Effect of Doxorubicin on Oxidative Phosphorylation in Brain Mitochondria

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In *in vitro* experiments, antineoplastic anthracycline antibiotic doxorubicine caused deenergization of rat brain mitochondria due to oxidation-phosphorylation uncoupling and inhibition of succinate oxidase system. Reduced production and oxidation of succinic acid is the most early effect of doxorubicin on mitochondrial oxidation.

Key Words: *brain mitochondria; doxorubicin; transamination*

Therapeutic and side effects of antineoplastic anthracycline antibiotics depend on their interaction with the energy production system, in particular, on their accumulation in cardiolipins of inner mitochondrial membrane [4]. The data on the mechanisms underlying disturbances energy metabolism induced by anthracycline antibiotics are contradictory [1], which complicates their pharmacological evaluation. We examined direct action of antineoplastic anthracycline antibiotic doxorubicin on the energy production system of rat brain mitochondria (MC) using the methods, which allowed to reveal the system state by MC reaction [2].

MATERIALS AND METHODS

Experiments were carried out on 2-month-old male Wistar rats (n=20) weighing 180-200 g (Rassvet Breeding Center, Tomsk). After transportation the animals were adapted for laboratory conditions during 2 weeks. Before and during the experiments the rats were kept in a vivarium in standard plastic cages (no more than 15 rats in each) at 20-25°C, humidity below 50%, 8:10 air efflux to influx ratio (ventilation volume), and day-night illumination.

The animals were decapitated under ether anesthesia. The brain was placed for 8 min into cold (0°C) isolation medium: 120 mM KCl, 10 mM HEPES, 0.2 mM EDTA (pH 7.2). Cold brain was freed from meninges and vascular plexuses and homogenized in the same medium for 1 min in a Teflon-plastic homogenizer (cooling jacket) at 1000 rpm. The homogenate was stored at 0°C and used within 40 min. Doxorubicin (Farmitalia Carlo Erba) was added to the isolation medium before homogenization to a final concentration of 0.6 mM. Respiratory activity of MC was measured polarographically by the rate of oxygen consumption in different metabolic states [6]. The following oxidation substrates were used: succinic acid (SA; 0.5, 1, and 2 mM), mixture of NAD-dependent substrates malate (3 mM) and glutamate (3 mM), mixture of succinate dehydrogenase (SDH) activators α-glycerophosphate (1.5 mM) and β-hydroxybutyrate (1.5 mM), SDH inhibitor malonate (2 mM), and transaminase inhibitor aminooxyacetate (AOA; 0.5 mM). ADP was added to incubation medium (120 mM KCl, 10 mM HEPES, 2 mM KH_2PO_4, 0.2 mM EDTA, pH 7.2) to a final concentration of 88 μM.

The results were processed by Student's t test for dependent variables.

RESULTS

In vitro experiments on the oxidation of 1 mM succinate by rat brain MC showed that doxorubicin increased MC respiration rate to ADP phosphorylation cycle (V_{4p}), 1.4-fold decreased the time of transition to

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a slow phase of phosphorylation respiration rate (V_3), and decreased ADP/O. Biphasic V_3 probably reflects SDH inhibition by oxaloacetate. An increase in substrate concentration to 2 mM eliminated SDH inhibition, in particular, the respiration rate in all metabolic states increased, biphasic phosphorylation respiration rate disappeared, and ADP/O returned to normal in both groups of MC. Similar effect was observed during oxidation of low SA concentration in the presence of SDH activators α-glycerophosphate and β-hydroxybutyrate (Table 1).

Doxorubicin markedly reduced V_3 during oxidation of NAD-dependent substrates (malate+glutamate) by MC. Malonate decreased V_3 in intact MC, thus levelling the differences between the control and experimental data. AOA produced a depriming effect on both groups of MC, but the difference in V_3 between the control and doxorubicin-treated groups persisted. SDH activators applied against the background of AOA increased respiration rate of MC of both groups and eliminated the differences between them (Fig. 1).

