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Effects of Adaptation to Periodic Hypoxia on Kinetic Parameters of Respiratory Chain Enzymes in Rat Brain

A. M. Dudchenko and L. D. Luk'yanova

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A study of kinetic parameters of brain respiratory enzymes revealed that the maximal velocity and the Michaelis apparent constant for NADH-cytochrome C-reductase are significantly lower in low-resistant rats than in rats with a high resistance to hypoxia. Adaptation to periodic hypoxia increases total resistance only in low-resistant rats. It is accompanied by an increase in the values of kinetic parameters for NADH-cytochrome C-reductase and cytochrome oxidase. Kinetic parameters for these enzymes in the brain of high-resistant rats are either unaltered or even decreased. It is suggested that the first enzymatic complex of the respiratory chain is one of the limiting or regulating links in energy metabolism determining the brain's resistance to hypoxia.

Key Words: *adaptation; hypoxia; enzymatic activity; respiratory chain; individual resistance*

We have shown that long-term periodic adaptation (LTA) to hypoxia is accompanied by a transformation in the activity of the brain respiratory chain which manifests itself in suppressed respiration and intensified oxidative phosphorylation, altered oxidative activity of respiratory substrates [3], a decreased concentration of cytochromes, and an increased number of brain mitochondria [2]. These data suggest that LTA modifies the activity of the respiratory chain enzymes. As no systematic data are available on this subject, in the present study we investigated the effects of LTA on the kinetic parameters of some enzymes of the brain respiratory chain in rats with various degrees of resistance to oxygen deficiency.

Laboratory of Bioenergetics, Research Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

The study was carried out on outbred male rats weighing 180-200 g. The animals were divided into high- and low-resistant groups (HR and LR, respectively) according to their resistance to acute hypoxia. Periodic LTA to hypoxia was produced by a technique described earlier [6]. Rats were decapitated and homogenates

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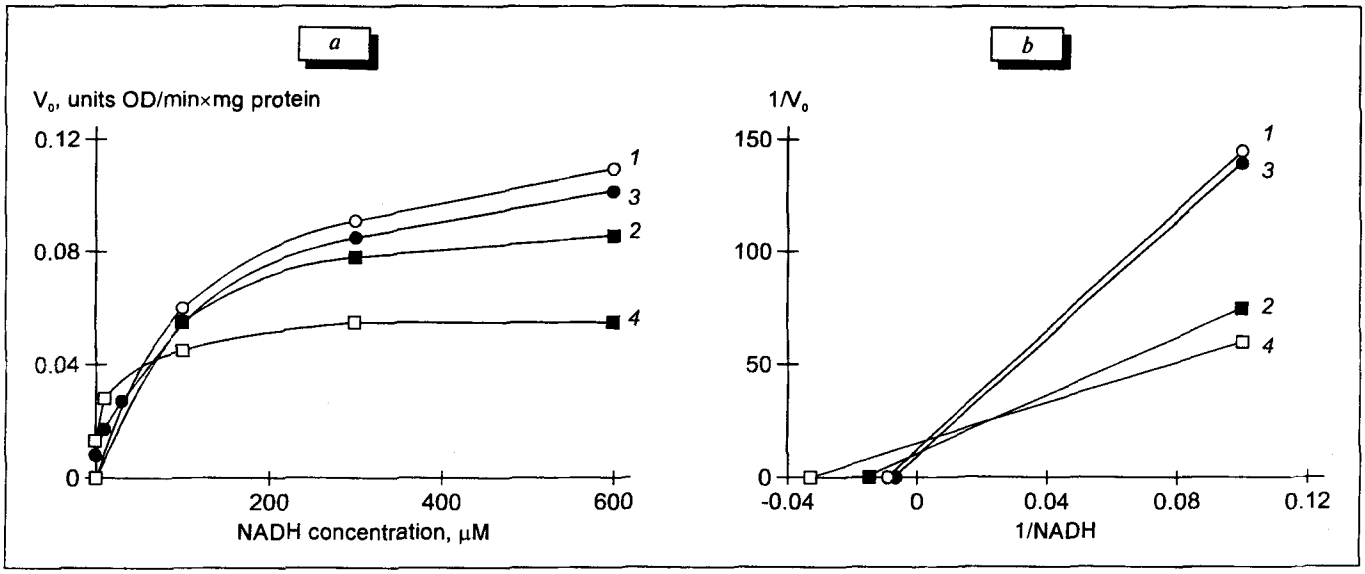

Fig. 1. Initial rate (V_0) of rotenone-sensitive NADH-cytochrome C-reductase (units of optical density/min×mg mitochondrial protein) as a function of NADH concentration in isolated mitochondrial fraction of LR (*3*, *4*) and HR (*1*, *2*) rats before (*1*, *4*) and after (*2*, *3*) adaptation in conventional (*a*) and Lainweaver-Burke (*b*) coordinates.

