Physicochemical Properties of Membranes and Functional Status of Liver Mitochondria in Rats with an Inherited Capacity for Increased Radical Formation

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Liver mitochondria of inbred W/SSM rats with inherited increased radical formation reveal the following anomalies: inhibition of oxidative phosphorylation, a lowered transmembrane potential, and alterations in protein-lipid interaction. The membrane viscosity and osmotic stability of mitochondria are unaffected.

Key Words: *rats of the W/SSM strain; oxygen radicals; mitochondria; oxidative phosphorylation; membrane structure*

Earlier a series of reports described the creation of a strain of rats with hereditary galactosemia (W/SSM strain) and biochemical, physiological, and morphological peculiarities of these animals were presented [2,3]. W/SSM rats show an increased activity of a carrier of the plasma membrane hexoses [3]. A high intracellular hexose content may cause the oxidation and autooxidation of monosaccharides, accompanied by the production of free radicals [9]. In the tissues of W/SSM rats a life-long increased capacity to generate HO· radicals is observed, possibly forming a basis for increased frequency of tumor development, premature aging, shortened life span, and development of chronic diseases [11].

Active forms of oxygen are known to induce oxidation of the biological membrane-associated proteins and lipids [4,11], which affects their physicochemical properties and leads to alterations of the cell membrane structures. Mitochondria are themselves sources of endogenous oxidizers and are characterized by an increased sensitivity to oxidative stress owing to the deficiency of protective histones and of DNA-repairing systems in their genome. Therefore, disturbances of mitochondrial functions represent one of the factors in the pathogenesis of chronic diseases that develop during a rise in free radical processes [12].

The goal of the present work was a study of the functional status and physicochemical properties of the liver mitochondrial (LM) membranes in inbred W/SSM rats with inherited increased formation of free radicals.

MATERIALS AND METHODS

The work was carried out on 20 male W/SSM rats. Animals 2-3 months old weighing 190-220 g and rats 10-12 months old weighing 300-330 g were used in the experiments. Wistar rats served as the control. LM were isolated from the liver using a routine method of differential centrifugation in the isolating solution containing 250 mM sucrose, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.4. Repeated LM sedimentation was performed in a solution without EDTA. The LM sediment was resuspended in the isolating solution so that the final protein concentration was 60-80 mg/ml. The LM protein content was measured after Lowry [8]. The rate of oxygen uptake was recorded polaro-

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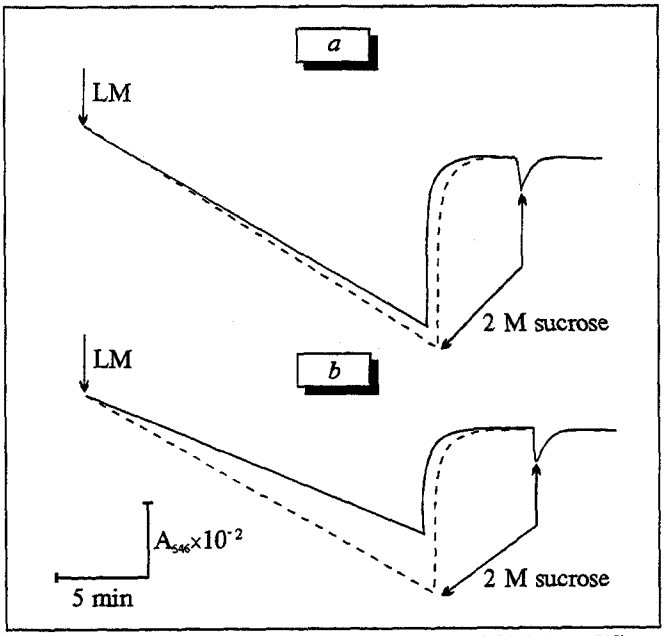