The revealed doxorubicin-induced changes in the MC oxidation system are probably connected with a low-energy shift [2] due to uncoupling of oxidative-phosphorylation. This is confirmed by a decrease in ADP/O, V_{4p}, and the duration of rapid V_3 phase during oxidation of 1 mM SA. It is likely that antioxidant system is unable to protect MC against free radicals generated by doxorubicin under these conditions [7,8], while saturation with substrate (2 mM SA) protects MC from deenergization and prevents SDH inhibition during phosphorylation cycle (monophasic V_3). Elimination of the low-energy shift by SDH activators also confirms this assumption. During oxidation of 2 mM SA, prooxidant effect of doxorubicin dominates over its inhibitory action on ATPase and adenylate translocase activity, which is confirmed by the absence of V_3 changes [1,5,11].

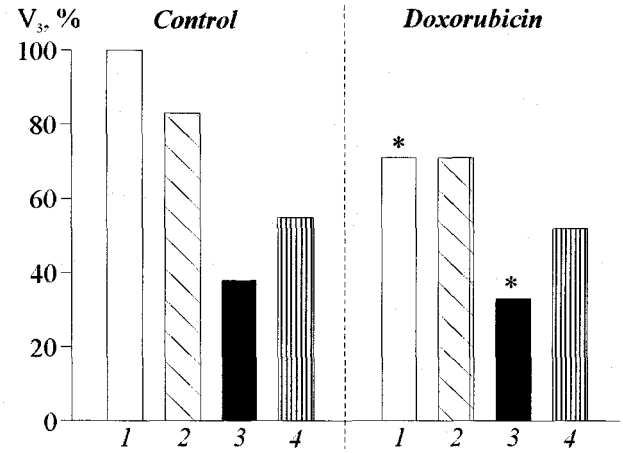

Fig. 1. Effect on doxorubicin of phosphorylation respiration rate in brain mitochondria during oxidation of NAD-dependent substrates. *1*) malate+glutamate; *2*) malate+glutamate+malonate; *3*) malate+glutamate+aminooxyacetate; *4*) malate+glutamate+aminooxyacetate+α-glycerophosphate+β-hydroxybutyrate. *$p<0.05$ compared to the control.

In contrast to previously reported data [9,11] doxorubicin-induced inhibition of malate and glutamate oxidation by MC is not associated with the impairment of NAD-dependent respiration, because SDH inhibitor malonate decreases the levels of V_3 in the control and experimental groups. The fact that AOA decreased V_3 in both groups, but preserved differences in the respiration rate suggests that doxorubicin limits SA production in transamination reactions. AOA in combination with SDH activators increased V_3 in these groups to the same level, which confirms this assumption. It is important to note that rapid metabolic cluster, in particular, endogenous SA production, appears to be vulnerable to prooxidant action of doxorubicin despite preserved oxidation phosphorylation (estimated by MC respiration rate and ADP/O ratio).

Thus, anthracycline antibiotic doxorubicin in a concentration of 0.6 mM on brain MC directly reduces

TABLE 1. Effect of Doxorubicin on Respiration Parameters of Rat Brain MC during Oxidation of SA (*M±m*, *n*=6)

Substrates of oxidation	V_{4p}	V_3	V_{40}	ADP/O	Time of phosphorylation, min
	ng-atom O_2/minxmg protein				
SA, 1 mM	12.1±0.4	22.8±2.4	13.2±0.6	2.24±0.09	1.12±0.13
	13.2±0.4*	21.5±1.5	13.4±1.2	1.78±0.09*	1.34±0.09
SA, 2 mM	12.8±0.8	31.4±1.3	15.1±0.8	1.98±0.09	0.86±0.07
	13.1±0.7	29.0±1.8	14.6±0.5	1.94±0.09	0.90±0.08
SA, 1 mM+α-glycerophosphate+β-hydroxybutyrate	13.6±1.2	28.2±3.1	16.5±1.7	2.0±0.13	1.02±0.16
	13.3±0.8	23.8±1.8	15.7±1.1	1.8±0.07	1.06±0.05

Note. Numerator — control; denominator — experiment; *$p<0.05$ compared to the control. V_{40} — rate of substrate oxidation after phosphorylation.

energy production in the reactions of rapid metabolic MC cluster, causes deenergization of MC due to oxidation-phosphorylation uncoupling and inhibition of SA production in the reactions of glutamate transamination. The damaging effect of doxorubicin inversely correlates with the intensity of energy metabolism in MC.

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