OXIDATIVE PHOSPHORYLATION IN BRAIN MITOCHONDRIA OF RATS DIFFERING IN THEIR SENSITIVITY TO HYPOXIA

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In any population of noninbred animals there are some individuals which differ considerably in their sensitivity to hypoxia [1-3, 9]. Differences in the response of the body to oxygen deficiency play an essential role in the development, course, and outcome of an accompanying pathological state. Individual differences in the response to hypoxia are manifested not only at the whole body level, but also at the organ and tissue levels [6-8, 12]. The brain is affected first and by the greatest degree in hypoxia, due to its high functional activity and high energy requirement, maintained chiefly by aerobic metabolism. It has been shown that differences in the resistance of the brain in animals highly resistant and sensitive to hypoxia correlates with the character of its energy-synthesizing function, which depends on oxidation of various substrates [2, 6, 8, 12]. Since this function is coupled with activity of the mitochondrial respiratory chain, the fundamental question arises: does the process of oxidative phosphorylation differ in the brain mitochondria of animals with unequal resistance to hypoxia and, if so, what is its role in the formation of the individual sensitivity of this tissue to hypoxia? The investigation described below was devoted to a study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200-250 g, kept on a standard diet. The animals were first separated on the basis of their resistance to hypobaric hypoxia into those with low resistance (LR) and with high resistance (HR) [19]. The animals' response to acute hypoxia was assessed on the basis of the duration of their stay at an "altitude" of 11,000 m ("ascent" in a pressure chamber), before the appearance of the second agonal inspiration. In the HR animals it was 10 min or more, in the LR it was under 3 min. The animals were decapitated. Mitochondria were isolated by differential centrifugation [16]. The isolation medium was: sucrose 0.32 M, Tris-HCl 5 mM, EDTA 1 mM, BSA 0.06%, pH 7.4. The incubation medium was: sucrose 0.32 M, Tris-HCl 10 mM, EDTA 1 mM, BSA 0.06%, KCl 10 mM, KH_2PO_4 10 mM, pH 7.4. Succinate (5 mM), glutamate with malate (4:1 mM), and also a mixture of these substrates, were used as the oxidation substrates. The following respiratory chain inhibitors were used: malonate (10 mM) and rotenone (10 μM). Oxidative phosphorylation processes were studied by a polarographic method [8]. The mitochondrial protein concentration was determined by the biuret method. Mitochondrial respiration was recorded in the following metabolic states (after Chance [15]): relative rest (V_2), in a state of activity during phosphorylation by 200 μM ADP (V_3), in the regulated state (V_4), and in the uncoupled state in the presence of 50 μM 2,4-DNP (V_u). The rate of phosphorylation (V_{ph}), the ratio ADP/O, and respiratory control coefficients after Lardy ($RC_L = V_3/V_2$) and Chance ($RC_C = V_3/V_4$) were calculated.

EXPERIMENTAL RESULTS

During oxidation of NAD-dependent substrates by the brain mitochondria, the velocities of respiration in states V_2 and V_4 and also the efficiency of work of the respiratory chain, estimated by the values of ADP/O, RC_L, and RC_C, did not

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TABLE 1. Parameters of Oxidative Phosphorylation of Brain Mitochondria of HR and LR Rats (M ± m)

