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Effects of Adaptation to Intermittent Hypoxia on Oxidative Phosphorylation in Brain Mitochondria of Rats with Different Sensitivities toward Oxygen Deficiency

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After long-term adaptation to intermittent hypoxia, rats with an initially low resistance to acute oxygen deficiency were 2 to 4 times more resistant to it, while highly resistant rats did not show a significant change in resistance. The adaptation was accompanied by weakening of the electron-transporting function of the respiratory chain and increasing efficiency of oxidative phosphorylation in the brain mitochondria oxidizing NAD-dependent substrates, indicating that energy was produced in a more economical way. The succinate oxidase pathway of oxidation was found to be utilized to only a limited extent as a compensatory mechanism in animals exposed to intermittent hypoxia over a prolonged period. The effects of adaptation were more marked in the brain mitochondria of rats initially highly sensitive to oxygen deficiency.

Key Words: *adaptation; intermittent hypoxia; individual resistance; brain mitochondria; oxidative phosphorylation; NADH-oxidase oxidation; succinate-oxidase oxidation*

In an earlier study we demonstrated, for the first time, differences in the work of the brain's mitochondrial respiratory chain between rats differing in susceptibility to acute hypoxia [6]. In the brain of rats with low resistance to hypoxia (LR rats) as compared to the brain of highly resistant (HR) rats, energy production in the mitochondria oxidizing NAD-dependent substrates was found to be less economical and the succinate oxidase pathway to be utilized to a greater extent as a compensatory mechanism during acute hypoxia. The question of how long-term (LT) adaptation to hypoxia influences the energy-synthesizing function of cells remains open. The available information is contradictory. According to Ozawa *et al.* [11], brain mitochondria are more sensitive to hypoxia than liver or heart mitochondria and exhibit reduced respiratory control and lowered efficiency of aerobic energy production as a result of LT adaptation to hypoxia. Other authors, however, failed

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TABLE 1. Effects of Long-Term Adaptation to Hypobaric Hypoxia on the Responses of HR and LR Rats to Acute Hypoxia Caused by Elevation to 11,000 m in a Pressure Chamber (Results from Three Series of Tests Carried Out in Different Seasons)

Parameter	Group	Control rats, range in min	Adapted rats, range in min	Efficiency of adaptation (adapted/control rats)
Time before postural loss	LR	1.00-2.83	1.67-5.75	1.7-2.0
	HR	1.50-5.17	2.28-10.87	1.5-2.1
Survival time	LR	1.17-3.72	2.53-16.40	2.2-4.4
	HR	7.08-13.42	4.28-15.45	0.6-1.1
Time taken to recover posture	LR	0.67-3.05	1.15-5.77	1.6-1.9
	HR	1.58-7.58	0.67-4.05	0.4-0.5

to detect any changes in the system of oxidative phosphorylation under the influence of LT adaptation [1]. This discrepancy may be attributed to differential effects of LT adaptation on oxidative phosphorylation in animals with initially different sensitivities to hypoxia, as is suggested by a study where the effect of LT adaptation to hypoxia on the [ATP]/Po_2 ratio in isolated hepatocytes was shown to depend on whether these cells were from HR or LR rats, and by similar findings from studies using sections of rat brain cortex [4,5,8]. The impact of LT adaptation to hypoxia on oxidative phosphorylation processes in brain mitochondria of rats with initially different sensitivities to oxygen deficiency has not been addressed and is the subject of the present study.

MATERIALS AND METHODS

The study was conducted on two groups of random-bred male rats (body weight 200-250 g), one of which consisted of LR animals and the other of HR ones, as found in preliminary tests for sensitivity to acute hypobaric hypoxia [6]. The criteria used to evaluate the response of rats to acute hypoxia in these tests (elevation to an "altitude" of 11,000 m in a pressure chamber) were the time elapsing before the rat lost its posture (fell on its side), survival time at this "altitude" (as estimated by the time elapsing before the second agonal inspiration), and the time taken by the rat to recover its posture after exposure to hypoxia was discontinued. Three weeks later, the rats began to be adapted to an "altitude" of 5000 m. The procedure involved their elevation to 2500 m on day 1, an increasingly higher elevation on each of the following 3 or 4 days to reach 5000 m, and daily exposure to this "altitude" for 6 h per day until day 30. Hypoxia-unadapted LH and HR rats served as controls (they stayed near the pressure chamber during the adaptation sessions). The resistance of rats to acute hypobaric hypoxia was checked on day 25 by elevating them to 11,000 m once again. After the 30-day adaptation period, all rats

TABLE 2. Oxidative Phosphorylation Parameters of Brain Mitochondria from Hypoxia-Adapted and Unadapted HR and LR Rats ($M \pm m$)

