Biochemical Indexes of the Skin and Blood Melatonin Concentration in Rats during Acute Stress and Treatment with Exogenous Melatonin

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We studied the effect of acute stress on serum melatonin concentration, content of major components in the connective tissue (uronic acids, hexosamines, and hydroxyproline), and β-galactosidase activity in the skin of rats with different activity in the open-field test receiving intraperitoneal injections of physiological saline or melatonin. Acute stress intensified catabolism of carbohydrate components and affected characteristics of the main skin biopolymers. The content of uronic acids in connective tissue carbohydrates decreased. Collagen structures of the skin underwent less pronounced changes. The observed changes were similar in behaviorally active and passive animals. Administration of melatonin increased the contents of uronic acids and hexosamines in the skin. Pretreatment with melatonin prevented the decrease in the content of glycosaminoglycans in rat skin during acute stress.

Key Words: *acute stress; exogenous melatonin; active and passive rats; skin glycosaminoglycans; blood melatonin*

At the present time, people are often exposed to acute stress (AS). Stress-induced neurohumoral changes modulate functions of peripheral organs and systems. It was believed that macromolecules of the connective tissue (*e.g.*, in the skin) are biochemically stable structures that react only to potent and long-acting exogenous factors. Recent studies showed that exposure to various stress factors modulates the content of connective tissue components [1]. However, biochemical changes in protein and carbohydrate components of the skin during AS remain unknown.

Published data show that the pineal gland is involved into the stress response [4]. Melatonin (MT) produced in the pineal gland is involved in the development of stress response. MT possesses antioxidant properties [9], is used to correct diurnal rhythms, and prevents ulcer formation in rat stomach during stress [2].

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Previous experiments showed that rats of various strains are characterized by different resistance to AS [3]. Individual resistance to stress also differs in animals of the same strain. The open-field test is extensively used to evaluate the prognostic resistance to stress factors.

Here we studied the effect of AS and exogenous MT on blood MT concentration, content of major components in the connective tissue (uronic acids, hexosamines, and hydroxyproline), and β-galactosidase activity in the skin of rats with different activity in the open-field test.

MATERIALS AND METHODS

Experiments were performed on 40 male Wistar rats weighing 195.25±2.97 g. The animals were adapted to laboratory conditions for 5 days.

Open-field behavior was studied for 5 min on day 6. We recorded the latencies of the first movement and entrance into the center of the open field, number of crossed squares (ambulations) and rearing postures in

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the peripheral and central zones, time of grooming (sec), and number of explored holes (Table 1). Index of activity was calculated. To this end, the sum of the numbers of crossed peripheral and central squares was divided by the sum of latencies of the first movement and entrance into the center of the open field. The rats were divided into groups of behaviorally active (n=21) and passive (n=19) animals.

Behaviorally active and passive rats were divided into 8 groups of 4-7 animals each. MT in a dose of 2 mg/kg in 1 ml physiological saline (PS) or PS alone were injected intraperitoneally to stressed animals immediately before AS. Control nonstressed rats received PS or MT 4 h before decapitation.

The rats were deprived of food, but had free access to water for 24 h before the experiment. Water-immersion stress served as the model of AS [8]. The animals were immobilized in plastic tubes (16.5×5.5 cm), immersed in water (23°C) to a level of the xiphoid process of the sternum for 2 h, and then returned to their home cages for 2 h. Stressed and control animals were decapitated.

The blood collected after decapitation was maintained for 45-60 min, and the serum was separated by centrifugation. Serum samples were frozen at -20°C. Serum MT concentration was measured by radioimmunoassay using ^{3}H-MT.

Skin samples (4×4 cm^2) were excised from the interscapular area after decapitation and frozen in liquid nitrogen. Subcutaneous fat and hair were removed. The samples were minced and lyophilized. For the analysis of structural components lipids were extracted with chloroform-methanol 2:1 mixture, organic solvents were removed, dry skin samples were extracted with 0.1 M NaCl. The contents of uronic acids, hexosamines, and hydroxyproline and β-galactosidase activity were measured in native skin and extracts.

The results were analyzed by Mann—Whitney test and multifactor ANOVA (AS/control×MT/PS×active/passive rats). We performed a multiple comparison LSD test when significant effect was revealed. Intergroup differences were evaluated by Wilcoxon test. Correlations were determined by Kendall test. The data are presented as means and standard errors.