Fig. 1. Comparison of osmotic properties of LM from Wistar and W/SSM rats of different age. *a*) 2–3-month-old rats; *b*) 10–12-month-old rats. Continuous line: Wistar rats; dotted line: W/SSM rats. Composition of incubation medium: 125 mM KCl, 5 mM Tris-HCl (pH 7.4), rotenone in a ratio of 1 μg/mg protein. Additives: 0.5 mg LM, 100 μl of 2 M sucrose per 3-ml cell.

graphically in a thermostatically controlled cell at 25°C using a Clark electrode. LM were incubated in the following solution: 125 mM KCl, 20 mM Tris-HCl (pH 7.4), 5 mM KH_2PO_4, and 1 mM $MgCl_2$. The substrates of oxidation were 10 mM succinate plus rotenone added in a ratio of 2 μg/mg LM protein, or 10 mM glutamate plus 2 mM malate. The following additives were used: 150 μM ADP and 0.5 μM chlorocarbonylcyanide phenylhydrazone. The transmembrane potential $\Delta\psi$ was measured using a fluorescent probe, 4-n(dimethylaminostyryl)-1-methylpyridinium (Zonde) [1], on a Hitachi - MPF-4 spectrofluorimeter. The osmotic stability of LM was assessed by recording their optical properties in a medium comsisting of 125 mM KCl and 5 mM Tris-HCl (pH 7.4) on a Hitachi-556 spectrophotometer at wavelength 546 nm [7].

The viscosity of LM membrane lipids was estimated using a pair of fluorescent probes, pyrene (Serva) and 4-(n-dimethylaminostyryl)-1-dodecylpyridinium (Zonde) [1]. The status of protein-lipid interaction was judged by recording the energy transfer from tryptophan residues of membrane proteins to lipid-localized pyrene [1].

RESULTS

In LM of young W/SSM rats during oxidation of substrates that provide reducing equivalents to complexes I and II of the LM respiratory chain (glutamate plus malate and succinate), lower rates of oxygen uptake in 3-metabolic and dissociated states, as well as a lower degree of respiratory control, are observed when compared with Wistar rats of the same age (Table 1). This provides evidence of the inhibition of LM respiratory chain enzymes in young W/SSM rats. However, the ADP /O_2 ratio does not differ from that in Wistar rats. The

Table 1. Parameters of the Functional Status of LM in Wistar and W/SSM Rats of Different Age ($M\pm m$)

Parameter	2-3 months old		10-12 months old	
	Wistar	W/SSM	Wistar	W/SSM
	Succinate, 10 mM			
V (MC–4)	28.6±2.0	23.0±1.7	25.6±1.5	25.6±1.9
V (MC–3)	127.1±6.5	81.1±8.6*	114.5±9.9	66.3±6.9*
V diss.	187.1±10.8	165.2±12.9	176.5±14.8	128.7±11.7*
Respiratory control	4.34±0.22	3.63±0.11*	4.48±0.2	2.65±0.24*,**
ADP/O_2	1.78±0.05	1.79±0.06	1.73±0.05	1.52±0.04*,**
V phosphorylation	225.0±10.0	157.1±9.5*	193.7±17.3	102.9±12.7*,**
	Glutamate, 10 mM, plus malate, 2 mM			
V (MC–4)	14.7±0.9	13.2±0.9	13.5±0.6	14.5±0.7
V (MC–3)	70.3±2.1	49.9±3.5*	69.9±9.5	43.4±6.1*
V diss.	87.4±2.7	66.2±4.9*	96.7±9.1	59.8±7.1*
Respiratory control	5.00±0.33	3.83±0.12*	5.34±0.47	2.98±0.35*,**
ADP/O_2	2.68±0.06	2.55±0.06	2.51±0.10	2.10±0.09*,**
V phosphorylation	183.5±8.8	131.2±9.7*	166.1±12.3	92.6±10.4*,**
$\Delta\psi$, mV	200.7±5.3	183.6±3.9*	192.1±4.6	177.3±2.9*,**

Note. Here and in Table 2: * – reliable differences ($p<0.01$) between indexes in W/SSM and Wistar rats; ** – reliable differences ($p<0.05$) between indexes in 2–3-month-old and 10–12-month-old W/SSM rats. The age-related differences in Wistar rats are unreliable. V (MC–4): rate of mitochondrial respiration in the 4th metabolic state; V (MC–3): the same in the 3rd metabolic state; V diss.: in the dissociated state. The rate of mitochondrial respiration is expressed in ng O_2/min/mg protein, the rate of phosphorylation in nmol ADP/min/mg protein.

