7. B. Halliwell and J. M. Gutteridge, Trends Biochem. Sci., 11, 372 (1986).
8. C. F. Nathan and S. Tsunawaki, Biochemistry of Macrophages, London (1986), pp. 211-230.
9. R. J. Smith, S. C. Speziale, and B. J. Bowman, Biochem. Biophys. Res. Commun., 130, No. 3, 1233 (1985).
10. M. Wasil, B. Halliwell, D. C. Hutchison, and A. Baum, Biochem. J., 243, No. 1, 219 (1987).
11. D. D. Wayner, G. W. Burton, K. U. Ingold, and S. J. Locke, FEBS Lett., 187, 33 (1985).
12. S. J. Weiss, Immunology of Inflammation, Vol. 4, Amsterdam (1983), pp. 37-75.
13. C. C. Winterbourn, Biochim. Biophys. Acta, 840, 204 (1985).

EXTERNAL OXIDATION PATHWAY IN NERVE TISSUE

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UDC 577.3+577.121:615.217:615.272

KEY WORDS: external oxidation pathway; brain; hypoxia; respiratory chain.

Besides the main respiratory chain, located in the inner membrane, intact mitochondria of the liver and heart are also known to have an additional electron-transport system for oxidation of extramitochondrial NADH, including flavine-containing NADH-cytochrome c-reductase of the outer membrane, and cytochrome b_5 and the labile fraction of cytochrome c, located in the inter membranous space. This redox chain shunts the flow electrons to the cytochrome oxidase of the inner mitochondrial membrane. The external oxidation pathway of NADH is resistant to amobarbital and antimycin A but sensitive to KCN and to low concentrations of mersalyl; it is activated under conditions leading to swelling and approximation of the membranes, and has been identified not only in the liver and heart, but also in mitochondria of skeletal muscles and in microorganisms, yeasts, and molds [3, 5-7, 9-14]. However, it is not yet clear whether this pathway functions in the intact mammalian cell.

The aim of this investigation was to study the presence and role of the external oxidation pathway in brain tissue under different conditions of oxygenation.

EXPERIMENTAL METHOD

Experiments were carried out on brain sections from noninbred male albino rats weighing 160-200 g, divided beforehand into those with high (HR) and low (LR) resistance to hypoxia. Sections were cut by the standard method on a microtome. Their respiration rate was determined polarographically [2]. To assess the relative contribution of the external oxidation pathway (EOP) to total respiration, mersalyl (10^{-5} M), a specific inhibitor of this pathway, was used. The concentration of the inhibitor was chosen on the basis of data in the literature [6, 13]. Our results obtained on isolated brain mitochondria are evidence that this concentration of mersalyl has no significant effect on the basic respiratory chain. Higher concentrations of mersalyl (10^{-4} M) inhibited mitochondrial respiration. The effect of inhibitors of various regions of the basic respiratory chain (amobarbital, malonate, antimycin A, KCN) on the mersalyl-sensitive component of respiration (MSR) of the brain preparations was estimated on the basis of its change under the influence of these inhibitors.

EXPERIMENTAL RESULTS

MSR in brain slices oxidizing glucose in carbogen-containing medium ("normoxic" conditions) accounted for 20-25% of the total tissue O_2 consumption (Table 1). The MSR of the brain was resistant to amytal and antimycin A and virtually completely inhibited in the presence of KCN (Table 1). This is evidence that the brain contains and EOP characterized by

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 431-433, April, 1989. Original article submitted March 26, 1988.