RESULTS

Under control conditions the content of uronic acids in the skin of active rats receiving PS was 1.8-fold higher than in passive animals (p<0.05, Table 2). AS decreased the content of uronic acids in the skin of active and passive rats receiving PS (by 1.48 and 1.22 times, respectively, p<0.05). Exogenous MT had no effect on the content of uronic acids in the skin of nonstressed animals. After administration of MT the content of uronic acids in the skin of active rats 1.2-fold surpassed that in passive animals (p<0.05). After AS and treatment with MT the content of uronic acids in the skin remained practically unchanged in active rats, but increased by 1.16 times in passive animals (p<0.05). Multifactor ANOVA revealed a significant effect of injection on the content of uronic acids in the skin of active (F=36.16, d.f.=1, p<0.00001) and passive rats (F=5.75, d.f.=1, p<0.03). After AS the content of uronic acids in active and passive rats receiving MT was higher than in animals of the PS group (by 1.49 and 1.36 times, respectively, p<0.05).

Under control conditions the content of hexosamines in the skin of active rats receiving PS and MT was higher than in passive animals (by 1.05 and 1.1 times, respectively, Table 2). AS slightly decreased the content of hexosamines in the skin of active rats receiving PS or MT. However, the content of hexosamines in the skin of passive animals increased after AS (statistically insignificant). Under control conditions and after AS the content of hexosamines in active and passive rats receiving MT was higher than in animals injected with PS (F=17.57, d.f.=1, p<0.0002).

No significant changes were revealed in the content of hydroxyproline after AS or administration of MT (Table 2). Probably, collagen structures do not undergo rapid degradation after AS. These results reflect structural lability of glycoproteins during stress.

Activity of β-galactosidase degrading macromolecules of proteoglycans and glycoproteins in the skin did not differ in active and passive rats not subjected to AS (Table 2). Administration of MT was followed by a slight increase in enzyme activity (statistically insignificant). After AS activity of β-galactosidase in the skin slightly increased in active and passive rats. Under control conditions and after AS activity of β-galactosidase in active and passive rats receiving MT was higher than in animals receiving PS (F=3.77, d.f.=1, p<0.06).

Serum MT concentration was similar in active and passive rats not subjected to AS and receiving PS (Ta-

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

Index	Active rats (n=23)	Passive rats (n=17)
Number of peripheral rearing postures	12.90±1.30*	9.05±1.09
Number of central rearing postures	2.29±0.56*	0.79±0.35
Number of explored objects	4.38±0.43	3.47±0.65
Time of grooming, sec	13.05±3.03	14.16±2.33
Index of activity	2.28±0.27**	0.41±0.06

Note. *p<0.05 and **p<0.01 compared to passive rats.

TABLE 2. Serum MT Concentration, Contents of Uronic Acids, Hexosamines, and Hydroxyproline, and b-Galactosidase Activity in the Skin of Rats ($M \pm m$)

Index	Active rats (n=23)		Passive rats (n=17)	
	PS	MT	PS	MT
Serum MT concentration, nmol/liter				
control	0.07±0.01	1.39±0.32^{++}	0.08±0.00	1.11±0.03^{++}
stress	0.38±0.20*	0.98±0.15^{+o}	0.66±0.38*	0.58±0.05*
Skin uronic acid content, mg/g				
control	2.44±0.08^{o}	2.60±0.07^{o}	2.25±0.03	2.16±0.16
stress	1.65±0.11*	2.46±0.06^{+}	1.85±0.12*	2.51±0.09^{*+}
Skin hexosamine content, mg/g				
control	3.38±0.03^{o}	3.72±0.05^{+}	3.23±0.07	3.35±0.34
stress	3.15±0.08*	3.66±0.11^{+}	3.28±0.09	3.75±0.07^{+}
Skin hydroxyproline content, mg/g				
control	53.43±5.24	56.48±1.31^{o}	55.88±1.51	50.90±2.56
stress	56.16±3.99	53.10±1.71	54.40±1.62	55.25±2.32
Skin β-galactosidase activity, U				
control	17.95±1.12	22.25±1.55^{+}	16.82±0.51	19.02±3.03
stress	21.34±2.39	20.32±1.47	18.63±2.54	23.88±1.62^{+}

Note. *p<0.05 compared to nonstressed rats; ^{+}p<0.05 and ^{++}p<0.01 compared to rats receiving PS; ^{o}p<0.05 compared to passive rats.

ble 2). AS increased blood MT level in active and passive rats of the PS group by 5.43 and 8.25 times, respectively (p<0.05).

