Effect of Melatonin on the Thymus, Adrenal Glands, and Spleen in Rats during Acute Stress

S. S. Pertsov

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We studied the effects of acute stress and exogenous melatonin on stress marker organs in rats. Administration of melatonin under normal conditions increased the relative weights of the thymus (active rats) and adrenal glands (active and passive rats). The relative weight of the spleen also tended to increase after melatonin treatment. Stress led to involution of the thymus and hypertrophy of the adrenal glands in active and especially in passive animals receiving physiological saline. Melatonin partially or completely prevented involution of the thymus under stress conditions. Stress had no effect on the relative weight of the adrenal glands in melatonin-treated rats. The relative weight of the spleen in active rats receiving melatonin in doses of 0.5 and 1 mg/kg decreased after stress exposure. Our results suggest that melatonin modulates the hemodynamics and function of stress marker organs.

Key Words: *stress load; melatonin; stress marker organs; active and passive rats*

Emotional overstrain associated with strengthening of the tempo of life, urbanization, and informational overload is the cause of neurotic, cardiovascular, and other diseases. Prevention of stress or decrease in the severity of stress consequences is an urgent problem of modern medicine.

The pineal hormone melatonin possesses biorhythmic [6], antioxidant [10], and immunomodulatory properties [12]. This substance plays an important role in the development of stress and stress-associated diseases [1]. Our previous experiments showed that melatonin prevents gastric ulceration [4], reduces the severity of changes in skin connective tissue [2], and regulates lipid peroxidation in the brain and liver of rats under stress conditions [3].

Rats of various strains are characterized by different resistance to stress [5]. The individual resistance to stress differs in animals of the same strain. The open-field test is extensively used to predict animal resistance to stress factors. Previous studies performed at the P. K. Anokhin Institute of Normal Physiology showed that rats resistant to emotional stress are characterized by short latency of the first movement and entrance into the center of the open field, pronounced orientation-and-exploratory behavior, and high index of locomotor activity.

Here we studied the effect of melatonin in various doses on stress marker organs (thymus, adrenal glands, and spleen) in active and passive rats exposed to acute stress.

MATERIALS AND METHODS

Experiments were performed on 80 male Wistar rats weighing 195±2 g. The animals were housed in cages (5 rats per cage) at 22-26°C and artificial light/dark regimen (8.00-20.00, day phase; 20.00-8.00, night phase). They had free access to water and food.

Five days after delivery to the laboratory, the behavior of rats was studied in the open-field test for 5 min. There were 34 active and 46 passive animals (Table 1).

P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** s.pertsov@mail.ru. S. S. Pertsov

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The rats were divided into 8 groups (4-7 rats per group). Melatonin in doses of 0.5, 1, and 2 mg/kg was dissolved in 1 ml physiological saline. Physiological saline or melatonin was injected intraperitoneally to stressed animals immediately before treatment. Unstressed 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. Water-immersion stress served as the model of acute stress [9]. 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 an additional 2 h. Control animals were maintained in home cages. The animals were decapitated.

Stress marker organs (thymus, adrenal glands, and spleen) were isolated immediately after decapitation. Surrounding tissues were removed. The organs were weighted on a VT-500 torsion balance. The relative weight of organs was calculated per 100 g body weight.

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

RESULTS

The relative weight of the thymus did not differ in unstressed active and passive rats receiving physiological saline (Table 2).

After acute stress the relative weight of the thymus in passive rats receiving physiological saline decreased by 1.25 times compared to unstressed animals ($p<0.05$). A stress-induced decrease in the relative weight of the thymus in active rats was statistically insignificant (compared to unstressed animals).

Administration of melatonin in doses of 0.5, 1, and 2 mg/kg increased the relative weight of the thymus in unstressed active rats compared to animals receiving physiological saline (by 1.16, 1.14, and 1.12 times, respectively, $p<0.05$). These changes were not found in passive rats.

We measured the relative weight of the thymus in rats receiving melatonin and exposed to acute stress. Stress slightly decreased the relative weight of the thymus in active animals. Stress-induced changes in the relative weight of the thymus were statistically insignificant in passive rats receiving melatonin in doses of 0.5 and 1 mg/kg. Administration of melatonin in a dose of 2 mg/kg prevented stress-induced involution of the thymus in passive rats.

After acute stress the relative weight of the thymus in rats receiving melatonin in doses of 0.5, 1, and 2 mg/kg was higher compared to animals receiving physiological saline. It was observed in active (by 1.28, 1.27, and 1.24 times, respectively) and passive rats (by 1.16, 1.02, and 1.27 times, respectively). However, these differences were statistically insignificant.

Under normal conditions the relative weight of the adrenal glands did not differ in active and passive rats receiving physiological saline (Table 2).

Acute stress slightly increased the relative weight of the adrenal glands in active rats receiving physiological saline. After stress exposure the relative weight of the adrenal glands in passive rats 1.33-fold surpassed the corresponding parameter in unstressed animals.

