Effect of a Phospholipid Hepatoprotector on Bioenergetics of the Brain in Experimental Inhibition of β-Oxidation of Fatty Acids

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Experimental encephalopathy induced by intoxication with 4-pentenoic acid, an inhibitor of β-oxidation of fatty acids, is accompanied by suppression of succinate- and NAD-dependent oxidative pathways in brain mitochondria, uncoupling of oxidative phosphorylation, and activation of LPO. Deenergization of mitochondria and LPO progressed 14 days after discontinuation of 4-pentenoic acid injections. In experimental encephalopathy, hepatoprotective agent Eplir containing essential phospholipids improves coupling of oxidative phosphorylation and energization in mitochondria and inhibits LPO.

Key Words: *essential phospholipids; mitochondria; brain; inhibition of β-oxidation of fatty acid*

Fatty acid metabolism is disturbed in alcohol- and drug-induced chronic hepatitis, congenital and acquired enzymopathies, and Reye's syndrome developing in children with viral infections treated with acetylsalicylic acid [2,6,7]. Inhibition of fatty acid β-oxidation (FAO) in the liver is accompanied by intensive release of long-chain and dicarboxylic fatty acids into the blood and by disturbances in ammonia, phenol, and bilirubin detoxification. These toxic substances cause severe encephalopathy [8]. Drug therapy of this encephalopathy includes hepatoprotective substances, which improve metabolic processes in the liver and attenuate the damaging effects of endogenous toxins on the brain [1].

Here we studied the effect of phospholipid hepatoprotector Eplir on bioenergetics of the brain in experimental encephalopathy caused by injection of FAO inhibitor 4-pentenoic acid. Eplir containing phosphatidylcholine, phosphatidylethanolamine, sulfolipids, and carotenoids, supplies phospholipids for replacement of damaged molecules in cell membranes and produces a pronounced antioxidant effect [4].

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MATERIALS AND METHODS

Experiments were carried out on 65 albino male rats weighing 180-200 g maintained under standard vivarium conditions with free access to food and water. For FAO inhibition the animals were intraperitoneally injected with 4-pentenoic acid (ISN, 20 mg/kg daily for 7 days) [9]. Eplir in an effective therapeutic dose of 30 mg/kg was administered into the stomach in 1% starch suspension starting from day 8 of the experiment and for the subsequent 14 days. Controls received starch suspension. The rats were decapitated under ether narcosis 12 h after the last dose of Eplir.

Functional state of mitochondria (MC) in brain homogenate was studied polarographically (RA-2 polarograph) using a Clark electrode made in our laboratory by the rate of O_2 consumption in different metabolic states after Chanse [5]. We mea-

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sured the rate of oxygen consumption by MC before (V_{4r}), during (V_3), and after (V_{4o}) the cycle of phosphorylation of added ADP (0.1 mmol) during oxidation of endogenous substrates, flavin-dependent substrate succinic acid (SA, 1 mM) and NAD-dependent substrates malate and glutamate (3 mM each) and the time of phosphorylation of added ADP (T_p). For evaluation of the energetic status of MC, we calculated the ADP/O ratio (coefficient of oxidative phosphorylation coupling). The contribution of oxidation of endogenous SA during oxidation of NAD-dependent substrates by mitochondria was determined by adding SDH inhibitor malonate (2 mM) to the incubation medium. LPO activity was evaluated by the rate of spontaneous and ascorbate-dependent MDA formation, content of conjugated dienes and Schiff bases [3].

The results were processed statistically using the method of paired comparisons by Wilcoxon—Mann—Whitney test, the probability of erroneous conclusion did not exceed 5% ($p<0.05$).

