Antioxidant System and Energy Provision of the Rat Heart during Aging Depend on Illumination Regimen and Are Resistant to Exogenous Melatonin

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 3, pp. 330-343, March, 2010
Original article submitted June 18, 2009

Activities of antioxidant enzymes, vitamin E level, lactate dehydrogenase isoenzyme spectrum, and effects of melatonin on these parameters were studied in the myocardium of rats aged 6, 12, 18, and 24 months exposed to different illumination regimens. The greatest number of changes was recorded in rats exposed to permanent illumination and light deprivation. Activity of SOD decreased with age, while catalase activity increased. Melatonin treatment did not modify activities of antioxidant enzymes and negligibly modified the level of tocopherol and isoenzyme spectrum of lactate dehydrogenase in rat heart.

Key Words: *antioxidant system; lactate dehydrogenase; melatonin; light regimens; aging*

According to the free-radical theory of aging, oxidative modification of macromolecules, caused by destructive effects of free radicals, including products of metabolism, reactive oxygen species (ROS), underlies this process. The cell antioxidant system (AOS) is responsible for the maintenance of a constant level of ROS concentration. A shift of the prooxidant/antioxidant balance towards excessive production of free radicals leads to a wide spectrum of disorders in vital activities, including heart diseases [6]. In addition, the aging process is associated with significant changes in the energy metabolism in the myocardium. Lactate dehydrogenase (LDH) isoenzymes catalyzing mutual transformations of lactate and pyruvate regulate the redox balance of pyridine nucleotides (indicator of cellular oxidation state).

Melatonin (pineal gland hormone) is characterized by antioxidant and geroprotective effects [1]. The mechanism of its action consists in direct capture of ROS and in inhibition of their generation in the cell, as well as in modulation of activities of antioxidant enzymes via stimulation of gene expression [8,10]. Age-associated involution of the pineal gland is associated with reduction of melatonin concentration in the body. Changes in the length of daylight period lead to either blockade of hormone production (under conditions of permanent illumination) or its hypersecretion (under conditions of light deprivation) [5,9]. Presumably, reduced activity of antioxidant enzymes and enhanced ROS generation during aging can be corrected by exogenous melatonin.

We analyzed changes in the integral parameters of ROS generation and quenching system, enzymatic and nonenzymatic components of AOS, and LDH isoenzyme profile in the hearts of rats exposed to different illumination regimens for a long time and treated with melatonin.

MATERIALS AND METHODS

Experiments were carried out on LIO (Leningrad Institute of Oncology) male and female rats kept on

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0007-4888/10/1493-0354

standard vivarium ration with free access to water. After 1 month, the animals were randomly divided into 4 groups kept under different illumination conditions: light deprivation (DD), fixed regimen (12:12 h light:darkness; LD), permanent light exposure (LL), and natural light (NL), characterized by long daylight period in summer and short in winter. From the age of 4 months, the rats of all groups except DD were divided into 2 subgroups and until the age of 2 years subgroup 1 received melatonin (Sigma) in a dose of 10 mg/liter with drinking water during the dark hours 5 days a week, while subgroup 2 were controls receiving water without melatonin. The rats kept in complete darkness received placebo.

The rats were decapitated at the age of 6, 12, 18, and 24 months and specimens of heart tissue were collected. Activity of SOD was evaluated by modified adrenochromic method, catalase was measured by spectrophotometry by the content of decomposed hydrogen peroxide. The status of the ROS quenching system was evaluated by induced chemiluminescence with two luminophores (luminol and lucigenin) and luminescence activators (hydrogen peroxide and bivalent iron). The concentration of vitamin E was measured by HPLC. The LDH isoenzymes were separated by horizontal enzyme electrophoresis on agar gel plates. The data were processed by methods of variation statistics, the values were compared using nonparametric Mann–Whitney test. The study was carried out with due consideration for the Helsinki Declaration on humane handling of animals.