Conditions of incubation	Animal group	V_2	V_3	V_4	V_u	ADP/O	RC_L	RC_C	V_{ph}
Glutamate + malate	HR	12,9±0,74	56,5±3,41	7,8±0,50	52,7±5,61	2,61±0,034	4,5±0,19	7,4±0,34	148±9,3
	LR	14,3±0,68	67,4±2,19	8,5±0,49	67,4±2,19	2,58±0,063	4,8±0,14	8,1±0,21	176±5,9
Succinate	HR	29,8±1,49	54,9±3,27	21,8±1,39	66,5±5,11	1,65±0,049	1,8±0,10	2,6±0,13	89±5,7
	LR	28,2±1,20	51,0±2,71	22,2±1,09	72,5±4,65	1,69±0,046	1,8±0,06	2,3±0,08	85±4,1
Mixture of substrates	HR	31,7±1,32	66,6±1,82	24,0±1,28	63,3±2,10	2,13±0,031	2,1±0,06	2,8±0,08	142±2,7
	LR	29,7±1,11	69,1±3,31	21,2±1,25	66,1±2,31	2,33±0,001	2,3±0,03	3,3±0,05	161±8,5
Glutamate + malate + malonate	HR	16,6±3,07	49,5±7,32	8,9±1,71	71,8±9,02	2,67±0,062	3,2±0,71	5,8±0,92	132±21,3
	LR	14,3±0,98	55,3±8,09	8,3±0,72	61,8±5,51	2,70±0,023	3,9±0,53	6,7±0,61	148±22,1
Succinate + rotenone	HR	28,1±3,05	59,8±6,82	21,8±2,81	64,4±9,82	1,54±0,086	2,2±0,16	2,8±0,27	92±8,7
	LR	33,6±3,01	63,1±1,34	28,0±2,91	68,0±2,91	1,55±0,131	1,9±0,01	1,3±0,19	97±5,9
Succinate + glutamate (2 mM)	HR	29,0±1,18	69,4±3,21	20,7±1,06	75,3±4,62	1,84±0,080	2,4±0,21	3,4±0,23	128±9,1
	LR	33,7±3,51	78,4±5,12	23,2±3,60	84,2±2,21	1,85±0,081	2,4±0,21	3,4±0,20	144±6,2
Mixture of substrates + rotenone	HR	33,3±1,27	47,1±0,24	22,8±0,14	44,4±0,97	1,44±0,003	1,4±0,06	2,1±0,02	68±3,5
	LR	29,1±1,86	43,3±3,02	18,0±1,70	41,5±2,99	1,80±0,051	1,5±0,03	2,4±0,06	77±3,5
Mixture of substrates + malonate	HR	14,5±0,12	32,8±0,33	8,7±0,01	38,6±0,85	2,90±0,030	2,3±0,06	3,8±0,04	96±6,2
	LR	15,6±0,59	34,9±1,96	8,7±0,59	43,2±2,15	3,10±0,030	2,2±0,05	4,1±0,17	106±6,0

Legend. Mixture of substrates means glutamate + malate + succinate.

differ significantly in the HR and LR animals (Table 1). Meanwhile, the intensity of phosphorylating (V_3) and uncoupled (V_u) respiration and also the velocity of phosphorylation (V_{ph}) in the brain mitochondria of the LR animals were significantly higher than in HR animals (Table 1). Thus the equal efficiency of work of the respiratory chain in the brain of the two groups of animals was achieved on account of the higher strain on energy-forming processes in the LR animals, evidence of the less economical nature of oxidative phosphorylation in these animals as a whole. It must also be pointed out that there was no difference between the velocities in states V_u and V_3 in the two groups of animals, or it was actually negative ($V_u - V_3 < 0$). This indicates that on oxidation of NAD-dependent substrates, activation of electron transfer in the respiratory chain is maximal and leads to exhaustion of the reserves of respiratory activity of the brain mitochondria. This state of affairs, together with the higher velocity of phosphorylating respiration in the brain mitochondria of LR rats compared with HR animals, may be one cause of the particular vulnerability of NAD-dependent oxidation, which we demonstrated previously on brain slices, inhibition of which during acute hypoxia in the brain of LR animals is expressed much more strongly than in HR rats [8, 9, 11, 12].

During oxidation of NAD-dependent substrates by brain mitochondria in state V_3, malonate-sensitive respiration, reflecting the contribution of oxidation of endogenous succinic acid, was 1.5 times higher in the brain mitochondria of LR than of HR animals (Table 1). This is in agreement with our previous data obtained in experiments on brain slices oxidizing glucose, for which a more marked malonate-sensitive component of respiration was demonstrated in LR animals [9, 12], a fact which evidently reflects higher succinate dehydrogenase activity in the brain of the latter than in that of HR rats.

During oxidation of endogenous succinate the velocity of nonphosphorylating respiration (V_2) of the brain mitochondria of LR and HR rats was 2-2.5 times faster than during oxidation of NAD-dependent substrates, in agreement with data in the literature [13, 14], but values of V_3 and V_{ph}, on the contrary, were lower. Since V_u under these circumstances did not change significantly, a reserve of respiratory activity ($V_u - V_3$) appeared. which was not present during oxidation of NAD-dependent substrates. In the brain mitochondria of LR animals it was twice as high as in HR animals (Table 1). This suggests the greater potential power of the succinate oxidase pathway of oxidation in the brain of the LR animals, which can be used as a compensatory pathway when activity of the NADH-oxidase pathway is suppressed or depressed. This also is in agreement with our previous data obtained on brain slices, showing that in acute hypoxia, leading to suppression of NAD-dependent oxidation, activation of the succinate oxidase pathway in the brain of LR animals is more strongly expressed than in HR rats [4, 8].