RESULTS

Acute period of experimental encephalopathy caused by FAO inhibition was characterized by decreased rates of respiration in all metabolic states during oxidation of endogenous substrates and exogenous SA and longer time and decreased coupling of phosphorylation of added ADP. The decrease in oxidative phosphorylation coupling was accompanied by partial loss of energetic respiration control. During utilization of endogenous substrates this manifested in an increase in the rate of MC respiration after a cycle of phosphorylation of added ADP ($V_{4o}>V_{4r}$). Energization of MC under the effect of SA restored the energetic respiration control ($V_{4o}=V_{4r}$) and leveled the decrease in oxidative phosphorylation coupling to 11% compared to 54% during utilization of endogenous substrates (Table 1).

Oxidation of NAD-dependent substrates under conditions of FAO inhibition in rat brain was characterized by decreased rates of respiration and phosphorylation and uncoupling of oxidative phosphorylation. The decrease in ADP/O ratio was more pronounced than during succinate oxidation (by 55 and 11%, respectively), signs of loss of energetic respiratory control appeared ($V_{4o}>V_{4r}$). These findings suggest that experimental encephalopathy is associated with primary disturbances in most active pathway of substrate utilization related to the formation and oxidation of endogenous SA (rapid metabolic cluster) [5]. Addition of SDH inhibitor malonate to brain MC incubation medium confirmed inhibition of succinate-dependent energy production: against the background of insignificant suppression of respiration and phosphorylation, oxidative phosphorylation coupling decreased only by 30%, while during utilization of NAD-dependent substrates and formation of endogenous SA in the Krebs cycle (oxidation in the absence of malonate on the incubation medium) this parameter decreased by 55%.

LPO in the brain tissue increased. Accumulation of underoxidized products and decreased energy supply accelerated ascorbate-dependent and spontaneous MDA generation by 2.1-2.3-times; the content of conjugated dienes and Schiff bases increased by 2.1 and 1.3 times, respectively, compared to the corresponding parameters in the intact group (Table 1). Changes in the metabolic state of rat brain after FAO inhibition manifested in suppression of rat locomotor activity, the animals looked sick and untidy.

Thus, experimental encephalopathy caused by FAO inhibition is accompanied by deceleration of substrate oxidation, uncoupling of oxidative phosphorylation, appearance of signs of deenergization, and exhaustion of substrate pools in brain mitochondria against the background of LPO activation. These changes primarily involve succinate-dependent energy production, which leads to inhibition of reactions of rapid metabolic cluster and restricts the adaptive capacities of the energy supply system [5,8]. These functional changes in MC are reversible (response to SA is preserved) and can be compensated after their energization.

The subjective state of experimental animals worsened and pathological changes in the brain tissues were augmented by the 14th day after 4-pentenoic acid withdrawal. Production of primary and secondary LPO products considerably increased, MC respiratory activity during oxidation of flavin- and NAD-dependent substrates was suppressed to a greater extent compared to acute period of experimental encephalopathy, uncoupling of oxidative phosphorylation progressed (ADP/O ratio decreased, T_p increased). It is evident, that new MC (MC renewal takes a week) gets into metabolic conditions disturbing oxidative phosphorylation due to accumulation of free fatty acids as a result of FAO inhibition. Fatty acids are known to uncouple oxidative phosphorylation [2]. Disturbed electron transport in the respiratory chain leads to generation of free radicals, which is accompanied by accumulation of LPO products.

Two-week therapy with essential phospholipids, Eplir components, considerably improved the subjective state of experimental animals; they became more tidy and active. Evaluation of the state

TABLE 1. Effect of Rplir on Oxidative Phosphorylation and LPO in Brain Homogenate against the Background of Experimental FAO Inhibition with 4-Pentenoic Acid ($M\pm m$; n=10)