or the fraction of isolated mitochondria [1] were prepared to determine the kinetic characteristics of enzymes (the maximum activity, V_{max} and the Michaelis apparent constant, K_m). The need to perform experiments on two different preparations stemmed from the fact that the isolation of mitochondria incurs a partial loss of organelles, which may affect the values of the parameters under study. On the other hand, although homogenates preserve all the subcellular structures, their proteolytic processes may be intensified, and that can also modify the enzymatic activity. The use of both preparations makes it possible to obtain comparable data and to prevent their misinterpretation. Kinetic parameters of the rotenone-sensitive NADH-cytochrome C-reductase (NCR), succinate-cytochrome C-reductase (SCR), and cytochrome oxidase were measured by the spectrophotometry technique in our modification [1], allowing for the use of saturating concentrations of the substrates. The apparent K_m values for NCR and SCR and cytochrome oxidase were assayed by NADH, succinate, and cytochrome C, respectively. The data were treated statistically with Student's test.

RESULTS

The study of the kinetic properties of the respiratory chain enzymes in the brain of intact HR and LR rats revealed significant differences in the values of the parameters. Thus, both V_{max} and K_m in homogenates and mitochondria from the brain of LR rats turned out to be significantly lower than those in HR rats (Tables 1 and 2). This means that LR brain NCR is saturated at a higher rate and oxidizes its substrate (NADH) at a lower rate as compared to HR brain, which is in agreement with previously obtained data on the lower activity of the NADH-oxidase pathway of substrate oxidation in the brain of LR animals [3-5]. These kinetic peculiarities may underlie the reduced efficiency of LR brain NCR under conditions of high NADH/NAD ratios, which are known to occur in hypoxia. The findings reinforce our previous conclusion that the first enzyme

TABLE 1. Maximal Activity (V_{max}) (units OD/min×mg protein) and Apparent K_m (10^{-5} M) of Mitochondrial Enzymes in Isolated Mitochondrial Fractions of Rat Brain (n=6, $M\pm\sigma$)

Parameter		Control			Long-term adaptation			HR_{LTA}/HR	LR_{LTA}/LR
		HR	LR	LR/HR	HR	LR	LR/HR		
NCR	V_{max}	0.143±0.045	0.061±0.018	0.43	0.100±0.025	0.167±0.083*	1.67	0.70	2.73
	K_m	13.30±4.31	2.90±0.74	0.22	5.40±1.70*	14.30±3.20*	2.16	0.41	1.43
SCR	V_{max}	0.11±0.026	0.10±0.027	0.99	0.11±0.03	0.12±0.069	1.09	1.00	1.20
	K_m	132.00±25.3	116.0±31.3	0.88	135.0±35.4	116.0±30.0	1.19	0.50	1.00
Cytochrome oxidase	V_{max}	0.77±0.15	0.67±0.22	0.87	1.06±0.48*	1.11±0.68*	1.05	1.38	1.66
	K_m	3.33±0.93	1.67±0.41	0.51	2.82±0.72	3.33±0.87*	1.18	0.85	1.99

Note. Here and in Table 2: *p<0.05.

TABLE 2. Maximal Activity (V_{max}) (cytochrome C, μmol/min×g Wet Weight) and Apparent K_m (10^{-5} M) of Mitochondrial Enzymes in Rat Brain Homogenates ($M\pm\sigma$)

Parameter		Control			Long-term adaptation			HR_{LTA}/HR	LR_{LTA}/LR
		HR (n=4)	LR (n=5-6)	LR/HR	HR (n=4-5)	LR (n=5)	LR/HR		
NCR	V_{max}	4.23±1.10	2.58±0.54	0.61	3.72±0.21	4.00±1.22*	1.08	0.88	1.55
	K_m	12.38±3.28	7.79±2.95	0.63	10.20±4.99	13.80±3.06*	1.36	0.82	1.77
SCR	V_{max}	3.44±0.82	3.28±0.69	0.95	3.33±0.74	3.64±0.91	1.09	0.96	1.11
	K_m	132.00±37.0	114.0±25.0	0.86	132.00±39.0	104.00±22.0	0.79	0.88	0.91
Cytochrome oxidase	V_{max}	34.78±6.06	33.85±7.75	0.97	54.95±16.25*	10.38±18.31*	0.92	1.57	1.49
	K_m	3.04±0.42	2.08±1.01	0.68	2.96±2.58	3.23±2.34	1.09	0.97	1.55

complex of the respiratory chain may be a limiting link in hypoxia, restricting the oxidation of NAD-dependent substrates - the main source of reducing equivalents for the brain respiratory chain. These limitations on NADH-oxidase oxidation in the brain of LR rats set in earlier and are more pronounced than in the brain of HR animals [3-5].

Just as for NCR, V_{max} and especially K_m for cytochrome oxidase were significantly lower in the brain of LR compared to HR rats (Tables 1 and 2). Therefore, LR brain cytochrome oxidase activity is controlled by the concentration of reduced cytochrome C within a more limited range than in the HR brain. By contrast, V_{max} and K_m for SCR do not differ significantly in the brain of LR and HR rats (Tables 1 and 2). Thus, we may assume that the initially different resistance of LR and HR rats to acute hypoxia is due to special features of the kinetics of the 1st and 4th enzyme complexes of the respiratory chain that regulate oxidative phosphorylation according to the thermodynamic model of respiration control [14].

