Effects of Adaptation to Hypoxia on Cytochrome Levels in the Brain and Liver of Rats

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After a prolonged (for 30 days) adaptation of rats to intermittent hypoxia, their brains contained lowered levels of mitochondrial cytochromes, despite an increase in the number of mitochondria in the brain tissue mass, along with similar levels of high-energy compounds and more protein as compaired to the brains of unadapted controls. A mitochondrial population with novel properties presumably emerged in the brain. These effects were all more strongly marked in rats with an initially low resistance to hypoxia. In the liver of hypoxia-adapted animals, unlike in their brain, cytochrome levels in the mitochondrial and microsomal redox chains were lowered and the biogenesis of mitochondria was much less intensive.

Key Words: *adaptation; hypoxia; cytochromes; brain; liver; individual resistance*

Long-term (LT) adaptation of animals to hypoxia has been shown to cause mitochondrial hyperplasia [4,15], activate protein synthesis [7,11], and raise mitochondrial cytochrome *c* [19] and cytochrome *c* oxidase levels [15] and the concentration of functional respiratory units [18] in the myocardium. Such changes may boost the power of the cardiac energy-synthesizing system, reduce the ATP and creatine phosphate deficits in the cardiac tissue [7,13], and mitigate the damaging effects of hypoxia on the functional and metabolic homeostasis of cardiac cells. As regards brain and liver cells, the existing information on changes which their mitochondrial enzyme systems undergo during LT adaptation to hypoxia is highly inconclusive [1,15], probably because the respiratory chain in animals with an initially low resistance to hypoxia works differently than in highly resistant animals [5], making it hard to unravel the mechanisms by which adaptive changes occur in the energy-synthesizing system of the brain and liver. In view of this, the purpose of the present study was to examine and compare the effects of LT adaptation to intermittent hypoxia on respiratory chain cytochromes and on cytochromes of other redox chains in rats with high and low resistance to acute hypoxia.

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MATERIALS AND METHODS

The study was conducted on random-bred male rats (body weight 180-200 g) pretested for responsiveness to acute hypobaric hypoxia in a pressure chamber (at an "altitude" of 11,000 m) [5] and considered, on the basis of test results, to be highly resistant (HR) or low-resistant (LR) to acute hypoxia. Thereafter, a proportion of rats from both groups were adapted, in daily 5-h sessions for a total of 30 days, to an "altitude" of 5000 m in the pressure chamber; after 25 days of the adaptation period, they were retested for their ability to survive at 11,000 m. The remaining HR and LR rats served as unadapted controls. One day after the last session, all rats were sacrificed to remove the brain and liver and obtain material for assays. These were run separately in homogenates and in isolated mitochondrial and microsomal fractions because, as shown earlier [16], the results for homogenates may differ from those for isolated fractions. The homogenates and isolated mitochondria were prepared as previously described [2], while isolated liver microsomes were prepared as detailed by Karuzina and Archakov [3]. Cy-

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TABLE 1. Effects of Long-Term Adaptation to Hypoxia on Cytochrome Levels in Brain Homogenates and Mitochondria of HR and LR Rats ($M \pm \sigma$)

Cytochrome	HR rats		LR rats	
	Unadapted	Hypoxia-adapted	Unadapted	Hypoxia-adapted
Homogenates, nmol/g wet weight				
b	4.36±0.57 [17] (101)	4.40±0.84 [17]	4.28±0.73 [16] (86)	3.68±0.64 [16]
$c+c_1$	7.10±1.20 [17] (147)	10.43±1.11* [17]	6.65±0.88 [19] (152)	10.12±0.96* [16]
aa_3	7.45±1.76 [18] (144)	10.75±1.26* [17]	6.91±1.03 [16] (152)	10.52±0.96* [16]
Mitochondria, nmol/mg protein				
b	0.044±0.012 [7] (82)	0.036±0.007 [6]	0.039±0.015 [9] (69)	0.027±0.005* [5]
$c+c_1$	0.141±0.029 [7] (65)	0.092±0.012* [6]	0.130±0.026 [9] (55)	0.072±0.012* [5]
aa_3	0.147±0.019 [7] (57)	0.084±0.011* [6]	0.119±0.038 [9] (55)	0.065±0.011* [5]

Note. Here and in Table 3: figures in brackets are the numbers of animals while figures in parentheses are percentages of the values obtained for unadapted controls and taken as 100%. *$p<0.05$ in comparison with unadapted controls.

tochromes in the isolated hepatic microsomal fraction were measured according to Omura and Sato [14] and microsomal cytochromes in the hepatic homogenates, by Jones' method in a universal buffer for spectrophotometry of turbid media [17]. The P-450 cytochrome level in the homogenates was estimated using a molar extinction coefficient of 104 $mM^{-1} \times cm^{-1}$ [12]. Mitochondrial cytochromes were measured as described by Mokhova and Zhigacheva [8]. Protein was estimated by the biuret method [9].

