GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Lipid Peroxidation in the Brain and Liver of Rats during Acute Stress and Melatonin Treatment

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 7, pp. 19-23, July, 2004
Original article submitted February 25, 2004

We studied the effects of acute stress and exogenous melatonin in various doses on the intensity of lipid peroxidation in emotiogenic structures of the brain and liver of rats with different activity in the open field. Stress had no effect on the content of malonic dialdehyde in the hypothalamus, sensorimotor cortex, and liver of active and passive rats receiving physiological saline. The influence of melatonin on malonic dialdehyde content depended on the dose of this substance. The amount of malonic dialdehyde in brain structures (active and passive rats) and liver (active rats) increased after administration of exogenous melatonin in doses of 0.5 and 2 mg/kg, but decreased after treatment with the hormone in a dose of 1 mg/kg. Melatonin in various doses decreased malonic dialdehyde content in the liver of passive rats. The effects of melatonin are partly related to modulation of lipid peroxidation in central and peripheral tissues of the organism.

Key Words: *acute stress; lipid peroxidation; melatonin; brain; liver*

The conditions of modern life contribute to the development of severe psychosomatic diseases in people, including coronary heart disease, arterial hypertension, peptic ulcer disease, tumor growth, and neuroses.

Acute stress-induced damage is closely related to free radical lipid peroxidation (LPO) [1]. LPO activation results in inflammation, reperfusion injury, and bronchopulmonary diseases [7,15]. Generation of free radicals and intensification of LPO should not be considered only as adverse factors [8]. These processes play a role in the synthesis of biological substances and renewal of lipid components in biological membranes.

The pineal gland is involved in the realization of stress response and counteracts stress-induced changes [2]. Melatonin is secreted in the pineal gland, plays an important role in the stress reaction, and possesses antistress activity. Administration of melatonin prevents the development of erosive and ulcerative lesions in the gastric mucosa of rats exposed to water-immersion immobilization stress [6] and shift in the natural light/dark cycle [5].

Rats of various strains are characterized by different resistance to stress [9]. The individual resistance to stress differs in animals of the same strain. The open-field test is extensively used to predict stress resistance in rats.

Here we studied the effects of acute stress and exogenous melatonin in various doses on the content of malonic dialdehyde (MDA, LPO end product) in rats with different behavioral activity in the open field. MDA content was measured in emotiogenic structures of the brain involved in the stress response (hypothalamus and sensorimotor cortex). MDA content was also measured in the liver characterized by most pronounced structural and functional changes during stress [10]. The differences in animal behavior were

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0007-4888/04/1381-0014

taken as the criterion for the resistance to emotional stress [9].

MATERIALS AND METHODS

Experiments were performed on 80 male Wistar rats weighing 195.51±2.04 g. The animals were kept in cages and had free access to water and food.

On day 6 the behavior of rats was studied in the open field for 5 min. The open field was an area (57×57 cm) divided into 16 central squares and 20 peripheral squares. There were 9 holes in the floor. We recorded the latency of the first movement and entrance into the center of the open field, number of crossed squares and rearing postures in the peripheral and central zones, time of grooming, and number of explored holes. To estimate the index of activity, the sum of the numbers of crossed peripheral and central squares was divided by the sum of the latencies of the first movement and entrance into the center of the open field. The rats were divided into groups comprising behaviorally active (n=34) and passive animals (n=46, Table 1).

Behaviorally active and passive rats were divided into 8 groups of 4-7 rats each. Melatonin in doses of 0.5, 1, and 2 mg/kg was dissolved in 1 ml physiological saline. Physiological saline and melatonin were injected intraperitoneally immediately before stress. Control rats received physiological saline or melatonin 4 h before decapitation.

The rats were deprived of food, but had free access to water for 24 h before the experiment. The animals were immobilized in plastic tubes (16.5×5.5 cm) and immersed in water (23°C) to a level of the xiphoid process of the sternum for 2 h. Then stressed rats were returned to home cages for 2 h. Control animals were maintained in home cages. Stressed and control animals were decapitated.

MDA content was measured in brain structures and liver. The brain was removed immediately after decapitation. The hypothalamus and sensorimotor cortex were rapidly isolated, washed with physiological saline, and frozen at -12-15°C.

Weighted tissue samples were homogenized in a glass homogenizer and stored in a refrigerator. Physiological saline with 0.1% Triton X-100 served as an isolation medium. Homogenates were clarified by centrifugation on a Beckman J9 centrifuge at 3000 rpm and 4°C for 10 min. The supernatant (50 μl) was dissolved in 0.5 ml physiological saline. MDA content was measured in 50 μl solution. The solution was placed in a centrifuge tube with 0.5 ml physiological saline and shaken. Centrifugation was performed at 2500 rpm for 10 min. The supernatant (50 μl) was placed in a centrifuge tube. H_2SO_4 (0.08 M, 4 ml) was added, and the mixture was agitated. Phosphotungstic acid (10%, 0.5 ml) was added. The mixture was agitated and kept at 20-22°C for 5 min. After centrifugation at 2500 rpm for 10 min, the supernatant was removed. H_2SO_4 (0.08 M, 2 ml) and phosphotungstic acid (10%, 0.3 ml) were added to the pellet. The mixture was agitated and centrifuged at 2500 rpm for 10 min. The supernatant was removed. The pellet was suspended in 4 ml distilled water and mixed with 1 ml thiobarbituric acid. The solution was heated in a water bath at 95°C for 1 h, cooled with flowing water, mixed with 5 ml n-butyl alcohol, and shaken. After centrifugation at 2500 rpm for 15 min, fluorescence was measured in butanol at 515 nm. The fluorescence wavelength was 553 nm. MDA content was expressed in relative units of fluorescence.