Periodic LTA to hypoxia resulted in a significant increase in the absolute values of the kinetic parameters of the LR brain enzymes. The greatest changes (a 1.5-2.5-fold increase) were observed for NCR and cytochrome oxidase and the smallest (insignificant) for SCR (Tables 1 and 2). On the other hand, the kinetic parameters of the HR brain enzymes were either unaltered or even decreased. As a result the absolute post-LTA values of V_{max} and K_m for the LR brain enzymes were close to the values for the HR brain. As for NCR, the values of its post-LTA kinetic parameters were significantly higher in the brain of LR rats than in that of HR rats (Table 1 and 2). Physiologically, these changes suggest that brain NCR and cytochrome oxidase of hypoxia-adapted LR animals gain the capacity to function in a wider range of substrate concentrations and at higher rates. This permits the enzymes not only to preserve their activity at high concentrations of NADH and reduced cytochrome C which are considerably in excess of their pre-LTA saturating levels, but even to enhance it. It can be seen from Fig. 1 that pre-LTA enzymatic activity peaked at 100 μM and post-LTA activity at 300-600 μM. Since hypoxia enlarges the pool of reduced pyridine nucleotides [13] and increases the reduction of cytochromes [11], including cytochrome C [7], the new kinetic properties of the enzymes utilizing NADH and cytochrome C as substrates may promote their more efficient functioning under conditions of oxygen deficiency. This in turn may bring about an increase in the resistance of brain mitochondria to oxygen deficiency in LR rats.

The alterations in the kinetic characteristics of the enzymes may be due to the formation of isoforms with new properties. Genetic information sufficient for the synthesis of multiple forms of specific proteins is a prerequisite for the appearance of new activities. For instance, NCR is known to be a flavin mononucleotide comprising several tens of subunits [9,10]. For some of them information is translated through the mitochondrial system, while for others it is relayed through the nuclear system [8]. The multipeptide origin of NCR may form the basis for the appearance of isoforms with new kinetic characteristics under long-term exposure to hypoxia. In fact, it has been shown that under anaerobic conditions the first respiratory enzyme complex does demonstrate some new properties [12]. Presumably, adaptive capacities of this kind are limited in the HR brain.

The absence of significant changes in SCR kinetic parameters most likely indicates that this enzyme is not involved in the adaptive changes in the respiratory chain of the brain of LR rats. This is in agreement with our previous data [5] demonstrating that the succinate oxidase pathway is not involved in the long-term adaptive responses of brain cells.

Since the observed changes in NCR and cytochrome oxidase kinetic parameters correlate with significant changes in the ability of LR rats to withstand acute hypoxia, we may speculate that they contribute to the cellular mechanisms underlying the formation of the brain's resistance to hypoxia during adaptation. Thus, the significant differences between the brain NCR kinetic parameters in rats with different degrees of sensitivity to hypoxia and the LTA-induced changes

in these parameters in LR rats, whose resistance to the oxygen deficit sharply increased, allow us to conclude that the first enzymatic complex of the respiratory chain, which helps regulate its activity, is of fundamental importance for the formation of the brain's resistance to hypoxia.

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The Effect of Extract from *Rhodiola rosea* on the Level of Inducible HSP-70 in the Myocardium during Stress

Yu. B. Lishmanov, A. V. Krylatov, L. N. Maslov, N. V. Naryzhnaya, and A. V. Zamotrinskii

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It is demonstrated that the cardioprotective activity of extract from *Rhodiola rosea* in stress reaches the maximum 5 days after the first dose. Heat shock proteins appear in the myocardium 3 days after an 8-day administration of the extract. It is believed that these proteins are not an important factor in the cardioprotective effect of the extract.

Key Words: *adaptation; Rhodiola rosea; heat shock proteins*

Rapid synthesis of heat shock proteins with a molecular weight of 70 kD (hsp-70) is a metabolic manifestation of the stress reaction [9,10]. Undoubtedly, generalized activation of hsp-70 synthesis plays an important role in the development of the cardioprotective effects induced by adaptation to stress [2]. It has been shown that *Rhodiola rosea* possesses marked antistressor and adaptogenic activities [3].

Department of Experimental Cardiology, Institute of Cardiology, Siberian Divison of the Russian Academy of Medical Sciences, Tomsk (Presented by R. S. Karpov, Member of the Russian Academy of Medical Sciences)

There is good reason to believe that the cardioprotective effect, which is probably realized with the involvement of hsp-70, is a manifestation of the adaptogenic effect of rhodiola during stress. The present study was designed to test this hypothesis.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 150-200 g. The animals were adapted by administering the conventional preparation of rhodiola extract for 8 days in a daily dose of 1 ml/kg *per os.*

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