The increase in blood MT concentration in stressed rats can be mediated by the hypothalamic-hypophyseal-adrenal mechanism. AS is accompanied by stimulation of the sympathetic nervous system that regulates functions of the pineal glands and activity of MT-synthesizing enzymes [5]. Moreover, AS initiates lipid peroxidation in the organism [5]. Probably, the increase in blood MT concentration during stress is related to its antioxidant properties and intensive release from the pineal gland.

Multifactor ANOVA revealed a significant effect of injection on blood MT concentration in active (F= 17.02, d.f.=1, p<0.0007) and passive rats (F=5.72, d.f.=1, p<0.03). Blood MT concentration in nonstressed active and passive rats receiving MT was higher than in animals receiving PS (by 19.86 and 13.87 times, respectively, p<0.01). Our results confirm published data that intravenous infusion of MT in low doses (10 and 30 μg/kg) to healthy volunteers increased its concentration in the serum and saliva [10].

Serum MT concentration decreased in active and, particularly, in passive rats subjected to AS and pretreated with MT (by 1.91 times, p<0.05, Table 2). Blood MT concentration in stressed active rats receiving MT was 2.58 times higher than in animals receiving PS (p<0.05). These differences were not revealed in passive rats. Under control conditions and after AS blood melatonin concentration in active rats receiving MT was 1.25 and 1.69 times higher, respectively, than in passive animals (p<0.05).

The decrease in serum MT concentration in stressed rats receiving exogenous MT (compared to control animals) can be mediated by several mechanisms. Addition of 2-iodomelatonin to the culture medium of the pineal gland suppresses the release of endogenous MT from pinealocytes [11]. Therefore, the release of MT from the pineal gland is regulated by the negative feedback mechanism. In our experiments intraperitoneal injection of MT probably also inhibited secretion of endogenous MT. It should be emphasized that AS was accompanied by consumption of MT circulating in the blood. However, the reserves of this hormone could not be replenished by endogenous MT released from the pineal gland. Published data show that the immunomodulatory effect of MT is mediated by cytokines secreted by T lymphocytes during the interaction of this hormone with specific receptors on the surface of cells [7]. Cytokines (*e.g.*, interleukin-1β) decrease the concentration of MT in rat plasma [6]. Moreover, the content of catecholamines (*e.g.*, dopamine) in the blood increases during stress. Stimulation of dopamine receptors inactivates serotonin-dependent N-acetyltransferase and suppresses MT synthesis in the retina [12]. These changes are followed by a decrease in blood MT level.

We determined the correlations between skin glycosaminoglycan content and serum MT concentration

in rats. In control active rats receiving PS blood MT concentration positively correlated with the contents of hexosamines (r=0.036) and hydroxyprolines in the skin (r=0.079). Blood MT concentration tended to correlate positively with skin hexosamine content in control active rats of the MT group (r=0.091). A negative correlation was revealed between blood MT concentration and skin β-galactosidase activity in control passive rats treated with MT (r=-0.041). In passive rats subjected to AS and treated with MT hexosamine content negative correlated with β-galactosidase activity (r=-0.041).

Our findings suggest that AS intensifies catabolism of carbohydrate components and affects characteristics of the main biopolymers in rat skin. The observed changes were similar in behaviorally active and passive animals. AS markedly decreases the content of uronic acids in connective tissue carbohydrates. It reflects depolymerization of skin biopolymers and, particularly, proteoglycans during AS. Collagen structures of the skin undergo less pronounced changes under these conditions.

Administration of MT increases the contents of uronic acids and hexosamines in the skin of active and passive rats and prevents the decrease in the content of glycosaminoglycans during AS. This is consistent with correlations between skin glycosaminoglycan content and blood MT concentration in rats.

Our results indicate that AS and MT produce various effects on biochemical indexes of the skin in rats. Further studies of cellular and extracellular components in the connective tissue are required for better understanding of the mechanism of these changes.

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