The results were analyzed by Mann—Whitney test. The data are presented as means and standard errors.

RESULTS

Under control conditions, hypothalamic MDA content in active rats receiving physiological saline was 1.15-fold higher than in passive animals (Table 2). Exogenous melatonin had a dose-dependent effect on MDA content in control rats (Table 2). MDA content in the hypothalamus of rats receiving melatonin in a dose of 0.5 mg/kg increased by 1.83 (p<0.01) and 1.45 times (p<0.05), respectively. Hypothalamic MDA content increased less significantly in animals treated with 2 mg/kg melatonin (by 1.08 and 1.12 times, respectively). Melatonin in a dose of 1 mg/kg decreased MDA content in the hypothalamus of nonstressed active and passive rats by 1.46 (p<0.05) and 1.27 times, respectively. Hypothalamic MDA content in active rats receiving melatonin in various doses surpassed that in passive animals.

MDA content in the sensorimotor cortex of control active rats receiving physiological saline was 1.17-fold higher than in passive animals (Table 2). The amount of MDA in the sensorimotor cortex of active rats increased after administration of melatonin in doses of 0.5 and 2 mg/kg (by 1.47 and 1.07 times, respectively), but decreased after treatment with the hormone in a dose of 1 mg/kg (by 1.63 times, p<0.05). Administration of 0.5 mg/kg melatonin increased MDA content in the sensorimotor cortex of passive rats by 1.11 times. However, MDA content in these animals decreased by 1.27 and 1.04 times after treatment with melatonin in doses of 1 and 2 mg/kg, respectively (statistically insignificant). Under control conditions, MDA content in the sensorimotor cortex of active rats receiving melatonin in doses of 0.5 and 2 mg/kg was higher than in passive animals (p<0.05). These data show that MDA content in emotiogenic structures of

TABLE 1. Open-Field Behavior in Rats (*M*±*m*)

Index	Active rats (*n*=34)	Passive rats (*n*=46)
Latency of the first movement, sec	3.12±0.48	3.87±0.45
Latency of entrance into the center of the open field, sec	80.76±9.12	255.74±12.14**
Number of peripheral squares	131.15±5.97	90.41±6.36*
Number of central squares	7.50±0.85	1.56±0.49*
Number of peripheral rearing postures	14.47±1.02	8.22±0.74*
Number of central rearing postures	2.59±0.53	0.52±0.17**
Number of explored objects	4.67±0.37	2.87±0.37*
Time of grooming, sec	10.53±2.33	13.56±2.09
Defecation rate	3.29±0.34	2.65±0.33
Urination rate	0.44±0.10	0.35±0.08
Index of activity	2.23±0.23	0.45±0.05**

Note. *$p<0.05$ and **$p<0.01$ compared to active rats.

the brain in control active rats receiving melatonin or physiological saline surpassed that in passive animals.

MDA content in the liver of nonstressed active rats receiving physiological saline was 1.94 times lower than in passive animals (Table 2). The amount of MDA in active rats increased after administration of melatonin in doses of 0.5 and 2 mg/kg (by 1.36 and 1.53 times, respectively, $p<0.05$), but decreased after treatment with the hormone in a dose of 1 mg/kg (by 2.25 times, $p<0.05$). Changes in MDA content in the liver of nonstressed active rats receiving melatonin were similar to those observed in the hypothalamus and sensorimotor cortex. Melatonin in doses of 0.5, 1, and 2 mg/kg decreased MDA content in the liver of passive animals by 1.40, 1.76 ($p<0.05$), and 1.77 times ($p<0.05$), respectively. It should be emphasized that MDA content in the liver of nonstressed active rats receiving melatonin in doses of 0.5 and, particularly, 1 mg/kg ($p<0.05$) was lower than in passive animals.

