PHARMACOLOGY AND TOXICOLOGY

Effect of Doxorubicin on Energy-Dependent Calcium Transport in Rat Brain Mitochondria *in Vitro*

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Calcium capacity of rat brain mitochondria oxidizing succinate and NAD-dependent substrates was reduced after incubation with doxorubicin as a result of decreased energy production due to inhibition of SDH and transamination processes.

Key Words: *doxorubicin; brain mitochondria; calcium*

Doxorubicin (DR) disturbs calcium metabolism [11, 13,14], inhibits tissue respiration and oxidative phosphorylation, and suppresses activity of succinate dehydrogenase (SDH) complex and NADH-oxidase [8, 9,15]. DR selectively binds to cardiolipins in the inner mitochondrial membrane and prevents the interaction of creatine kinase and adenylate cyclase with adenine nucleotide translocase, which disturbs energy transformation and transport of macroergic phosphates [4,15] and destabilizes Ca^{2+} homeostasis [2]. DR inhibits the symport of phosphates by isolating cardiolipines from other membrane components [3], which also affects Ca^{2+} sequestration by mitochondria (MC). Thus, DR exerts multiple effects on mitochondrial function. It should be noted that these changes are produced in different organs by different DR concentrations. Therefore, it was of interest to study the effect of the lowest toxic concentrations of DR on the parameters of energy-dependent Ca^{2+} transport taking into account the state of mitochondrial membranes, membrane-bound enzymes, and the system of oxidative phosphorylation.

MATERIALS AND METHODS

The study was carried out on brain MC with cardiolipin-enriched inner membranes. Preliminary experiments showed that the brain concentration of DR after its peripheral administration in a maximum permissible dose (4 mg/kg for male rats) is about 0.6 mM. This concentration corresponds to the minimum effective concentration of DR modulating oxidative phosphorylation. Therefore, this concentration was used in the study of the DR effect on energy metabolism and energy-dependent Ca^{2+}-transport in rat brain MC.

The experiments were carried out on 2-month-old male Wistar rats (180-200 g) from the Collection of the Institute of Experimental Biomedical Modeling, Tomsk Research Center. The rats were decapitated under ether anesthesia, the brains were removed and placed in ice-cold isolation medium for 8 min, then released from external membranes and vascular plexuses and homogenized for 1 min in a teflon-glass homogenizer at 1000 rpm. In the experimental group, 0.6 mM DR (Farmitalia Karlo Erba) was added to the isolation medium before homogenization. Energy-dependent Ca^{2+} transport was assessed by measuring the decrease in external Ca^{2+} concentration with an EA-920 ion-selective electrode (Orion Research). Ca^{2+} was introduced into the medium in 10 nmol portions until saturation (release of accumulated Ca^{2+} from MC). Respiratory activity of MC was measured by polarography, oxygen consumption rate was determined in different metabolic states [5]. The following substances were used as the substrates: succinate (0.5, 1, and 2 mM), its combination with SDH activators α-gly-

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cerophosphate and β-hydroxybutyrate (1.5 mM each), the mixture of NAD-dependent substrates (malate and glutamate, 3 mM each), and its combination with SDH inhibitor malonate (2 mM) or transaminase inhibitor aminooxyacetic acid (0.5 mM). The data were analyzed statistically using paired Student's *t* test.

RESULTS

DR increased the rate of MC respiration at rest (*4R*) and decreased the ADP/O ratio during oxidation of 1 mM succinate. These changes were reversed by increasing substrate concentration (2 mM succinate) or by combined application of SDH activators. During oxidation of NAD-dependent substrates, DR reduced the rate of phosphorylating respiration (V_3), however, the SDH inhibitor was effective only in the control group. Aminooxyacetic acid decreased V_3 in both the control and experimental groups, but in the latter this effect was more pronounced. During oxidation of 2 mM succinate or NAD-dependent substrates, DR did not affect phosphorylation time (Tr) and ADP/O ratio. The analysis of Ca^{2+} transport revealed that DR reduced Ca^{2+} capacity of MC during oxidation of succinate (0.5 mM) and NAD-dependent substrates by 42 and 20%, respectively. It was found that Ca^{2+} capacity gradually decreased with time (Table 1), while respiration and oxidative phosphorylation indices remained relatively stable: the only significant change was a decrease in the respiratory rate in state *4R* in the DR-treated group after 60 min (Table 2). In the control group, the Ca^{2+} capacity of MC oxidizing succinate and NAD-dependent substrates decreased by 76% and 37% after 1 h, respectively, in the DR group — by 89% and 34%, respectively.