 0007-4888/89/0004-0482$12.50

TABLE 1. MSR of Brain Tissue Preparations Oxidizing Glucose (10 mM) and Succinate (10 mM) in the Presence of Various Inhibitors (pO_2 = 100%; M ± m)

Substrate	Inhibitor	MSR	
		nmoles O_2/mg net weight of tissue per min	% of V_{orig}
Glucose	Control (glucose)	0,25±0,044 (16)	21±3,8
	Amobarbital (1 mM)	0,24±0,044 (11)	20±3,7
	Antimycin A (20 μM)	0,42±0,048 (7)	35±4,1
	KCN (1 mM)	0,024±0,012 (5)	2±1,2
	Malonate (40 mM)	0,34±0,090 (5)	28±0,2
Succinate	Control (succinate)	0,38±0,027 (10)	31±3,1
	Amobarbital (1 mM)	0,12±0,037 (5)	10±2,0
	Malonate (40 mM)	0,19±0,014 (8)	16±1,2
	Amobarbital 91 mM)+ malonate (40 mM)	0,094±0,046 (5)	8±4,4
	Antimycin A (20 μm)	0,23±0,025 (5)	19±1,8

Legend. Here and in Table 2: number of experiments shown in parentheses.

TABLE 2. MSR of Brain Tissue Preparations Oxidizing Glucose (10 mM) and Succinate (10 mM) in the Presence of Different Inhibitors (pO_2 = 25%; M ± m)

Substrate	Inhibitor	MSR	
		nmoles O_2/mg net weight of tissue/min	% of V_{orig}
Glucose	Control (glucose)	0,13±0,010 (54)	21±1,4
	Amobarbital (1 mM)	0,11±0,013 (29)	18±2,8
	Antimycin A (20 μM)	0,15±0,017 (13)	24±1,5
	KCN (1 mM)	0,013±0,005 (11)	2±0,8
	Malonate (40 mM)	0,08±0,004 (11)	12±2,2
	Amobarbital (1 mM) + malonate (40 mM)	0,03±0,004 (8)	5±1,1
Succinate	Control (succinate)	0,20±0,026 (22)	32±3,6
	Amobarbital (1 mM)	0,14±0,021 (12)	22±3,7
	Malonate (40 mM)	0,15±0,012 (12)	23±1,8
	Amobarbital (1 mM) + malonate (40 mM)	0,013±0,005 (12)	2±1,0
	Antimycin A (20 μM)	0,14±0,012 (11)	22±1,8

equal rates of oxygen consumption in HR and LR animals. Antimycin A increased MSR by 1.7 times. Consequently, inhibition of electron transport in the cytochrome b-cytochrome g_1 region intensified the flow of reducing equivalents along EOP. This suggests that the substrate region of the respiratory chain is their supplier.

Succinate had clear advantages for maintaining activity of the EOP. In its presence, this activity was increased by 1.5 times (Table 1). However, if succinate was added after amobarbital, inhibition of EOP and not its increased activity was observed (Table 1). Consequently, reversed electron transfer from succinate, increasing the pool of mitochondrial NADH, promoted oxidation of NADH along the mersalyl-sensitive pathway.

This hypothesis was confirmed by the following facts (Table 1): 1) malonate, which inhibits oxidation of succinate, reduced MSR by half; 2) amobarbital, which interrupts the pathway for reverse electron transfer during oxidation of succinate, reduced MSR by two-thirds; 3) in the presence of antimycin A and succinate, energy-dependent electron transfer was reduced, and EOP was reduced by 40%; 4) if simultaneously the pathway for reverse electron transport was closed by amobarbital and succinate dehydrogenase was inhibited by malonate, EOP was reduced by three-quarters in the presence of succinate. Thus reversed electron transport during oxidation of succinate may be the source of reducing equivalents for EOP in brain tissue. This fact becomes understandable in the light of data in [8], showing that

succinate facilitates malate generation and its transport from mitochondria into cytosol, and the oxidation of the mitochondrial NADH associated with this. Meanwhile, malate is known to be able to reduce cytochrome b_5 of the EOP and thus to activate the latter [3].