Melatonin in doses of 0.5 and 2 mg/kg increased MDA content in brain structures (active and passive rats) and liver (active rats). Therefore, this hormone

TABLE 2. MDA Content in the Hypothalamus, Sensorimotor Cortex, and Liver of Control and Stressed Rats with Different Activity in the Open Field (optical density units, *M*±*m*)

Group		Active rats (*n*=34)		Passive rats (*n*=46)	
		control	stress	control	stress
Hypothalamus					
physiological saline		16.86±1.90	16.89±1.75^{x}	14.66±1.21	12.38±1.23
melatonin, mg/kg	0.5	30.82±1.49^{+x}	25.37±2.74^{+x}	21.22±3.70^{+}	15.62±2.43
	1	11.56±0.50^{+}	10.99±1.24^{+}	11.50±1.38	9.82±2.96
	2	18.22±2.05	13.56±0.55*^{x}	16.47±4.31	19.69±2.47^{+}
Sensorimotor cortex					
physiological saline		17.49±1.40	13.98±4.01	14.92±2.34	16.20±1.60
melatonin, mg/kg	0.5	25.79±6.65	27.16±5.17^{+x}	16.56±2.82	44.12±7.61^{***+}
	1	10.74±0.50^{+}	16.52±3.32*	11.76±1.94	17.16±1.91*
	2	18.79±2.76^{x}	17.49±1.74^{x}	14.39±0.65	25.29±4.14^{**+}
Liver					
physiological saline		7.62±1.65^{x}	8.44±2.30	14.78±3.46	11.82±2.97
melatonin, mg/kg	0.5	10.38±2.62	8.89±0.95	10.59±1.19	8.01±0.15
	1	3.39±0.30^{+x}	6.89±1.17*	8.42±1.84^{+}	4.69±0.81*$^{+}$
	2	11.65±0.67^{+}	11.37±1.18	8.37±0.74^{+}	9.33±1.10

Note. *$p<0.05$ and **$p<0.01$ compared to the control; $^{+}p<0.05$ and $^{++}p<0.01$ compared to physiological saline; $^{x}p<0.05$ compared to passive rats.

intensified the process of LPO. Reactive oxygen metabolites maintain vascular tone [14], play an important role in cell proliferation, prostaglandin synthesis [12], microbicidal effect of phagocytes [4], and oxidation of catecholamines, and act as intracellular messengers in the regulation of metabolic processes [13]. Activation of free radical processes with melatonin probably contributes to organism's adaptation to stress [3,11].

After stress hypothalamic MDA content remained practically unchanged in active rats receiving physiological saline, but slightly decreased in passive animals. MDA content in active rats was 1.36-fold higher than in passive animals. Our results are consistent with published data that 6-h immobilization has no effect on the intensity of LPO and state of the antioxidant system in the liver, lungs, and intestine of rats.

After acute stress hypothalamic MDA content in rats receiving exogenous melatonin was lower than in nonstressed animals (except for passive rats treated with 2 mg/kg melatonin). We compared hypothalamic MDA content in rats exposed to stress and receiving melatonin in various doses or physiological saline. MDA content in the hypothalamus of stressed active rats receiving 0.5 mg/kg melatonin was higher than in animals treated with physiological saline (by 1.5 times, $p<0.05$). However, after administration of melatonin in doses of 1 and 2 mg/kg hypothalamic MDA content in these rats was lower than in animals receiving physiological saline by 1.54 and 1.25 times, respectively. In passive rats exposed to stress and receiving melatonin in doses of 0.5 and 2 mg/kg hypothalamic MDA content was higher than in animals treated physiological saline by 1.26 and 1.59 times, respectively ($p<0.05$). By contrast, MDA content in the hypothalamus of stressed passive rats receiving 1 mg/kg melatonin was 1.26-fold lower than in animals treated with physiological saline.

After stress MDA content in the sensorimotor cortex slightly decreased in active rats receiving physiological saline or 2 mg/kg melatonin (statistically insignificant), but increased in animals treated with the hormone in doses of 0.5 and, particularly, 1 mg/kg ($p<0.05$). In passive rats exposed to stress and receiving physiological saline or melatonin in doses of 0.5 ($p<0.01$), 1 ($p<0.05$), and 2 mg/kg ($p<0.05$) MDA content in the sensorimotor cortex was higher than in control animals (by 1.09, 2.66, 1.46, and 1.78 times, respectively). MDA content in the sensorimotor cortex of active rats was lower than in passive animals. After stress MDA content in the sensorimotor cortex of active and passive rats receiving exogenous melatonin in various doses was higher than in animals treated with physiological saline. The observed differences were most pronounced in passive rats receiving melatonin in doses of 0.5 ($p<0.01$) and 2 mg/kg ($p<0.05$).

Stress had no effect on MDA content in the liver of active and passive rats receiving physiological saline. After stress MDA content in the liver of active rats was 1.4-fold lower than in passive animals (Table 2).

MDA content in the liver of stressed rats receiving 0.5 mg/kg melatonin was lower than in nonstressed animals. After acute stress and pretreatment with 1 mg/kg melatonin MDA content in the liver increased by 2.03 times in active rats ($p<0.05$), but decreased by 1.8 times in passive animals ($p<0.05$). Moreover, MDA content in the liver decreased in active rats receiving 2 mg/kg melatonin, but increased in passive animals (statistically insignificant).

Our results show that stress is not accompanied by changes in MDA content in brain structures and liver of rats receiving physiological saline. The influence of melatonin on MDA content in the hypothalamus, sensorimotor cortex, and liver of animals depended on the dose of this substance. The direction of changes in MDA content was similar in brain structures, but differed in the liver of behaviorally active and passive rats. These data indicate that the effects of melatonin are partly related to modulation of LPO in central and peripheral tissues of the organism.

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