Parameter	Intact animals	FAO inhibition, day		Eplir+4-pentenoic acid
		7	14	
Oxidation of endogenous substrates				
V_{4r}, nanoatom O_2/min/mg MC protein	38.0±1.7	25.8±0.8[1]	12.4±0.3[1,2]	22.5±0.2[1-3]
V_3, nanoatom O_2/min/mg MC protein	52.7±0.6	45.6±2.2[1]	41.6±0.5[1,2]	51.9±0.4[1,3]
V_{4o}, nanoatom O_2/min/mg MC protein	38.4±1.1	31.4±2.9[1]	13.8±0.8[1,2]	34.7±2.4[2,3]
ADP/O	1.65±0.02	0.90±0.40[1]	0.90±0.10[1]	1.50±0.10[1-3]
T_p, min/mg MC protein	1.20±1.10	2.30±0.30[1]	2.80±0.10[1,2]	0.90±0.10[2,3]
SA oxidation				
V_{4r}, nanoatom O_2/min/mg MC protein	37.9±0.3	28.9±0.8[1]	16.5±0.4[1,2]	26.2±1.2[1,3]
V_3, nanoatom O_2/min/mg MC protein	91.7±3.7	77.3±6.5[1]	32.1±1.6[1,2]	66.7±0.8[1-3]
V_{4o}, nanoatom O_2/min/mg MC protein	36.3±1.3	25.5±1.9[1]	15.3±0.5[1,2]	38.5±2.4[1-3]
ADP/O	2.10±0.20	1.87±0.21[1]	1.35±0.03[1,2]	2.10±0.20[3]
T_p, min/mg MC protein	0.80±0.02	1.63±0.09[1]	1.60±0.02[1,2]	0.80±0.02[3]
Malate+glutamate oxidation				
V_{4r}, nanoatom O_2/min/mg MC protein	29.2±1.9	24.8±0.6[1]	16.1±0.9[1,2]	41.7±2.1[1-3]
V_3, nanoatom O_2/min/mg MC protein	70.6±2.6	53.7±4.8[1]	28.1±0.5[1,2]	65.4±3.7[1-3]
V_{4o}, nanoatom O_2/min/mg MC protein	29.4±0.4	30.1±1.3	13.9±0.1[1,2]	43.3±2.5[1-3]
ADP/O	2.02±0.07	1.42±0.40[1]	1.27±0.05[1,2]	1.80±0.08[1-3]
T_p, min/mg MC protein	0.70±0.05	0.80±0.02	1.40±0.14[1,2]	0.80±0.04[3]
LPO parameters				
MDA, nmol/mg protein/min				
spontaneous	0.13±0.01	0.28±0.02[1]	0.36±0.04[1,2]	0.21±0.05[1-3]
ascorbate-dependent	0.25±0.03	0.58±0.01[1]	0.98±0.05[1,2]	0.48±0.01[1-3]
Conjugated dienes, U/mg lipids	0.24±0.03	0.51±0.07[1]	1.15±0.09[1,2]	0.55±0.07[1-3]
Schiff bases, U/mg lipids	1.85±0.10	2.38±0.12[1]	2.75±0.10[1,2]	2.19±0.09[1-3]

Note. p<0.05 compared to [1]intact animals, [2]FAO disturbances for 7 days, [3]14 days.

of brain MC revealed shifts towards normalization of respiratory activity and oxidative phosphorylation during oxidation of both NAD-dependent substrates and SA. The signs of improvement were more pronounced, when SA was used as the oxidation substrate (normalization of ADP/O ratio), which attested to the integrity of the inner MC membrane. Abolition of SDH inhibition probably suggests recovery of the energetic potential of MC and components of antioxidant defense system (pyridine nucleotide, glutathione). This is confirmed by pronounced decrease in the concentration of primary and secondary LPO products in the brain tissue (Table 1).

Thus, essential phospholipids of Eplir as effective antioxidants attenuate deenergization of brain MC caused by FAO inhibition, restore the rate of utilization of Krebs cycle substrates and oxidative phosphorylation coupling, normalize activity of rapid metabolic cluster, and inhibit LPO. It should be noted, that Eplir phospholipids produce their therapeutic effect under conditions of altered lipid metabolism, which attests to the possibility of using this drug for the treatment of states characterized by disturbed FAO.

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