Administration of melatonin in doses of 0.5, 1, and 2 mg/kg increased the relative weight of the adrenal glands in unstressed rats compared to animals of the physiological saline group. These changes were observed in active (by 1.41, 1.34, and

TABLE 1. Open-Field Behavior of Rats ($M\pm m$)

Parameter	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, 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

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

TABLE 2. Relative Weight of the Thymus, Adrenal Glands, and Spleen in Control and Stressed Rats with Different Activity in the Open Field after Administration of Physiological Saline or Melatonin (mg/100 g body weight, $M \pm m$)

Experimental conditions	Active rats (n=34)		Passive rats (n=46)	
	control	stress	control	stress
Relative weight of the thymus				
Physiological saline	235.08±9.49	188.52±24.51	242.58±12.90	194.84±14.97*
Melatonin, 0.5 mg/kg	273.04±11.17+	241.97±25.28	242.50±23.84	225.68±28.06
Melatonin, 1 mg/kg	268.70±8.50+	239.56±14.65	240.49±17.39	198.74±22.62
Melatonin, 2 mg/kg	263.38±17.02	234.67±22.77	229.31±21.19	246.82±27.02
Relative weight of the adrenal glands				
Physiological saline	17.69±1.07	20.21±1.21	16.57±1.04	22.05±1.05*
Melatonin, 0.5 mg/kg	25.03±3.03+	22.64±1.61	21.71±0.84+	23.72±2.31
Melatonin, 1 mg/kg	23.78±0.50+	22.02±2.19	21.84±0.99+	21.59±2.54
Melatonin, 2 mg/kg	22.70±1.41+	23.31±1.63	22.00±1.33+	20.91±2.08
Relative weight of the spleen				
Physiological saline	418.97±34.89	443.40±6.90	405.58±24.11	445.04±25.29
Melatonin, 0.5 mg/kg	452.86±41.17	349.76±35.85+	401.74±37.44	513.04±85.22
Melatonin, 1 mg/kg	425.65±24.50	341.79±19.71+	454.38±36.58	431.53±76.23
Melatonin, 2 mg/kg	443.55±16.86	435.65±54.41	411.67±101.61	368.65±35.84

Note. *p<0.05 compared to unstressed animals; +p<0.05 compared to physiological saline.

1.28 times, respectively) and passive rats (by 1.31, 1.32, and 1.33 times, respectively, p<0.05).

The relative weight of the adrenal glands in melatonin-treated rats underwent little changes after acute stress.

Under normal conditions the relative weight of the spleen did not differ in active and passive rats receiving physiological saline (Table 2).

Stress did not significantly changed the relative weight of the spleen in rats receiving physiological saline.

Melatonin slightly increased the relative weight of the spleen in unstressed active (0.5 and 2 mg/kg) and passive rats (1 mg/kg).

The relative weight of the spleen in active rats receiving melatonin in doses of 0.5 and 1 mg/kg and exposed to acute stress was lower compared to animals of the physiological saline group (by 1.27 and 1.30 times, respectively). The relative weight of the spleen in passive rats remained practically unchanged after stress exposure.

Our results show that under normal conditions, administration of melatonin is accompanied by an increase in the relative weight of the thymus in active, but not in passive rats. Melatonin significantly increased the relative weight of the adrenal glands and slightly increased the relative weight of the spleen in unstressed active and passive animals. There are several causes for the increase in the relative weight of organs in melatonin-treated rats. Published data show that melatonin increases the diameter of arteries in skeletal muscles and improves blood circulation in capillaries under conditions of ischemia [13]. Experiments on rats with middle cerebral artery occlusion showed that melatonin improves blood circulation due to inhibition of endothelin-1-converting enzyme and suppression of endothelin formation (one of the most potent vasoconstrictors) [8]. Moreover, melatonin consumption with drinking water led to hypertrophy of arteriolar walls, increased vascular distensibility, and decreased the lower limit of blood flow self-regulation in rats [7]. In our experiments melatonin increased the relative weight of the thymus, adrenal glands, and spleen. These changes are probably associated with the influence of melatonin on the vascular system, modulation of hemodynamics, and increase in blood volume in the examined organs.

In our experiments acute stress is followed by involution of the thymus and hypertrophy of the adrenal glands in active and, especially, in passive rats receiving physiological saline. The observed changes in the relative weight of the thymus and adrenal glands in animals are typical of the stress reaction [11]. Our findings agree with published data on the existence of individual differences in stress resistance [5].

Exogenous melatonin decreased the severity or abolished involution of the thymus in rats under stress conditions. The relative weight of the adrenal

glands in rats remained unchanged after stress exposure.

These data show that exogenous melatonin has a protective effect on stress marker organs in rats during acute stress.

The observed differences in the relative weight of organs in active and passive animals upon acute stress and melatonin treatment suggest that individual characteristics of the organism should be taken into account during correction of stress-induced disturbances.

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