PHYSIOLOGY

Role of the Hypothalamic Suprachiasmatic Nucleus in the Effect of Melatonin on the Thymus, Adrenal Glands, and Spleen in Rats

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We studied the role of the hypothalamic suprachiasmatic nucleus in realization of the effect of melatonin on stress marker organs in rats under normal conditions and during acute stress. Stress induced involution of the thymus in active rats and adrenal gland hypertrophy in active and passive animals. Electrocoagulation of the suprachiasmatic nucleus induced a more pronounced decrease in the weight of the thymus and greater increase in the weight of the adrenal glands. Melatonin administration after electrocoagulation of the suprachiasmatic nucleus had no effect on the relative weight of the thymus, adrenal glands, and spleen in control and stressed animals. The influence of melatonin on the thymus, adrenal glands, and spleen is partly mediated by this structure of the brain.

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

Activation of the hypothalamic-pituitary-adrenal (HPA) axis during stress is a general physiological process that plays a role in the maintenance of homeostasis and adaptation of the organism. The hypothalamic suprachiasmatic nucleus (SCN) is the major rhythm-generating structure [6] determining rhythmic activity of the HPA axis under normal conditions and during stress.

Functional studies showed that the pineal gland is a stress-limiting structure of the brain. The pineal gland secretes bioactive compounds determining the stress response [7], *e.g.* melatonin produced primarily in cells of the pineal gland (pinealocytes).

The major manifestations of stress are involution of the thymus and lymph nodes, ulceration in the gastrointestinal tract, and hypertrophy of the adrenal cortex [10]. Our previous studies showed that melatonin produces a strong protective effect on the gastric mucosa [4] and prevents involution of the thymus and hypertrophy of the adrenal glands in rats under stress conditions [3]. It remains unclear whether the stress-protective effect of melatonin is associated with its direct influence on peripheral tissues or mediated by the hypothalamic SCN.

High importance of individual approach in the studies of the mechanisms of stress is proved [5]. The open-field test is used to predict animal resistance to stress exposure. Previous experiments showed that rats demonstrating active behavior in the open field are more resistant to stress compared to passive animals [2].

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Here we studied the role of SCN in the effect of melatonin on the thymus, adrenal glands, and spleen in active and passive rats under normal conditions and during acute stress.

MATERIALS AND METHODS

Experiments were performed on 92 male Wistar rats weighing 306±6 g. The animals were maintained in a vivarium under standard conditions.

Five days after delivery to the laboratory, rat behavior was studied in the open-field test for 5 min. To calculate the index of activity, the sum of crossed peripheral and central squares, peripheral and central rearing postures, and explored objects was divided by the sum of latencies of the first movement and entrance into the center of the open field. There were 46 active and 46 passive animals differing in the index of activity in the open field (4.02±0.62 and 0.39±0.06, respectively, $p<0.01$). Active and passive rats were divided into 8 groups (5-6 rats per group).

Bilateral electrocoagulation of SCN was performed under urethane anesthesia (1 g/kg intraperitoneally) on the next day after open field testing. Stainless steel electrodes (diameter 0.2 mm) were introduced into the brain according to stereotaxic coordinates (AP=-0.5, L=±0.2, H=7.5). Electrocoagulation was performed with anode current (1 mA) for 20 sec [9]. SCN destruction was verified on brain sections stained with cresyl violet.

Some rats were sham-operated (scalping and insertion of electrodes with no delivery of electric current) to exclude possible effects of surgery on the weight of organs. Some animals remained intact.

The tests were conducted after 6 days. Physiological saline (1 ml) was injected intraperitoneally to intact and sham-operated animals and rats subjected to SCN electrocoagulation. Melatonin in a dose of 2 mg/kg was dissolved in 1 ml physiological saline and administered to rats subjected to SCN electrocoagulation. Unstressed rats received injections 1 h before decapitation. The animals exposed to acute stress were treated immediately before stress. Immobilization in plastic cages (16.5×5.5 cm, 1 h) with simultaneous electrocutaneous stimulation served as the model of acute stress. Stochastic electrocutaneous stimulation was delivered via metal needle electrodes fixed in the skin on the back of animals (30-60 sec, 50 Hz). The strength of stimulation was selected by vocalization threshold during electrostimulation. The rats were decapitated after stress.

The thymus, adrenal glands, and spleen were isolated 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 using Mann—Whitney test. The data are presented as means and standard errors.

RESULTS

The relative weight of organs did not differ in sham-operated and intact rats (Table 1). We concluded that surgery (scalping and insertion of electrodes) had no effect on the weight of the thymus, adrenal glands, and spleen in rats. Further study of changes in the relative weight of organs under conditions of SCN electrocoagulation and melatonin treatment was performed relative to unoperated animals (Table 1).

SCN electrocoagulation was followed by a significant decrease in the relative weight of the thymus in active and passive rats (by 2.31 and 2.08 times, respectively, compared to unoperated animals; $p<0.05$). Melatonin administration after SCN electrocoagulation had no effect on the relative weight of the thymus in unstressed animals compared to rats receiving physiological saline. The relative weight of the thymus in active and passive rats receiving melatonin after SCN electrocoagulation was lower than in unoperated animals (by 2.2 and 1.83 times, respectively).