Table 2. Parameters of the Status of LM Membranes in W/SSM and Wistar Rats of Different Age ($M \pm m$)

Parameter	2-3 months old		10-12 months old	
	Wistar	W/SSM	Wistar	W/SSM
Lipid viscosity, poise	0.99±0.17	1.04±0.08	1.43±0.08	1.28±0.11
Protein–lipid interaction, % of proteins that do not make contact with lipids	46.4±1.2	43.1±1.3	47.2±0.9	52.2±1.2*,**

reduced rate of oxidation of substrates present in an active metabolic form leads to a marked drop of the phosphorylation rate in the LM of young W/SSM rats. The inhibition of respiratory chain activity is reflected in the transmembrane potential $\Delta\psi$.

In 10-12-month-old W/SSM rats the alterations in the function of the mitochondrial apparatus are aggravated. The values of respiratory control and of ADP/O_2 are lowered, leading to a marked drop in the phosphorylation rate. The $\Delta\psi$ index is also lowered (Table 1).

The derangements in the functional status of LM in W/SSM rats may be connected with the modification of respiratory chain enzymes, since it is known that the activity of NADH-dehydrogenase, NADH-oxidase, succinate dehydrogenase, and F_0-F_1-ATPase is inhibited by superoxide radicals and by hydroperoxides [13,14]. On the other hand, one of the main targets of HO· radicals is DNA [6]. W/SSM rats, despite stringent inbreeding for more than 40 generations and a high level of homozygosis, show frequent alterations of the nuclear DNA [11]. Mitochondrial DNA, which encodes several polypeptides of the respiratory chain, is more sensitive to oxidants as compared to nuclear DNA [12]. Therefore, the decreased efficacy of respiratory chain functioning in W/SSM rats observed by us may be attributed to oxidative damage of both mitochondrial proteins and DNA.

Among the primary oxidative stress-induced alterations in the mitochondria are a reduction of osmotic stability and an increase in membrane fragility [10]. Our results concerning the optical properties of LM, in particular the equal degree of the sucrose hypertonic solution-induced matrix contraction of W/SSM and Wistar rat mitochondria (Fig. 1), argue against an increased nonspecific permeability of LM inner membranes in W/SSM rats.

The study of LM membrane structure using fluorescent probes failed to reveal reliable differences in the viscosity of membrane lipids between Wistar and W/SSM rats. The only tendency detected was an age-related increase in lipid viscosity in the rats of both strains (Table 2).

The measurements of the efficacy of luminescence-free transfer of excitation energy from tryptophan residues of mitochondrial proteins to the membrane lipid-residing pyrene (Table 2) in young W/SSM rats point to a tendency toward a reduction of the percentage of proteins that do not come in contact with lipids, i.e., those which are separated from lipids by a distance exceeding some critical one, which is equal to 3.6 nm for the tryptophan-pyrene pair [1]. This parameter significantly increases with age, which implies a reduced degree of mitochondrial protein insertion into the lipid bilayer and/or an increased amount of aggregated proteins in the mitochondrial membranes of W/SSM rats. Alteration of the protein-lipid interaction in W/SSM rats is consistent with the ability of free radicals to induce the formation of protein aggregates [5].

Thus, investigation of the membrane physicochemical properties and functional status of LM in W/SSM rats with increased capacity for radical formation revealed marked alterations in oxidative phosphorylation associated with inhibition of the respiratory chain in LM. These results may serve as a basis for further study of W/SSM rats as a model of mitochondrial disorders.

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