RESULTS

The level of ROS generation by enzymatic systems and by nonenzymatic modes gradually decreases in the myocardium with aging. This process is paralleled by an increase in the intensity AOS functioning. The work of the enzyme component (antioxidant enzymes) of the ROS quenching system increases in the myocardium of rats exposed to light regimens of all kinds, except fixed illumination (LD), starting from the age of 12 months. Starting from this age, generation of ROS by nonenzymatic systems in the myocardium decreased in rats exposed to fixed illumination and light deprivation, while enzymatic ROS generation decreased under conditions of fixed and permanent illumination. Nonenzymatic generation of ROS in rat heart decreased

TABLE 1. Cardiac SOD Activity in Rats of Different Age under Conditions of Different Light Exposure and Melatonin Treatment

Light exposure	Age of rats, months	SOD, arb. units/g tissue	
		placebo	melatonin
LD	6	152.25±30.55	243.37±65.32
	12	55.37±19.20	63.65±18.13
	18	75.27±2.48	102.95±33.57
	24	89.06±5.65	64.49±13.73
NL	6	211.22±54.81	111.79±25.76
	12	75.9±14.89	60.77±17.43
	18	84.96±23.48	93.59±5.71
	24	80.5±14.11	87.24±11.79
DD	6	241.48±43.82	
	12	40.69±11.41°^	
	18	118.78±55.34	
	24	67.81±13.32^	
LL	6	179.09±25.55	264.67±65.03
	12	86.69±8.82^	58.52±9.08
	18	91.59±11.39^	88.07±7.18
	24	99.06±10.81^	99.85±6.636

Note. Here and in Tables 2, 3: $p<0.05$ compared to: *placebo group of the same age and respective light exposure; +no melatonin, LD mode; ×no melatonin, NL mode; °no melatonin, LL mode; ^6-month animals at similar illumination mode, placebo; ^^12-month animals at similar illumination mode, placebo; ^^^18-month animals at similar illumination mode, placebo.

by the age of 12 months under conditions of natural and permanent illumination and increased in aging (18 month-old) and old (24 months) rats to the level of young (6-month) animals. The changes in ROS generation systems were parallel, while the dynamics of enzymatic and nonenzymatic AOS components was asynchronous because of different light regimens. A drop of SOD activity (the main component of enzymatic AOS) was detected in the myocardium by the age of 12 months (Table 1) in animals exposed to permanent illumination and light deprivation; age-associated changes in tocopherol level in animals exposed to these light regimens manifested later. Activity of another antioxidant enzyme, catalase, increased in rats exposed to permanent illumination starting from the age of 18 months. Presumably, this was due to the fact that the sources of catalase substrates were reactions with various oxidases [4], in addition to superoxide dismutation into hydrogen peroxide.

The impact of light exposure for the content of vitamin E (Table 2) was detected in 18-month animals. Tocopherol level decreased in rats of this age exposed to permanent darkness and fixed illumination, while under conditions of natural illumination it increased in comparison with animals aged 6 and 12 months. Exposure of old (24 months) animals to light deprivation in comparison with other illumination modes led to a 2-fold decrease in vitamin concentration.

Melatonin modifies gene expression and activities of SOD and catalase in mammalian liver, kidneys, and brain [1,11]. In contrast to other organs we studied [2,3], no effect of the hormone on activities of antioxidant enzymes in the myocardium were detected (Table 1). No differences in these parameters were detected even between the groups of rats kept under conditions of permanent illumination (when melatonin synthesis is suppressed) and in complete darkness (with its hyperproduction). The hormone virtually did not modify tocopherol concentrations in the rat heart during all periods of age in all groups kept under conditions of different illumination. Just a slight increase of the vitamin level was observed in the myocardium after its injection to rats exposed to permanent illumination (Table 2).

