Prediction of the Resistance to Hypoxia on the Basis of Modifications of the Adenine Nucleotide System

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An empirical dependence allowing one to identify rhythmic fluctuations of stable and unstable state in respect of hypoxia in the process of adaptation has been developed on the basis of long-term investigation of changes in brain content of adenine nucleotides upon "hypobaric training" of rabbits. The function approximating this dependence enables one to predict the effective number of "hypobaric training" sessions providing for the adaptive reaction.

Key Words: *hypoxia; AMP; ATP; energy metabolism; adaptation*

The response to hypoxia of organs and tissues with intense energy metabolism (for instance, brain and muscles) has several aspects. Versatile biochemical mechanisms of this response possess characteristic differences and tissue specialization. Adenosine triphosphate is a direct energy substrate for the majority of cellular functions. Proceeding from this, it seems reasonable to suggest the balance of adenine nucleotides for the basic component in analysis of adaptive mechanisms and estimation of adaptation efficiency.

The creatine kinase reaction is an extremely effective mechanism of emergency correction of ATP level upon substantial (up to 3-fold) decrease in the rate of oxygen consumption [12]; this mechanism provides for not more than 5% modulations of ATP level. Although this mechanism has limited reserves, it is associated with regulatory systems of energy metabolism (respiratory chain enzymes and reduced equivalents in the mitochondria) operating at other levels to prevent energy deficit upon hypoxia. Since these systems are very complex, it is impossible to describe their function even with the use of a simplified kinetic model. Meanwhile, empirical dependences characterizing changes in the concentrations of adenine nucleotides, guanidine phosphates, and other energy metabolites can be employed for solution of some practical problems, for instance, for definition of criteria of the resistance to hypoxia. The set of dependencies can be varied in the search for optimal decision taking into consideration the informativeness of parameters, the difficulties of their experimental measurements, etc.

The state of the adenine nucleotide system, specifically, the ATP and AMP concentrations, is a sensitive marker of brain resistance to hypoxia [10,11, 13]. Is has been shown that brain ATP content decreases after "hypobaric trainings" which provide a 2-4-fold increase in the resistance to hypoxia.

We studied the relationships between the concentration of major energy metabolites (ATP, AMP, ADP, and creatine phosphate) in hypobaric hypoxia and ischemia and approximated them in an attempt to predict the state of the brain energy metabolism.

In the present work we measured brain concentrations of adenine nucleotides and obtained an empirical dependence describing changes in the AMP concentration relative to brain ATP content. The

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function approximating this dependence enabled us to determine the optimal number of training sessions.

MATERIALS AND METHODS

Experiments were performed on male rabbits weighing 2.5-3 kg. A training session consisted in elevation of rabbit in a flow barocamera at a height of 7000 m (atmospheric pressure 310 mm HG) for 1 h during 2, 4 and 8 days. Concentration of adenine nucleotides was measured microchromatographically on ECTEOLA-cellulose columns [3]. Empirical dependence was obtained using polynomial approximation.

RESULTS

Mathematical processing of experimental data showed that the dependence between AMP and ATP concentrations is sufficiently smooth. It can be approximated as follows: $y=a_0x^3+a_1x^2+a_2x+a_3$. The coefficients a_0, a_1, a_2, and a_3 were determined by the method of least squares. The dependence between the coefficients and the number of days was described as follows: $a_\iota=\phi_\iota(n)$, where $\iota=1, 2, 3$. This dependence was approximated by the function $\phi_\iota(n)=(A_2)_i+((A_0)_i cos(A_1)_i/n)$, where ι is the number of days. The form of the function depended on experimental data. Parameters $(A_0)_i$, $(A_1)_i$, and $(A_2)_i$ (i=1, 2, 3) were determined by interpolation. Changes in brain content of AMP relative to this of ATP and the number of "elevations" was described by equation:

$$y=(2542.8333+5886.4041\ cos(489.1449/n))x^3+(1420.6595+2837.5352\ cos(489.14/n))x^2-(37.988152+70.45783\ cos(16.4/n)x-(188.38578+379.80187\ cos(16.8/n),$$

where y is brain AMP concentration, n is the number of training sessions, x is the content of ATP. $(A_0)_i$, $(A_1)_i$ and $(A_2)_i$ coefficients are replaced by their values.

Taking into consideration the fact that fluctuations in the ATP concentration reflect brain resistance to hypoxia, we analyzed the behavior of the function, i.e., its increase or decrease relative to $y=N$ (brain ATP concentration in control animals), calculated for a 90-day period (Fig. 1). The value of x (ATP concentration) varied in the interval of experimental ATP concentrations under hypoxic conditions (Table 1). From the behavior of the function it follows that brain ATP concentration varies within the period of "hypobaric training", which may determine the organism's resistance to hypoxia. Within the first three weeks, 1-2 days of high resistance are followed by 2-3 days of low resistance to hypoxia. Then these periods become longer (5-12 days). From day 57 till day 90 the resistance to hypoxia decreases.