During acute stress the relative weight of the thymus decreased by 1.29 times in unoperated passive rats, but remained practically unchanged in active animals. After SCN electrocoagulation, stress induced no significant changes in the weight of the thymus in active and passive rats.

SCN electrocoagulation was followed by an increase in the relative weight of the adrenal glands in unstressed active and passive rats (by 1.46 and 1.31 times, respectively, compared to unoperated animals; $p<0.05$). Melatonin administration after SCN electrocoagulation had no effect on the relative weight of the adrenal glands in unstressed animals compared to rats receiving physiological saline. The relative weight of the adrenal glands in active and passive rats receiving melatonin after SCN electrocoagulation was higher than in unoperated animals (by 1.38 and 1.56 times, respectively, $p<0.05$).

The relative weight of the adrenal glands in unoperated active rats and, particularly, in passive animals increased after acute stress (by 1.29 and 1.41 times, respectively, compared to unstressed specimens; $p<0.05$). Stress had no effect on the weight of the adrenal glands after SCN electrocoagulation.

The relative weight of the spleen in unstressed active rats tended to increase after SCN electro-

TABLE 1. Relative Weight of Organs in Rats under Various Experimental Conditions (mg/100 g body weight, *M*±*m*)

Group	Active rats (*n*=46)		Passive rats (*n*=46)	
	control (no stress)	stress	control (no stress)	stress
Relative weight of the thymus				
Intact, physiological saline	75.01±4.70	79.55±5.81^{o}	66.96±4.79	51.81±3.25*
Sham operation, physiological saline	68.74±3.58	67.91±7.28^{o}	58.88±4.82	45.12±2.89*
SCN coagulation, physiological saline	32.43±1.36^{+}	31.85±2.99^{+}	32.13±2.61^{+}	26.54±2.00^{+}
SCN coagulation, melatonin	35.45±2.18^{+}	28.35±1.62^{+}	36.63±2.81^{+}	34.41±3.17^{+}
Relative weight of the adrenal glands				
Intact, physiological saline	6.84±0.31	8.86±0.56*	6.64±0.44	9.39±0.83*
Sham operation, physiological saline	7.33±0.60	8.27±0.39	7.55±0.57	10.59±0.99*
SCN coagulation, physiological saline	9.98±0.86^{+}	9.97±0.47	8.69±0.80^{+}	9.49±0.34
SCN coagulation, melatonin	9.43±0.67^{+}	10.62±0.94	10.34±0.88^{+}	8.55±0.74
Relative weight of the spleen				
Intact, physiological saline	342.60±27.55	374.05±20.31	356.93±34.69	420.07±46.61
Sham operation, physiological saline	300.93±35.46	347.63±30.11	372.49±43.05	390.89±37.03
SCN coagulation, physiological saline	398.92±30.45	376.41±42.08	368.29±26.62	310.31±28.69
SCN coagulation, melatonin	351.23±33.46	295.74±25.58	398.40±29.58	344.58±20.05

Note. *$p<0.05$ compared to unstressed rats; $^{+}p<0.05$ compared to intact rats; $^{o}p<0.05$ compared to passive rats.

coagulation (by 1.16 times compared to unoperated animals, statistically insignificant). These changes were absent in passive rats. Melatonin administration after SCN electrocoagulation had no significant effect on the relative weight of rat spleen.

Acute stress was followed by a slight increase in the relative weight of the spleen in unoperated passive and active rats (by 1.09 and 1.18 times, respectively, compared to unstressed animals). The weight of the spleen in active and passive rats receiving physiological saline after SCN electrocoagulation tended to decrease under stress conditions.

Our previous experiments showed that intraperitoneal injection of melatonin under normal conditions is followed by an increase in the relative weight of the thymus, adrenal glands, and spleen in animals [3]. Exogenous melatonin had also a protective effect on stress-marker organs in rats during acute stress. The present study indicates that administration of melatonin after electrocoagulation of SCN has no effect on the relative weight of thymus, adrenal glands, and spleen in rats maintained under normal conditions or exposed to stress. This specific feature is probably related to high density of melatonin receptors in SCN [8].

Acute stress induced involution of the thymus in active rats and hypertrophy of the adrenal glands in active and passive animals. This stress response was not observed after SCN destruction. SCN electrocoagulation was followed by a decrease in the relative weight of the thymus and increase in the weight of the adrenal glands in active and passive rats. It should be emphasized that changes SCN electrocoagulation produced more significant changes in the weight of organs than stress. Published data show that stability of rhythmic processes in the organism is mainly determined by the interrelations of the pineal gland and other structures of the brain with SCN [1]. Hence, SCN destruction has a negative effect on circadian organization of physiological reactions and is followed by changes typical of the stress response. Probably, further exposure to stress under these conditions has no effect on study organs.

Our results indicate that the hypothalamic SCN plays an important role in the realization of stress response. The effects of melatonin on the thymus, adrenal glands, and spleen in rats under normal and stress conditions is partly mediated by this structure of the brain.

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