Animals	V_2	V_3	V_4	V_{DNP}	V_p, nmol ADP/mg protein/min	ADP/O	V_3/V_2	V_3/V_4
	natoms O/mg protein/min							
Glutamate (4 mM)+malate (1 mM)								
Unadapted LR rats	14.3±0.68	67.4±2.19	8.5±0.49	67.4±2.19	176.0±5.91	2.58±0.063	4.8±0.14	8.1±0.21
Adapted LR rats	8.4±0.57	38.0±1.73	5.7±0.27	40.4±0.91	108.7±6.50	2.86±0.050	4.6±0.12	6.7±0.26
	(59)	(56)	(67)	(60)	(61)	(111)	(96)	(83)
Succinate (5 mM)								
Unadapted LR rats	28.2±1.20	51.0±2.71	22.2±1.09	72.5±4.65	85.0±4.11	1.69±0.046	1.8±0.06	2.3±0.08
Adapted LR rats	22.9±1.41	44.7±2.63	18.3±1.24	47.4±3.02	73.7±4.21	1.65±0.030	1.9±0.07	2.5±0.04
	(81)	(88)	(82)	(65)	(86)	(98)	(106)	(109)
Glutamate (4 mM)+malate (1 mM)								
Unadapted HR rats	12.9±0.74	56.5±3.41	7.8±0.50	52.7±5.61	148.0±9.30	2.61±0.034	4.5±0.19	7.4±0.34
Adapted HR rats	9.3±0.48	40.3±1.85	5.9±0.33	42.5±1.49	116.1±5.89	2.89±0.040	4.4±0.10	6.8±0.20
	(72)	(71)	(76)	(81)	(78)	(111)	(98)	(92)
Succinate (5 mM)								
Unadapted HR rats	29.8±1.49	54.9±3.27	21.8±1.39	66.5±5.11	89.0±5.68	1.65±0.049	1.8±0.10	2.6±0.13
Adapted HR rats	25.6±0.90	48.6±2.50	19.6±0.75	54.6±2.15	76.9±4.21	1.57±0.030	1.9±0.07	2.5±0.06
	(86)	(89)	(90)	(82)	(87)	(95)	(106)	(96)

Note. Figures in parentheses are percentages of the control values.

were decapitated and mitochondria were isolated from their brains by differential centrifugation [10]. Oxidative phosphorylation processes were studied polarographically [9]. The media used for isolating and incubating mitochondria are described in our earlier article [6]. The functional state of brain mitochondria was evaluated by measuring the following parameters: respiration rates in different metabolic states according to Chance [9] and in the presence of 50 μM 2,4-dinitrophenol (2,4-DNP) (V_{DNP}); ADP phosphorylation rate (V_P); ADP/O ratio; and respiratory control coefficients as defined by Lardy (V_3/V_2) and Chance (V_3/V_4). NADP oxidase oxidation was evaluated using glutamate (4 mM) with malate (1 mM) and succinate-oxidase oxidation, using succinate (5 mM). Protein levels in the mitochondria were estimated by the biuret method.

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

RESULTS

The LT adaptation to hypoxia increased the total resistance to oxygen deficiency in LR rats 2 to 4 times (as evidenced by their longer survival at the 11,000 m "altitude") without altering, or even lowering, this resistance in HR rats (Table 1). The times elapsing before postural loss were longer in both groups, indicating that the thresholds of responses to hypoxia became higher, whereas the times taken to recover posture increased in the LR group and decreased in the HR group (Table 1). In the latter group, therefore, the prolonged adaptation to intermittent hypoxia accelerated restorative processes without increasing the resistance to hypoxia. In the LR group, the increase in resistance appears to have been due to a greater utilization of the body's compensatory potentials, which were more depleted in this group, as is indicated by the longer times taken to recover normal posture. These findings suggested that the mechanisms by which cells develop resistance to oxygen deficiency in LR rats differ fundamentally from those in HR animals.