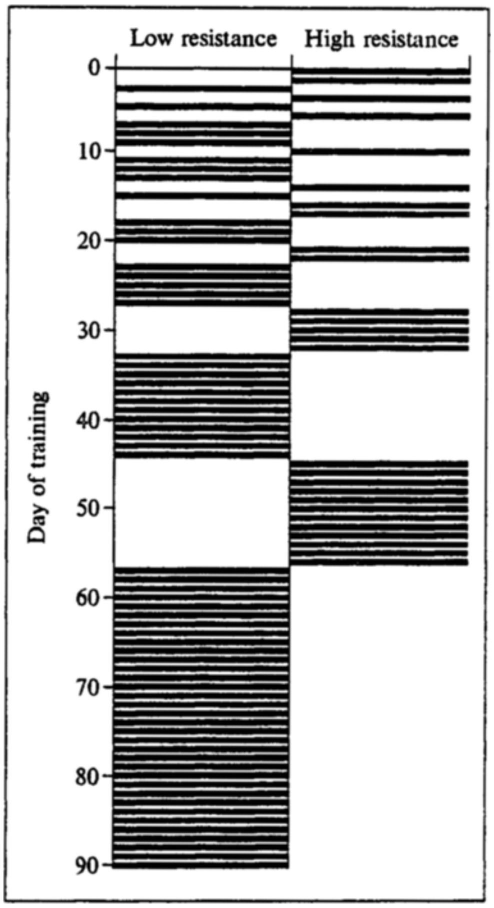

Fig. 1. Fluctuations in the resistance to hypoxia.

Our experimental results and published data were used to check up analytical prediction. Our experiments showed that the resistance to hypoxia increases

TABLE 1. Concentration of Adenine Nucleotides in Rabbit Brain under Hypoxic Conditions (mmol/g Tissue, $M\pm m$)

Experimental conditions	ATP concentration	AMP concentration
Intact animals (n=36)	1.76±0.06	0.27±0.02
Two training sessions+1-h hypoxia (7000 m, n=7)	1.74±0.06**	0.10±0.03***
Four training sessions+1-h hypoxia (7000 m, n=10)	1.92±0.107*	0.17±0.030*
Eight training sessions+1-h hypoxia (7000 m, n=9)	1.51±0.073**	0.29±0.036**

Note. *p<0.05, **p<0.01, ***p<0.001 compared with intact animals.

after 4 days of hypobaric training (7000 m, 1 h every day), which coincided with a decrease in the AMP content. After 8 days of training, the resistance to hypoxia decreased, the AMP content being higher than in control animals (Table 1). The days designated in Fig. 1 as high and low resistance (days 4 and 8, respectively) are consistent with experimental data.

The results of other researchers were also analyzed. For instance, after hypobaric training of rats (2000-7000 m, 5 h every day) their resistance to hypoxia increased on days 10, 21 and 30 and decreased on the 3rd day [2]. In rats and dogs maintained in the mountains at a height of 3200 m above the sea level, high resistance to hypoxia was observed on days 10, 14, 20 and 30 and low resistance on day 3 [5,8].

Thus, analytical prediction of high and low resistance of animals to hypoxia depending on the number of "elevations" has been confirmed experimentally. From these findings it follows that adaptation is an oscillatory process.

On the basis of modern concepts [4,9], the adaptive process has the following phases: mobilization (activation of protective processes), reorganization of adaptive mechanisms (which is often accompanied by a decrease in the resistance to hypoxia), involvement of new mechanisms, and stabilization (at a new level or return to the original level).

The proposed mathematical model reflects oscillatory nature of adaptative process and enables one to predict the development of adaptation in animal organism. From our observations (Fig. 1) it follows that 4- and 6-day hypobaric training is optimal. Presumably, this period coincides with the first phase of adaptive process (mobilization).

Metabolic adaptation in this phase manifests itself in activation of the respiratory chain enzymes and oxidative phosphorylation [6].

In long-term training (1 month), the resistance to hypoxia is increased on days 28-32). We think, however, that a 2-month training is optimal. In this case, high resistance to hypoxia is observed from the 44th till the 56th day. It can be suggested that this period corresponds to the third phase — involvement of new mechanisms in the process of adaptation.

Metabolic adaptation consists in enhanced synthesis of purine nucleotides and increased activity of the respiratory chain enzymes [1]. These modifications create conditions for transition to the stabilization phase. Our studies have shown that training longer than two months is unreasonable, since it can abolish adaptation and exhaust energy resources of the organism.

The dependence between ATP level and rate of oxidative phosphorylation as well as between brain ATP and creatine kinase concentrations under conditions of hypoxia will be investigated in our further research.

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