LDH isoenzymes participate in lactate and pyruvate metabolism and therefore are involved in energy metabolism processes. The synthesis of LDH1 in the hearts of old rats increases in comparison with that in 12-month-old animals under conditions of all studied light regimens except light deprivation (Table 3), while the synthesis of LDH2 increased at natural and permanent illumination. The percent of LDH3 in the myocardium dropped in 24-month rats under

TABLE 2. Vitamin E Content in the Hearts of Rats of Different Age Exposed to Different light Regimens and Melatonin Treatment

Light mode	Age of rats, months	Vitamin E, μg/ml	
		placebo	melatonin
LD	6	1.23±0.19	1.39±0.18
	12	1.77±0.08	1.76±0.04
	18	0.19±0.05^,^^	0.28±0.04
	24	1.43±0.15^^^	1.87±0.31
NL	6	1.24±0.19	2.55±0.63
	12	1.28±0.14	1.31±0.01
	18	3.08±0.87+^,^^	1.57±0.41+
	24	1.63±0z+1	1.62±0.34
DD	6	1.03±0.26	
	12	1.59±0.19	
	18	0.96±0.21+x	
	24	0.58±0.09+xo^^	
LL	6	1.45±0.21	1.38±0.19
	12	1.27±0.04	1.63±0.19*+x
	18	0.46±0.16^,^^	0.36±0.02
	24	1.73±0.34^^^	0.73±0.29

TABLE 3. Effects of Melatonin and Light Exposure Modes on LDH1 Isoenzyme Content in Rat Myocardium during Aging

Light mode	Age of rats, months	LDH1, % of all isoenzymes	
		placebo	melatonin
LD	6	35.55±1.34	32.37±1.05
	12	33.35±1.21	43.15±7.73
	18	33.25±1.96	35.91±2.87
	24	43.69±5.42^^	39.94±1.05
NL	6	35.73±0.89	35.59±1.44
	12	34.01±0.81	27.43±1.61*
	18	36.86±1.19	34.89±1.06
	24	38.53±0.52^,^^	37.04±2.08
DD	6	35.52±0.84	
	12	34.83±1.97°	
	18	36.61±1.24	
	24	46.09±7.83	
LL	6	35.01±1.07	34.93±1.01
	12	28.08±1.28^	32.22±0.68+x
	18	36.81±1.53^^	35.08±1.34
	24	39.71±1.67^^	44.95±7.33

conditions of all the studied illumination regimens, except permanent illumination. These changes attest to predominace of lactate transformation into pyruvate, which can serve as the source for the maintenance of aerobic metabolism at a high level in the hearts of old animals. This can be paralleled by the increase in ROS generation, which, along with reduction of SOD activity in old rats can lead to cardiomyocyte damage and development of heart diseases. Changes in isoenzyme ratio in the myocardium depended on the illumination mode. Exposure of animals to permanent light led to a decrease in relative content of LDH1 in the myocardium of 12-month rats (Table 3). At natural and permanent light, the myocardial level of LDH2 in 18-month rats increased in comparison with animals living under conditions of fixed light/darkness schedule. Injection of melatonin to rats of all groups virtually did not modify isoenzyme spectrum of myocardial LDH in the course of their natural aging.

Hence, activities of SOD and catalase, vitamin E concentration, and LDH isoenzyme spectrum in the hearts of LIO rats depend not only on their age, but are also determined by the illumination mode. AOS of rat heart is less sensitive to melatonin than that of other organs and tissues. Injection of this pineal gland hormone increased its concentration in the hearts of mice, but the level of melatonin was significantly lower than in other organs. Melatonin metabolite 6-hydroxymelatonin sulfate (6-HMS) predominates in the heart. The 6-HMS/melatonin ratio in the heart is 134 and after melatonin injection it drops to 49, while in the liver and kidneys these values are significantly lower [7]. It can be hypothesized that, due to the fact that melatonin concentration in the heart is lower than in other organs (even after its injection), treatment with this hormone did not modify activities of the studied antioxidant enzymes and just negligibly modified tocopherol content and LDH isoenzyme ratio. With aging the cardiac antiradical defense system is changing because of a decrease in SOD activity. Changes in activities of LDH isoenzymes during aging consist in rearrangement of energy metabolism in the myocardium towards predominance of aerobic modes of energy production, which is fraught with negative consequences under conditions of reduced SOD activity.

The study was supported by the Russian Foundation for Basic Research (grant No. 07-04-00546) and Program of the President of the Russian Federation (NSh-306.2008.4).

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