Effect of Interleukin-1β on the Thymus, Adrenals, and Spleen in August, Wistar, and WAG Rats in Stress

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Emotional stress causes a decrease in the relative mass of the thymus, adrenals, and spleen in August, Wistar, and WAG rats, which is the greatest in animals with a low motor activity in the open field test. Intraventricular administration of interleukin-1β diminishes the decrease in the relative mass of spleen in August and Wistar rats, has no significant effect on the relative mass of thymus in all rats, and leads to an increase in the relative mass of adrenals in immobilized WAG rats.

Key Words: *interleukin-1β; emotional stress; August, Wistar and WAG rats; behavior*

According to Selye, the "general adaptation syndrome" or the "syndrome of biological stress" includes involution of the thymus and lymph nodes, ulceration of the gastrointestinal tract, and hypertrophy of adrenal medulla [16].

Different rat populations are characterized by different resistance to emotional stress (ES) [4]. Moreover, difference were observed in autonomous nerve system of stressed rats. Individual resistance to ES has been also established. The open field test has been widely used to assess individual resistance to ES [9]. Rats resistant to ES have a short latency of the first movement, high motor activity, and low vegetative balance in the open field [1].

Cytokines, specifically interleukin-1β (IL-1β), play an important role in normally functioning organism and in ES [8]. Emotional stress stimulated secretion of cytokines by cells [12] and synthesis of the IL-1 mRNA in the brain [13]. On the other hand, IL-1β plays a stress-protecting role. Previously, we showed that in acute ES IL-1β suppresses formation of peptic ulcers [2], inhibits lipid peroxidation in the brain [3], and induces functional changes in platelets.

In the present study we examined the effect of central administration of IL-1β on relative mass of thymus, adrenals, and spleen of stressed August, Wistar, and WAG rats.

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MATERIALS AND METHODS

Experiments were performed on 17 male August rats (body weight 196.1±4.1 g), 15 male Wistar rats (180.4± 11.2 g), and 13 male WAG rats (268.5±13.9 g). The animals were maintained in standard cages (4 rats) at 20-22°C under natural light and had free access to food and water.

Five days before experiment, steel tubes (length 3 mm, inner diameter 0.8 mm) were implanted into the skull bone under Nembutal anesthesia (40 mg/kg intraperitoneally). The tube was located 1 mm rostral relative the lambda and 1 mm right to the sagittal suture and did not penetrate the brain and the lateral ventricle. Four days after the surgery, the rats were tested in the open field. The open field was a 57×57-cm square divided into 36 squares: 20 peripheral and 16 central with 9 "holes" 2.5 cm in diameter. A rat was placed in the field and observed for 15 min. The following parameters were recorded: latency of the first movement and reaching the center of the field, number of rearings and crossed squares, duration of grooming, number of explorations of the "holes," and number of droppings and urinations.

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Interleukin-1β was injected into the lateral ventricle on day 5 after surgery immediately before ES. The needle was inserted into the tube 3 mm above the brain surface. Control rats were injected with normal saline.

The rats were divided into 4 groups: group 1 rats were given IL-1β in 10 μl normal saline (10 μg/ml) and subjected to acute ES. Group 2 rats were given IL-1β and placed in their cages. Group 3 rats were injected with 10 ml normal saline and subjected to ES, and group 4 rats received normal saline and were not stressed. Human recombinant IL-1β (activity 3×10^7 U/μl) was received from the Institute of Immunology.

Twenty-four hours before experiment, the rats were deprived of food and had free access to water. After administration of IL-1β or normal saline, they were subjected to ES [14] by immobilization in plastic boxes (length 16.5 cm, inner diameter 5.5 cm) and immersed into water (23°C) up to the xiphoid process and left there for 2 h. Then the rats were returned to their cages and sacrificed after 2 h. The thymus, adrenals, and spleen were weighed.

The data were analyzed by multifactor analysis of variance: (stress/control×IL-1β/normal saline×population). The LSD test was used for multiple comparison. The Kendall test was used for construction of correlation matrix. The values in text and tables are the means±standard error of the mean (mg organ mass/100 g body weight).