Under hypoxic conditions (25% O_2) during oxidation of glucose by brain slices MSR was reduced almost by half (Table 2). This can be understood because, despite swelling of the mitochondria, which could promote activation of EOP, in this case oxidation of the substrates along the NAD-dependent pathway is limited [1, 4], possibly due to a deficiency of mitochondrial NADH. Otherwise during hypoxia, the circumstances characteristic of EOP described above were preserved. MSR of brain preparations, resistant to amobarbital and antimycin A, was completely inhibited by KCN (Table 2). Antimycin A strengthened this pathway, but by a lesser degree (than in "normoxia"), further confirmation of a deficiency of mitochondrial NADH (Table 2). Inhibition of oxidation of the endogenous succinate by malonate inhibited MSR by half. Addition of exogenous succinate under hypoxic conditions strengthened EOP, although it did not bring it up to the "normoxic" values (Table 2). Consequently, when oxidation of NAD-dependent substrates was limited, reversed electron transport, on account of which mitochondrial NADH is reduced and MSR activated, was possible. Naturally malonate and antimycin A reduced MSR of the brain a little, during oxidation of succinate, whereas the combined use of malonate and amobarbital suppressed EOP virtually completely (Table 2).

The principles governing function of EOP in the brain tissue were the same in both HR and LR animals, i.e., this pathway is not involved in the formation of the individual resistance of the brain to oxygen deficiency. We likewise were unable to discover any significant differences, in the values of MSR in animals adapted and not adapted to hypoxia. However, an increase in MSR in the presence of amobarbital in the brain of the adapted LR animals must be noted, which was not found in the unadapted rats and was evidently connected with enhancement of the role of the NAD-dependent oxidation pathway, established by the writers previously, in the main respiratory chain after adaptation [4]. Limitation of this pathway by amobarbital naturally led to alternative strengthening of EOP.

The presence of an amobarbital- and antimycin-insensitive, and also cyanide- and mersalyl-sensitive external oxidation pathway, which can utilize mitochondrial NADH, and which functions parallel to the main respiratory chain and can reduce cytochrome oxidase during inhibition of the first and third enzyme complexes, i.e., in a situation developing in acute hypoxia, was thus demonstrated in brain tissue, confirming the view expressed by the writers previously that cytochrome oxidase is not the limiting region of the respiratory chain in hypoxia [1]. With a reduction of the electron flow in the main respiratory chain, its activity can evidently be maintained by alternative flows, including EOP.

LITERATURE CITED

1. L. D. Luk'yanova, B. S. Balmukhanov, and A. T. Ugolev, Oxygen-Dependent Processes in the Cell and Its Functional State [in Russian], Moscow (1982).
2. L. D. Luk'yanova, Metabolic Regulation of the Physiological State [in Russian], Pushchino (1984), pp. 23-24.
3. V. P. Skulachev, Accumulation of Energy in the Cell [in Russian], Moscow (1969).
4. G. N. Chernobaeva, "Features of regulation of brain oxidative metabolism in animals differing in individual sensitivity to oxygen deficiency," Author's Abstract of Dissertation for the Degree of Candidate of Biological Sciences [in Russian], Moscow (1985).
5. P. Bernardi and G. F. Azzone, J. Biol. Chem., 256, 7187 (1981).
6. A. De Santis and B. A. Melandri, Arch. Biochem. Biophys., 232, 354 (1984).
7. L. Ernster, G. Dallner, and G. F. Azzone, J. Biol. Chem., 238, 1124 (1963).
8. W. D. Holtzclaw, R. G. Thurman, and F. C. Kauffman, Arch. Biochem. Biophys., 22, 345 (1984).
9. A. L. Lehninger, J. Biol. Chem., 190, 345 (1951).
10. E. N. Mokhova, V. P. Skulachev, and I. V. Zhigacheva, Biochim. Biophys. Acta, 501, 415 (1977).
11. D. G. Nicholls, Eur, J. Biochem., 62, 223 (1976).
12. G. L. Sottocasa, B. Kuylenstierna, L. Ernster, et al., J. Cell Biol., 32, 415 (1967).
13. A. Szczesna-Kaczmarek, D. Litwinska, and J. Popinigis, Int. J. Biochem., 16, 1231 (1984).
14. A. A. Yasaitis and Z. J. Krivickiene, First European Bioenergetic Conference: Reports, Bologna (1980), pp. 111-112.