The results were statistically analyzed by Student's t test.

RESULTS

After the adaptation to hypoxia, the estimated survival time of LR rats at the critical "altitude" in the pressure chamber (11,000 m) increased by a factor of 4 on average, whereas no significant change was recorded in the survival time of HR rats at this altitude. Individual differences in this parameter between LR and HR animals before the adaptation ranged from 3- to 7-fold *vs.* 25% to 30% after it.

Cytochrome levels in the brain mitochondrial membranes of LR rats unadapted to hypoxia did not differ significantly from those of unadapted HR rats. The LT adaptation to hypoxia lowered the levels of mitochondrial cytochromes in both groups. Thus, the levels of cytochromes aa_3, $c+c_1$, and b fell by 45, 45, and 31%, respectively, in the LR group and by 43, 35, and 18% in the HR group (Table 1). In both groups, the decreases in cytochrome levels correlated with those in the mitochondrial respiration rates, i.e., with reductions in the electron-transporting function of the respiratory chain, and these were more marked in the LR group [6]. However, the adaptation to hypoxia did not significantly alter brain levels of high-energy compounds, which were similar in the unadapted HR and LR animals (Table 2).

The findings presented above may be explained by assuming that LT adaptation to hypoxia results in a greater efficiency of oxidative phosphorylation and in increased mitochondrial mass per unit tissue weight. Evidence supporting the first assumption is adduced in the preceding article. The validity of the second assumption was checked in separate assays for

TABLE 2. Effects of Long-Term Adaptation to Hypoxia on Brain Levels of Creatine Phosphate and Adenine Nucleotide Levels in the Brains of HR and LR Rats ($M \pm \sigma$)

Animals	Creatine phosphate	ATP	ADP	AMP
	μmol/g wet weight			
Unadapted HR rats	3.98±0.26	2.93±0.22	0.269±0.024	0.058±0.018
Adapted HR rats	3.86±0.24 (97)	2.78±0.21 (95)	0.297±0.028 (110)	0.070±0.021 (120)
Unadapted LR rats	3.82±0.21	2.79±0.27	0.285±0.055	0.068±0.018
Adapted LR rats	4.28±0.29 (112)	2.48±0.26 (89)	0.254±0.031 (89)	0.061±0.022 (90)

Note. Each group consisted of 8 animals. Figures in parentheses are percentages of the values obtained for unadapted controls and taken as 100%.

TABLE 3. Effects of Long-Term Adaptation to Hypoxia on Cytochrome Levels in Liver Homogenates, Mitochondria, and Microsomes of HR and LR Rats ($M \pm \sigma$)

Cytochrome	HR rats		LR rats	
	Unadapted	Hypoxia-adapted	Unadapted	Hypoxia-adapted
Homogenates, nmol/g wet weight				
aa_3	12.57±3.49 [18] (93)	11.69±1.58 [18]	11.08±3.11 [21] (110)	12.13±2.04 [24]
b	10.28±2.81 [18] (64)	6.56±3.03* [18]	9.94±3.04 [21] (70)	6.96±3.19* [22]
$c+c_1$	19.12±3.63 [18] (82)	15.65±4.29* [20]	18.39±3.31 [21] (88)	16.17±5.07 [23]
P-450	37.71±5.68 [18] (77)	29.07±7.60* [24]	36.37±7.40 [20] (82)	29.95±6.51* [22]
Mitochondria, nmol/mg protein				
aa_3	0.151±0.036 [6] (69)	0.104±0.023* [5]	0.143±0.032 [6] (60)	0.086±0.021* [6]
b	0.081±0.016 [6] (103)	0.083±0.018 [5]	0.094±0.025 [6] (72)	0.068±0.012* [6]
$c+c_1$	0.151±0.031 [6] (82)	0.123±0.028 [5]	0.154±0.044 [6] (70)	0.107±0.025* [6]
Microsomes, nmol/mg protein				
P-450	0.56±0.12 [8] (104)	0.58±0.14 [7]	0.56±0.11 [8] (116)	0.65±0.17 [6]