Support for this assumption was provided by the tests with brain mitochondria, which showed that the LT adaptation to hypoxia had substantial but unequal effects on oxidative phosphorylation in the brain mitochondria of HR and LR rats. For example, the respiratory rates in all metabolic states and V_P values during the oxidation of NAD-dependent substrates by mitochondria were 30-45% lower in the LR group but only 20-30% lower in the HR group, as compared to the brain mitochondria from unadapted controls (Table 2); as a result, the differences in V_3, V_{DNP}, and V_P values between hypoxia-adapted LR and HR rats became insignificant while being significantly higher in unadapted LR rats than in their unadapted HR counterparts (Table 2). Despite the reduced oxygen consumption, ADP/O values after the adaptation were higher than before it (2.9 *vs.* 2.6 on average), which is an indication of increased oxidative phosphorylation efficiency (Table 2). The absence of significant changes in the V_3/V_2 and V_3/V_4 ratios under the influence of adaptation provides further evidence that oxidation and phosphorylation were strongly coupled in the brain mitochondria of adapted rats. The LT adaptation to hypoxia thus caused the respiratory chain oxidizing NAD-dependent substrates to work more efficiently while depressing its electron-transporting function; hence, it may be concluded that energy was produced more economically in the brain mitochondria of adapted rats than in those of unadapted controls.

This conclusion is supported by other findings. For instance, the difference between respiratory rates in metabolic states 3 and 4 (V_3-V_4), regarded as "the physiological range of respiratory activity" [7], was found to have decreased after the LT adaptation, particularly in LR rats (by 45% *vs.* 29% in HR rats; Table 3). Also, the mitochondria from adapted rats, unlike those from unadapted controls, had a respiratory reserve, defined by the difference between respiratory rates in the uncoupled and phosphorylating states (V_{DNP}-V_3) (Table 3). Consequently, the rate of electron transfer during the oxidation of NAD-dependent substrates in the mitochondrial respiratory chain was not maximal as in the mitochondria from unadapted rats [6]. The changes elicited by LT adaptation in the NADH oxidase pathway, which are more marked in the mitochondria of LR animals, attest to an increased contribution of this pathway to energy metabolism, which agrees with the results obtained for brain sections [4,5,8]. This effect of LT adaptation may be a major determinant of the increased resistance to hypoxia in LR animals, since the maintenance of high activity of the NADH oxidase pathway has been shown to be a crucial factor in the development of the brain's resistance to oxygen deficiency [3,5,8].

During succinate oxidation by brain mitochondria from adapted rats, respiratory rates in all metabolic states were lower than in control tests but the differences were insignificant; in fact, ADP/O, V_3/V_2, V_3/V_4, and V_3-V_4 values recorded for adapted rats and unadapted controls were at the same levels (Tables 2 and 3). The LT adaptation, therefore, had little effect on the activity of the succinate oxidase pathway in the brain mitochondria of LR and HR rats. However, the decrease in V_{DNP} was much greater in LR rats than in HR rats (35% *vs.* 18%; Table 2), with the result that the respiratory reserve increased

TABLE 3. Functional Indicators of Respiratory Activity Shown by Brain Mitochondria from Hypoxia-Adapted and Unadapted LR and HR Rats

Substrate	Animals	LR		HR	
		V_3-V_4	V_{DNP}-V_3	V_3-V_4	V_{DNP}-V_3
		natoms O/mg protein/min			
Glutamate (4 mM)+malate (1 mM)	Unadapted	58.9	0	48.7	0
	Adapted	32.3	2.4	34.4	2.2
Succinate (5 mM)	Unadapted	28.8	21.9	33.2	11.6
	Adapted	26.4	2.7	29.0	6.0

8-fold in the former group and only 1.4-fold in the latter (Table 3). Thus, although the LT adaptation to hypoxia did not have a significant impact on the succinate-oxidizing phosphorylation in brain mitochondria, it reduced the potential for heightened activity of the succinate oxidase pathway and did so to a much greater extent in the brain of LR animals. This statement is consistent with our earlier studies using brain sections, which showed that a compensatory activation of the succinate oxidase pathway, particularly in the brain of LR animals, occurred under conditions of acute hypoxia leading to suppression of NAD-dependent oxidation, and that after a prolonged adaptation to hypoxia, when the NADP oxidase pathway made a greater contribution to energy metabolism in the brain, the succinate oxidase pathway played a lesser role in the brain's compensatory responses to oxygen deficiency [4,5,8]. Activation of the latter pathway is therefore one of the emergency mechanisms by which the brain responds to an acutely arising oxygen deficit. In animals exposed to intermittent hypoxia over a prolonged period, this pathway is used by the cell to only a limited extent as a mechanism of adaptation to oxygen deficiency.

In summary, the reduced external respiration and more economical energy expenditure known to occur at the organismic level after LT adaptation correlate with the changes occurring at the level of the mitochondrial respiratory chain and manifested in the ability of brain mitochondria from hypoxia-adapted rats to oxidize NAD-dependent substrates more efficiently at lower rates of oxidative processes. These changes are much more pronounced in the brain of LR rats, which is in accord with the finding that the LT adaptation increased severalfold the resistance of these animals to acute hypobaric hypoxia while exerting little effect on the resistance of HR rats.

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