TABLE 1. ATP Concentration in the Brain and mCK Activity in Different Experimental Conditions (*M*±*m*)

Experimental conditions		ATP (experiment), μmol/g protein	ATP (calculated)	mCK (experiment), μg-eq H^+/mg protein/min	mCK (calculated)
Intact animals		2.08±0.07	2.139804	2.79±0.13	2.706328
		n=21	(2.88)	*n*=23	(2.98)
Ischemia	30 min	1.40±0.13*	1.470974	1.50±0.14*	1.452681
		p<0.001	(1.94)	*p*<0.05	(3.15)
		n=8		*n*=12	
	18 h	1.52±0.10*	1.721237	2.00±0.06*	1.884483
		p<0.001	(13.2)	*p*<0.05	(5.78)
		(*n*=9)		*n*=14	
Adaptation	single		2.042515	2.05±0.03*	
				p<0.003	
				n=10	
	4 days		1.811021	2.33±0.05*	
				p<0.003	
				n=10	
	single+30 min ischemia		1.996885	2.590±0.053+	
				p<0.002	
				n=8	
	4-fold+30 min ischemia	1.796±0.100+	1.982560	2.57±0.15+	2.308280
		p<0.05	(10.24)	*p*<0.002	(10.12)
		n=10		*n*=10	
	4-fold+18 h ischemia	1.592±0.070			1.952813
		p<0.001			
		n=9			

Note. Significant changes compared to: *intact animals, +30-min ischemia in intact animals. Mean error of the formula (%) is shown in parentheses.

animals and rats exposed to 30-min and 18-h ischemia. Relative error of estimations varied from 1.9 до 13.2% and was within the range of permissible error (<20%) of the approximating function (Table 1). This suggests that regression model **(1)** and **(2)** adequately united the dependent parameters of mCK reaction. Functions (1) and (2) were used for prediction and evaluation of the studied parameters in other experiments. Thus, under conditions of single and 4-fold hypoxic preconditioning, mCK activity was measured experimentally, while ATP concentration was calculated by the formulas. We found that after single preconditioning, CK activity in the total mitochondrial fraction significantly decreased by 27% compared to that in intact animals; 4-fold preconditioning increased mCK activity by 13% compared to single preconditioning, but it remained below the initial level (Table 1). Analysis of estimated ATP concentrations in the brain of experimental animals showed that ATP content in these cases was comparable with that of intact animals (Table 1).

Hence, interval hypoxic preconditioning produced a positive effect on brain adaptation to hypoxia. The stability of the adaptive state was verified in subsequent hypoxic sessions. To this end, we studied mCK activity and ATP content under conditions of 30-min ischemia after single and 4-fold hypoxic preconditioning (Table 1). In this experimental series we also used our regression model for calculation of the chosen parameters. We found that mCK activity after 30-min ischemia in animals adapted to hypoxia did not decrease (in contrast to non-adapted rats), but even increased (by 25% compared to single and by 10% compared to 4-fold preconditioning) and approached the intact level. The content of ATP in these experiments corresponded to normal (Table 1). The resistance of animals to hemodynamic disturbances was still observed 18 h after bilateral ligation of the common carotid arteries. When 18-h ischemia developed against the background of 4-fold preconditioning, 100% survival of experimental animals was observed, whereas acute long-term ischemia caused death of 38% experimental rats. ATP concentration and calculated activity of mCK in adapted and non-adapted animals were similar.

Thus, our study of the dependence of total mCK and ATP content during ischemia of varying duration showed that ATP content and mCK activity decreased at early terms of brain circulatory disturbances and with increasing the time after surgery. Hypoxic preconditioning formed stable resistance of the brain to oxygen deficiency, which was confirmed by recovery of the studied parameters to the level observed in intact animals during subsequent ischemia exposure. At the same time, 18-h ischemia exposure can be considered as an adaptive procedure leading to complete recovery of animal lifespan and additional preconditioning little changed the studied parameters.

The use of numerical methods of analysis allowed us to approximate the empirical dependence of mCK activity and ATP content. The method of approximation of empirical dependence was previously tested by us for other parameters of energy metabolism in the brain: for analysis and prognosis of final concentration of ATP from activity of mitochondrial enzyme complexes [14]; for evaluation of kinetic characteristics of oxidative phosphorylation in the brain of experimental animals [13]; for prediction of the limits of organism's resistance to hypoxia [8], *etc.* The use of the method of approximation of empirical dependencies in this study makes it possible to predict mCK activity by calculation methods and to determine ATP content in the brain under extreme conditions.

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