Inhibitory analysis revealed a number of basically new data on the general principles of interaction between metabolic flows in the substrate region of the respiratory chain in the brain, playing a regulatory role in oxidative phosphorylation. In fact it has been found that suppression of the succinate oxidase pathway by malonate in mitochondria oxidizing NAD-dependent substrates (glutamate with malate) did not affect V_2, V_4, or V_u, but depressed V_3 and, as a result, RC_L and RC_C, and also slightly increased the ADP/O ratio from 2.6 to 2.7 (Table 1). Consequently, oxidation of

endogenous succinate limits the process of ATP formation linked with oxidation of NAD-dependent substrates, and thereby reduces the efficiency of phosphorylation in this case.

Meanwhile, in brain mitochondria oxidizing exogenous succinate, the specific inhibitor of the NADH-oxidase pathway, rotenone, did not significantly affect the absolute values of the different parameters of oxidative phosphorylation. However, in its presence V_u was reduced, the reserves of respiratory activity disappeared, and the ADP/O ratio fell a little (from 1.7 to 1.5). This indicates that during oxidation of exogenous succinate, a small flow of endogenous NAD-dependent substrates was preserved, and maintained activity of the NADH-oxidase pathway, permitting a higher degree of energization of the mitochondria and efficiency of oxidative phosphorylation.

Direct confirmation of this conclusion is given by data obtained on mitochondria oxidizing succinate in the presence of glutamate (2 mM). Under these conditions glutamate did not significantly change values of V_2, V_4, and V_u characteristic of succinate, but it did increase the velocity of respiration of brain mitochondria of the HR and LR animals in the active state and, consequently, it changed the values of RC_L and RC_C, by 1.3 and 1.5 times respectively (Table 1). These effects of glutamate were not realized after administration of rotenone. Consequently glutamate, by activating the NADH-oxidase pathway, promoted activation of succinate dehydrogenase, and thereby increased the degree of energization of the mitochondria.

Preservation of activity of the NADH-oxidase pathway during oxidation of succinate and of the succinate oxidase pathway during oxidation of exogenous NAD-dependent substrates, and also their undoubted mutually corrective influence on the process of oxidative phosphorylation, are evidence of the complex interaction of these two pathways of oxidation of energy substrates in the respiratory chain of brain mitochondria, and under conditions in vivo this can be used by the cell as a mechanism of regulation of succinate dehydrogenase activity. Testing this hypothesis showed that under certain conditions mitochondria can in fact "choose" their oxidation substrate. For instance, during utilization of a mixture of substrates (succinate, glutamate, malate) the values of V_2 and V_4 were virtually identical for brain mitochondria of HR and LR animals, and did not differ significantly from the corresponding values during oxidation of a single substrate alone (Table 1). However, V_3, V_u, and V_{ph} were the same (or even greater) both during oxidation of glutamate with malate, and the phosphorylation time was significantly shorter than during oxidation by the mitochondria both of NAD-dependent substrates and, in particular, of succinate. Hence it follows that the succinate oxidase pathway of oxidation is utilized in the brain mitochondria predominantly in Chance's states 2 and 4, whereas in the phosphorylating state the role of NAD-dependent oxidation becomes more important. This not only is in complete agreement with data obtained by Singer and co-workers [18], but it also supplements them. These workers showed that a switch from oxidation of succinate to oxidation of NAD-dependent substrates takes place in mitochondria in the active state, and is connected with the need to increase the rate of ATP synthesis.

Additional proof of the possibility that such a mechanism is in fact realized is given by experiments with inhibition of one oxidation pathway. Inhibition of the succinate oxidase pathway, by malonate, for example, in mitochondria oxidizing a mixture of substrates, reveals the particular features of oxidation of NAD-dependent substrates immediately (Table 1): V_2 and V_4 are diminished by half, ADP/O is increased to 2.9-3.0. However, under these same conditions, V_3, V_4, and V_{ph} were reduced compared with their values during oxidation both of NAD-dependent substrates and of succinate, confirming that activity of the succinate oxidase pathway is essential for activation of the NADH-oxidase pathway during the period of phosphorylation.

Meanwhile rotenone, which inhibits the NAD-dependent pathway and must apparently promote manifestation of succinate oxidase oxidation, reduced all parameters characterizing oxidative phosphorylation, and actually to lower values than during oxidation of exogenous succinate alone. Under these conditions reserves of respiratory activity ($V_u - V_3$) were absent. Consequently, to maintain optimal conditions for succinate oxidation, the present of NADH-oxidase activity is in fact necessary.

Thus the characteristics of function of the NAD-dependent and succinate oxidase regions of the respiratory chain in brain mitochondria of HR and LR animals, revealed by this investigation, point to the existence of a fine regulatory mechanism, which in the intact cell can exercise a corrective influence, through interaction of metabolic flows, on the processes of oxidation phosphorylation and may be responsible for differences in the sensitivity of the brain of these animals to hypoxia.

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