cytochromes in brain homogenates. When the content of cytochromes was calculated per unit tissue weight, the brains of hypoxia-adapted rats were found to contain more of aa_3 and $c+c_1$ cytochromes than did those of unadapted controls, but virtually the same levels of cytochrome *b* (Table 1). The brain content of total protein increased by an average of 20%. These results suggest that the LT adaptation to intermittent hypoxia led to the appearance of a mitochondrial population with novel properties, namely reduced levels of respiratory carriers in the cytochrome part of the respiratory chain and lower oxygen consumption rates and yet greater efficiency because of an increased ADP/O ratio [6]. This reorganization undergone by the brain's system of aerobic energy synthesis in HR and, to a greater extent, LR rats enables them to maintain high-energy compounds at the high levels necessary for normal functioning of the brain and for sustaining the energy-dependent processes therein under conditions of oxygen deficiency.

As shown in Table 3, the LT adaptation to hypoxia also lowered cytochrome levels in the liver mitochondria (the difference from control values being greatest for aa_3, less for $c+c_1$ and still less for *b*) as well as the levels of cytochrome P-450 in liver homogenates (approximately by 20%). Falls in cytochromes thus occurred in both liver and brain homogenates, but in the liver, in contrast to the brain, the content of $c+c_1$ and aa_3 per unit tissue weight did not change significantly as a result of adaptation, whereas that of *b* even decreased by 30-35% (Table 3). In the liver, too, the LT adaptation to intermittent hypoxia appears to have given rise to a new mitochondrial population with lowered cytochrome levels, but, unlike in the brain, the biogenesis of these organelles in the liver of adapted animals was much less intensive.

REFERENCES

1. V. I. Dedukhova and E. N. Mokhova, *Kosm. Biol.*, **5**, № 2, 31-38 (1971).
2. A. M. Dudchenko, G. N. Chernobaeva, and V. V. Belousova, *Byull. Eksp. Biol. Med.*, **115**, № 3, 251-254 (1993).
3. I. I. Karuzina and A. I. Archakov, in: *Current Topics in Biochemistry* [in Russian], Moscow (1977), pp. 49-52.
4. L. D. Luk'yanova, N. N. Kleimenova, V. N. Arefolov, *et al.*, *Byull. Eksp. Biol. Med.*, **112**, № 8, 148-151 (1991).
5. L. D. Luk'yanova, V. E. Romanova, and G. N. Chernobaeva, *Byull. Eksp. Biol. Med.*, **112**, № 7, 49-51 (1991).
6. L. D. Luk'yanova, G. N. Chernobaeva, and V. E. Romanova, *Byull. Eksp. Biol. Med.*, **120**, № 12, 572-575 (1995).
7. F. Z. Meerson, in: *Mechanisms and Protective Effects of Adaptation* [in Russian], Moscow (1993), p. 331.
8. E. N. Mokhova and I. V. Zhigacheva, in: *Mitochondria. Accumulation of Energy and Regulation of Enzymatic Processes* [in Russian], Moscow (1977), pp. 138-143.
9. H. U. Bergmeyer, *Methods of Enzymatic Analysis*, Vol. 3, Weinheim (1983), pp. 86-88.
10. P. D. Jones, *J. Biol. Chem.*, **255**, 2383-2385 (1980).
11. H. J. Leenders and P. J. Bechers, *J. Cell Biol.*, **55**, 257-265 (1972).
12. T. Matsubara and M. Koike, *Anal. Biochem.*, **75**, 596-599 (1976).
13. P. R. Moret and F. Duchosal, *Schweiz. Med. Wochenschr.*, **103**, 1796-1797 (1973).
14. T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2379-2385 (1964).
15. L. C. Ou and S. M. Tenney, *Respir. Physiol.*, **8**, 151-159 (1970).
16. J. Patkin and E. J. Masoro, Am. J. Physiol., **199**, 201-204 (1960).
17. A. Rutten, *Arch. Toxicol.*, **61**, 27-31 (1987).
18. E. H. Strickland, E. Ackerman, and A. Anthony, *J. Appl. Physiol.*, **17**, 535-538 (1962).
19. D. V. Tappan, V. R. P. Reynafarje, and A. Hurtado, *Am. J. Physiol.*, **190**, 93-98 (1957).