RESULTS

Thymus. The relative mass of the thymus was significantly different in August, Wistar and WAG rats (F=3.3(2), p<0.05): 129.61±18.59 August rats, 141.14±12.06 Wistar rats, 78.88±11.04 WAG rats (LSD test, p<0.05).

After ES, the relative mass of thymus decreased in all rats (Table 1), which is consistent with Selye's definition of stress [16]. In stressed animals given normal saline the relative mass of the thymus decreased by 33.7% (August), 9.5% (Wistar), and 50% (WAG) (F=9.8(1), p<0.02).

Interleukin-1β had no significant effect on the relative mass of thymus in control and stressed rats, which may be associated with the high content of IL-1β in the thymus [10].

In August rats injected with normal saline, the relative mass of the thymus after ES positively correlated with the number of rearings at the periphery and in the center of the open field, while in IL-1β-treated August rats it correlated with the number of appearings in the center and urinations and with exploring activity. This confirms the observation that involution of the thymus after acute ES in more pronounced in animals with low activity in the open field [17]. In rats with high activity in the open field, the relative mass of the thymus was greater than that in rats with low activity [1].

Adrenals. The relative mass of adrenals significantly (F=10.9(2), p<0.0003) differed in August, Wistar, and WAG rats: 16.79±1.50 (Wistar), 15.14±0.74 (August), 10.05±0.43 (WAG) (LSD test, p<0.05).

In WAG rats, two-factor dispersion analysis revealed significant (F=25.9(1), p=0.001) effect of IL-1β on the relative mass of adrenals, which has increased by 22.2% (LSD test, p<0.01) compared with rats given normal saline (Table 2). In IL-1β-treated Wistar rats, the relative mass of adrenals increased, the increase being statistically insignificant.

Presumably, the increase in the relative mass of adrenals caused by IL-1β is due to enhanced release of glucocorticoids by adrenals resulting from stimulation of secretion of corticotropin-releasing factor and adrenocorticotropic hormone by IL-1β [7]. In addition, IL-1β can directly influence the adrenals, stimulating the release of glucocorticoids from them [6]; hypertrophy of *zona fasciculata* in adrenal cells has been observed after administration of IL-1β [11].

After ES, a tendency toward a decrease in the relative mass of adrenals was observed in all rats (F=3.99(1), p<0.05). In rats treated with normal saline, the relative mass of adrenals decreased by 6.3% in August rats, remained unchanged in Wistar rats, and increased by 12.5% in WAG rats. These changes were statistically insignificant.

In IL-1β-treated rats the relative mass of adrenals after ES decreased by 18.9% (August), 39.1% (Wistar), and 8.3% (WAG). However, the decrease was statistically insignificant.

Generally, adrenal hypertrophy has been observed in ES. The decrease in the relative mass of adrenals observed in our experiments may be due to polyphasal functioning of the hypothalamo-adrenocortical system during stress [5] or insufficient stimulation of adrenals, judging from reduced release of corticotropin and corticoliberin in ES.

In IL-1β-treated Wistar rats and WAG rats given normal saline, the relative mass of adrenals after ES positively correlated with the number of appearances in the center of the open field (p<0.05). In August rats injected with physiological saline, the relative mass of adrenals after ES negatively correlated with the latency of appearings in the center of the open field (p<0.06). Presumably, in rats with high motor activity the relative mass of adrenals after ES changes to a lesser extent than in rats with low motor activity.

Spleen. The relative weight of spleen was significantly (F=58.8(2), p<0.0001) different in the studied

TABLE 1. Relative Mass of Thymus of August, Wistar and WAG Rats After Acute Emotional Stress (mg/100 body weight, *M±m*)

Group		Relative mass of thymus	
		Normal saline	IL-1β
August	control	168.76±69.72	110.08±33.46
	stress	111.84±28.11	147.33±38.55
Wistar	control	168.03±19.86	124.90±16.50
	stress	152.16±28.53	117.51±22.79
WAG	control	107.50±7.50	130.37±50.60
	stress	53.44±11.16*	67.19±10.19*

Note. *$p<0.02$ compared with the control. For all groups the differences between populations are significant ($p<0.05$, multifactor analysis of variance).