These data show that the drug produced a minor uncoupling effect on the oxidative phosphorylation system, potentiates SDH inhibition, and inhibits endogenous succinate production. DR in test concentration exerts no inhibitory effects on the respiratory chain and ATP-synthase and does not modulate activity of membrane-bound enzymes (adenylate kinase, creatine kinase, adenine nucleotide translocase) and $H_2PO_4^-/H^+$ symporter providing phosphate transport (no changes in V_3, ADP/O, and Tr). The changes in the respiratory chain and oxydative phosphorylation system were reversible and could result from activation of lipid peroxidation (LPO) [6] rather than from interaction with cardiolipins in MC membrane. The effect of DR on energy production can be explained by modification of SH-groups [12] disturbing the integrity of MC membranes (uncoupling) and inactiva-

TABLE 1. Effect of DR and Time on Calcium Capacity of Brain Mitochondria (nmol Ca/mg protein) Oxidizing Succinate and NAD-Dependent Substrates (*M±m*, *n*=5)

Time, min	Succinate		NAD-dependent substrates	
	control	DR	control	DR
0	8.0±0.8	4.7±0.5*	26.6±2.6	21.3±1.9*
20	4.7±1.2	2.8±0.5	23.5±2.6	21.3±1.9
40	3.3±1.0	1.3±0.6	20.2±2.2	17.7±2.1
60	1.9±1.0+	0.5±0.3+	16.8±1.3+	14.1±2.9+

Note. Here and in Table 2: $p<0.05$: *in comparison with the control; +in comparison with the initial value (0 min).

TABLE 2. Effect of DR on Respiration Rate of Brain Mitochondria (ngatom O_2/min/mg protein) Oxidizing 1 mM Succinate (*M±m*, *n*=5)

Parameters	Control		DR	
	0 min	60 min	0 min	60 min
V_{4R}	12.1±0.4	10.8±1.3	13.2±0.4*	11.4±0.5+
V_3	22.8±2.4	19.0±2.5	21.5±1.5	19.7±1.0
V_{4O}	13.2±0.6	12.7±1.9	13.4±1.2	14.1±1.1
V_3/V_{4R}	1.86±0.15	1.78±0.20	1.60±0.08	1.70±0.05
V_3/V_{4O}	1.08±0.03	1.16±0.05	1.04±0.11	1.22±0.12
ADP/O	2.24±0.09	2.14±0.07	1.78±0.09*	1.96±0.02
Tr	1.12±0.13	1.22±0.15	1.34±0.09	1.22±0.07

ting membrane-bound SDH (SH-groups of creatine kinase are highly sensitive to LPO products in the presence of DR [7]). SDH activation in the presence of higher substrate concentration or specific activators (α-phosphoglycerate and β-hydroxybutyrate) restored activity of respiratory chain, inhibited LPO and reactivated SH-groups of the inner membrane proteins. Thus, the integrity of MC membranes was preserved, when SDH inhibition was reversed with high substrate concentration or enzyme activators. DR-induced inhibition of transamination processes can be attributed to both inactivation of aspartate aminotransferase by LPO products and to reduced activity of glutamate/aspartate antiporter due to reduced inner membrane potential.

The data on the functional state of MC in the presence of DR suggest that the decrease in MC calcium capacity caused by DR is due to inhibition of energy production after SDH inactivation and reduced production of endogenous succinate rather than to disturbed transport of adenine nucleotides and phosphates.

Considerable reduction of calcium capacity without alterations in energy production observed during aging of the homogenate can be explained by the fact that Ca^{2+} transport requires higher activity of system maintaining membrane potential in MC than phosphorylation of added ADP. Therefore, changes occurring in MC during aging are more pronounced under conditions of more intense function, *i.e.* during Ca^{2+} sequestration. The most pronounced reduction of calcium capacity in the DR group (89% *vs.* 76% in the control group during oxidation of succinate) indicate more serious damage to MC membranes which can be attributed to the prooxidant properties of DR.

Thus, DR in a concentration of 0.6 mM destabilized inner MC membranes *in vitro*, thus potentiating the inhibition of SDH and transamination which impaired energy production and disturbed energy-dependent Ca^{2+}-transport.

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