Chronobiological Aspects of Melatonin-Produced Antioxidant Effects in Senescent Rats

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Melatonin in low doses of (0.05 and 0.5 mg/kg) administered to senescent rats in winter and summer inhibited free radical oxidation and LPO. The antioxidant effect of melatonin increased with increasing the dose of the hormone. Changes in test parameters were more pronounced in winter, but the directionality of changes did not depend on the duration of the light period.

Key Words: *melatonin; free radical processes; lipid peroxidation; antiradical and antioxidant systems*

The pineal gland plays an important role in the regulation of biological rhythms. Hormones secreted by the pineal gland, in particular melatonin, possess immunomodulatory, anticarcinogenic, and geroprotective properties [2,11,14]. Studies performed in the past decade showed that melatonin possesses pronounced antioxidant activity, which probably determines its geroprotective effects [1,2,14]. However, the antioxidant effect was observed after treatment with high (above physiological) doses of melatonin without consideration of the rhythmicity of melatonin-induced changes. Previous studies showed that melatonin produces various or sometimes opposite effects depending on the time of treatment and duration of the light period [12,13]. Melatonin in pharmacological doses induces not only positive, but also negative changes in physiological functions [2,11]. To obtain the most pronounced corrective effects, the dose and scheme of treatment with melatonin should be selected with due consideration for its circadian and seasonal rhythms. Here we studied changes in free radical processes, lipid peroxidation (LPO), and activity of the antiradical and antioxidant systems induced by low doses of melatonin depending on the duration of the light period.

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MATERIALS AND METHODS

Experiments were performed on 18-month-old male Wistar rats weighing 230-280 g in January and July (light periods 9 and 17 h, respectively). The rats received daily intraperitoneal injections of melatonin (Sigma) in doses of 0.05 and 0.5 mg/kg for 10 days (1 h before darkness). Control animals were treated with vehicle (distilled water and ethanol in trace concentrations). The rats were decapitated during nighttime (00.00-03.00), which corresponds to maximum activity of the pineal gland. The plasma and liver homogenates were examined. The intensity of free radical processes was estimated by spontaneous chemiluminescence (CL) recorded on a KhLM1Ts-01 chemiluminometer. The intensity of LPO was evaluated by fast flash amplitude of Fe^{2+}-induced CL reflecting the content of lipid hydroperoxides (LHP) and the integral CL intensity (area under CL curve over 240 sec after fast flash) reflecting the rate of LHP oxidation [4]. The content of secondary LPO products (thiobarbituric acid-reactive substances, TBARS) was measured on a SF-41 spectrophotometer [10,15]. Activity of the antiradical and antioxidant systems was estimated by the flash amplitude and integral CL induced by H_2O_2, which reflected the total content of biological antioxidants and the state of antioxidant enzyme systems [3]. Superoxide dismutase (SOD) [6], glutathione per-

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oxidase [7], and glutathione reductase activities [7] and the content of reduced glutathione [8] in liver homogenates were measured spectrophotometrically. The results were processed statistically using Student's *t* test.

RESULTS

Intraperitoneal injection of 0.05 mg/kg melatonin to senescent rats with age-related hypofunction of the pineal gland [2] in short daytime period decreased the intensity of spontaneous CL in liver homogenates (by 28%) and plasma (insignificant, Table 1). After increasing the dose of melatonin to 0.5 mg/kg these changes became more pronounced: the intensity of spontaneous CL in the plasma and liver homogenates decreased by 34 and 37%, respectively, compared to the control. These results indicate that melatonin even in near-physiological doses (0.05 mg/kg) inhibits free radical oxidation in rats with functional insufficiency of the pineal gland. In summer the intensity of spontaneous CL decreased only in rats receiving 0.5 mg/kg melatonin. In the plasma and liver homogenates this parameter decreased by 21 and 10%, respectively, which was less pronounced than in winter (Table 1). Thus, under conditions of long daytime melatonin was less potent in modulating free radical processes.

It should be emphasized that the fast flash amplitude and the integral Fe^{2+}-induced CL decreased in rats treated with 0.5 mg/kg melatonin in winter and summer. Therefore, melatonin decreased the content of LHP and intensified their oxidation. In winter these processes primarily occurred in the liver, while in summer melatonin induced most pronounced changes in the plasma. It could be expected that the content of secondary LPO products will increase. However, TBARS content in the plasma and liver homogenates from melatonin-treated rats did not differ from the control (irrespective on melatonin dose and daytime duration). This can be explained by the fact that only 20-25% LHP formed during LPO undergo oxidation to malonic dialdehyde (the main TBARS) [5]. Moreover, our experiments demonstrated that accumulation of secondary LPO products was prevented by activation of the antioxidant system.