TABLE 2. Relative Mass of Adrenals of August, Wistar and WAG Rats After Acute Emotional Stress (mg/100 body weight, *M±m*)

Group		Relative mass of adrenals	
		Normal saline	IL-1β
August	control	16.46±0.25	15.79±1.05
	stress*	15.28±2.30	13.43±1.19
Wistar	control	14.95±0.92	22.98±4.83
	stress*	15.26±1.33	13.90±1.05
WAG	control	7.87±0.81	12.18±0.50**
	stress*	9.27±0.39	10.68±0.34**

Note. *$p<0.06$ compared with the control; **$p<0.01$ between rats given IL-1β and normal saline; $p<0.0003$ between rat populations.

TABLE 3. Relative Mass of Spleen of August, Wistar and WAG Rats After Acute Emotional Stress (mg/100 body weight, *M±m*)

Group		Relative mass of spleen	
		Normal saline	IL-1β
August	control	352.82±33.70	286.54±26.77
	stress*	231.72±15.49	241.76±26.43
Wistar	control	823.76±31.37	691.41±129.38
	stress*	552.89±52.54	513.70±58.45
WAG	control	403.58±52.99	418.22±30.42
	stress*	330.31±11.49	305.97±20.36

Note. *$p<0.02$ compared with the control; $p<0.0001$ between rat populations.

rat populations: 628.51±46.30 (Wistar), 270.80±15.35 (August), 345.75±16.68 (WAG) (LSD test, $p<0.01$).

Emotional stress had a significant effect ($F=19.3(1)$, $p=0.0001$) on the relative mass of spleen in all rats (Table 3). After ES, this parameter decreased by 23.3% in August rats (LSD test, $p<0.01$), by 28.5% in Wistar rats (LSD test, $p<0.02$), and by 22.9% in WAG rats (LSD test, $p<0.007$). Thus, Wistar rats were more sensitive to ES than August and WAG rats.

In IL-1β-treated August and Wistar rats, the ES-induced decrease in the relative mass of the spleen was smaller than in rats given normal saline. After ES, the relative mass of the spleen in August rats given normal saline decreased by 34.3%, while in IL-1β-treated rats it decreased by 15.7%. In control and IL-1β-treated Wistar rats the decrease was, respectively, 32.9 and 25.6%. This indicates that IL-1β protects the spleen against ES. A smaller decrease in the relative mass of spleen caused by ES in IL-1β-treated rats may be associated with activation of the sympathetic innervation of this organ. It was shown that IL-1β selectively enhances norepinephrine turnover in the spleen, indicating an increase in sympathetic activity [15]. Thus, IL-1β directly modifies immune and metabolic functions of the spleen.

After ES, the decrease in the relative mass of spleen was smaller in WAG rats given normal saline compared with that in IL-1β-treated WAG rats: 18.3% *vs.* 26.8%. The differences, however, were statistically insignificant.

After acute ES, the relative mass of spleen in Wistar rats given normal saline positively correlated with the number of appearances in the center and the number of droppings in the open field, and in IL-1β-treated Wistar rats it correlated with the number of the appearances in the center and the number of explored "holes" ($p<0.05$). Thus, the decrease in the relative mass of spleen in acute ES is smaller in rats with higher motor activity in the open field.

Our findings indicate that ES decreases the relative mass of thymus, adrenals, and spleen in August, Wistar, and WAG rats, the decrease being more pronounced in the animals with low motor activity in the open fields test. Injection of IL-1β in the lateral brain ventricle diminishes the ES-induced decrease in the relative mass of spleen in August and Wistar rats, has no significant effect on the relative mass of thymus in none studied rat population, and increases the relative mass of adrenals in unstressed WAG rats. The mechanisms of action of IL-1β will be elucidated in our further investigations.

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