Melatonin in doses of 0.05 and 0.5 mg/kg significantly increased SOD activity in liver homogenates. Glutathione reductase activity increased by 30 and 44%, respectively. Melatonin decreased the integral intensity of H_2O_2-induced CL in the plasma, which attested to an increase in its antioxidant activity. Thus, winter experiments showed that melatonin in near-physiological doses acts as a free radical scavenger. Melatonin did not change flash amplitude of H_2O_2-induced CL in the plasma and liver homogenates (antiradical activity). Previous studies showed that various endogenous and exogenous pro- and antioxidants modulate the intensity of CL. Therefore, melatonin in low doses did not change this parameter under conditions of short daytime.

In summer melatonin displayed pronounced antioxidant and antiradical properties. Melatonin increased SOD activity, but decreased total H_2O_2-induced CL in the plasma and liver homogenates and flash amplitude

TABLE 1. Effects of Melatonin on Free Radical Processes and LPO Depending on Daytime Duration (*M*±*s*, *n*=6-9)

Parameter	9-h light period			17-h light period		
	control	melatonin, mg/kg		control	melatonin, mg/kg	
		0.05	0.5		0.05	0.5
Plasma						
Spontaneous CL, pps/ml	45±4	37±3	30±2**	47±2	47±1	37±2**
Fe^{2+}-induced CL, pps/ml						
fast flash amplitude	553±8	546±27	507±24*	555±20	558±23	447±19**
integral CL	284±33	282±9	231±11	267±7	261±10	213±4**
TBARS, nmol/ml	2.4±0.3	3.0±0.3	2.2±0.3	2.6±0.2	2.5±0.2	2.3±0.2
Liver homogenates						
Spontaneous CL, pps/10 mg tissue	40±1	29±1**	25±1**	42±1	42±1	38±1**
Fe^{2+}-induced CL, pps/10 mg tissue						
fast flash amplitude	81±5	70±4	59±2**	83±1	82±2	78±1**
integral CL	86±4	80±4	65±1**	93±3	89±2	79±1**
TBARS, nmol/mg protein	2.8±0.5	2.2±0.3	2.9±0.2	3.0±0.3	3.3±0.2	3.0±0.2

Note. Here and in Table 2: *$0.05<p<0.01$; **$p<0.05$.

TABLE 2. Effects of Melatonin on Antiradical and Antioxidant Systems Depending on Daytime Duration ($M \pm m$, n=6-9)

Parameter	9-h light period			17-h light period		
	control	melatonin, mg/kg		control	melatonin, mg/kg	
		0.05	0.5		0.05	0.5
Plasma						
H_2O_2-induced CL, pps/ml						
fast flash amplitude	6376±289	6332±269	6807±249	5569±27	5123±68**	5073±92**
integral CL	1636±94	1264±148*	1085±92**	1519±27	1456±32*	1333±23**
Liver homogenates						
H_2O_2-induced CL, pps/10 mg tissue						
fast flash amplitude	866±75	799±43	834±45	903±18	911±24	898±12
integral CL	94±7	98±6	100±5	100±1	97±2	84±2**
SOD, arb. U/mg protein	1346.0±30.0	1535.4±88.9*	1545.4±80.1**	1410.0±93.3	1421.1±55.2	1675.1±61.1**
Glutathione peroxidase, nmol NADPH/mg protein	176.5±21.1	215.7±23.3	164.8±23.0	—	—	—
Reduced glutathione, mg/100 g	—	—	—	52.7±3.7	54.0±5.4	47.6±2.8
Glutathione reductase, nmol NADPH/mg protein	37.5±2.1	48.9±4.5**	54.0±3.3**	58.2±7.0	38.5±2.6**	43.2±3.7*

in the plasma (Table 2). These changes were more pronounced after treatment with melatonin in a dose of 0.5 mg/kg.

It should be emphasized that melatonin induced opposite changes in glutathione reductase activity in summer and in winter. Even in a dose of 0.05 mg/kg this hormone markedly decreased glutathione reductase activity in summer. However, in winter melatonin dose-dependently increased enzyme activity (Table 2). Melatonin did not change the content of reduced glutathione in liver homogenates (summer) and glutathione peroxidase activity (winter). It is difficult to evaluate the causal relationship between changes in these parameters. Our experiments [9] and published data [14] indicate that the glutathione-dependent system mediates the antioxidant effect of melatonin. We found that melatonin in a dose of 1 mg/kg administered for 5 days increased the content of reduced glutathione in the plasma, but had no effect on this parameter in liver homogenates.

Our results indicate that melatonin in low (near-physiological) doses dose-dependently inhibits free radical oxidation, including LPO, in senescent rats. The directionality of melatonin-induced changes does not depend on the duration of daytime. The antioxidant effect of melatonin in a dose of 0.05 mg/kg is more pronounced in winter, *i.e.*, under conditions of a short daytime. In winter significant changes in test parameters are observed after treatment with melatonin in a dose of 0.5 mg/kg.

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