Morphological Changes in Immune and Endocrine Organs of Stressed Mice after Administration of a Gonadotropin-Releasing Hormone Analogue

I. I. Bobyntsev, A. A. Dolzhikov, and L. A. Sever'yanova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 11, pp. 590-593, November, 2007
Original article submitted November 28, 2006

Administration of Surfagon, a gonadotropin-releasing hormone analogue, in doses of 0.1 and 5.0 μg/kg before emotional nociceptive stress increased lymphocyte migration from the thymus, decreased the volume of lymphoid tissue in the spleen and thymus, reduced the width of the zona fasciculata and increased the width of the zona glomerulosa in the adrenal cortex of male CBA mice. These effects of the peptide persisted in castrated animals. Surfagon prevented stress-induced activation of the adrenal glands and accidental transformation of the thymus and spleen in castrated animals.

Key Words: *gonadotropin-releasing hormone analogue; immune and endocrine systems; castration; stress*

Gonadotropin-releasing hormone (GnRH) and its analogues produce non-endocrine effects, which indicates that these compounds are involved in the interaction between regulatory systems of the organism (nervous, endocrine, and immune systems) [4-8,11]. GnRH analogue Surfagon produces a strong modulatory effect on the immune system under basal conditions and during emotional nociceptive stress [2,3]. Morphological study of immune and endocrine organs in unstressed animals receiving repeated injections of the peptide revealed significant structural changes [1]. Surfagon possesses stress-limiting activity [2,5]. Hence, it is important to perform similar morphological study under conditions of stress exposure.

Here we studied structural changes in the thymus, spleen, and adrenal glands of stressed mice after repeated administration of Surfagon.

Department of Pathophysiology, Kursk State Medical University. ***Address for correspondence:*** bobig@mail.ru. I. I. Bobyntsev

MATERIALS AND METHODS

Experiments were performed on male CBA mice weighing 20-22 g. The mice were divided into 8 groups of 10 animals each. Surfagon (pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-ethylamide) was synthesized at the Laboratory of Peptide Synthesis (Russian Cardiology Research-and-Production Center, Russian Ministry of Health). The peptide was injected intraperitoneally in daily doses of 0.1 and 5.0 μg/kg 15 min before stress (for 4 days). Control animals received an equivalent volume of physiological saline. Emotional nociceptive stress was induced daily at the same time of the day. Pairs of animals were subjected to gradually increasing stimulation in a chamber with electrified floor. The maximum voltage of electric current applied to the grid was 40 V. Two consecutive stimulations (total time 2 min) were performed with a 1-min interval.

The mice were killed by ether overdose. The weight index of the thymus, spleen, and adrenal

glands was calculated as the ratio of the weight of the organ to body weight (mg/g). The organs were fixed in 10% formalin. The material was imbedded into paraffin. Sections were stained with hematoxylin and eosin. Serial sections of the adrenal glands and thymus were prepared in the frontal plane. Serial sections of the spleen were made by cutting through the middle part of the pulp and tangentially to the surface. Morphometry was carried out on digital images of microstructures. They were obtained using a Biomed-2 microscope equipped with a standard adapter and digital cameras Nikon Coolpix 4500 and RoverShot- 515z. The data were processed with WCIF Image J-1.341 software (National Institute of Public Health, USA). We measured the diameter of splenic follicles, width of zones in the adrenal cortex, width of the cortex of the thymus, and diameter of thymic corpuscles (Hassall's corpuscles). Each series involved at least 30 measurements of each object. Midsections were used for morphometry of the adrenal glands. Splenic follicles were assayed only when they contained transverse arterial sections.

Experiments were performed on intact and castrated animals. The mice were castrated through midsection of the scrotum under hexenal anesthesia. Study was conducted 12 days after surgery.

The results were analyzed by Student's *t* test.

RESULTS

Morphological changes in animals after emotional nociceptive stress corresponded to reconstruction of lymphoid organs and adrenal glands due to stress-induced accidental transformation of the thymus, hyperactivity of the adrenal glands, and hyperplasia of peripheral lymphoid tissue (Table 1). The structure of splenic follicles in stressed mice was similar to that in unstressed animals. Nonspecific changes mainly included red pulp plethora, which is consistent with published data [7]. Most significant structural changes were observed in the thymus and manifested in a 3-fold increase in the diameter of Hassall's corpuscles in the medullary layer.

Significant morphometric changes in the adrenal glands included widening of the zona glomerulosa and, particularly, of the zona fasciculata and reticularis (by 53%) due to vacuolization of cortical cells and increased blood filling. Significant activation of this zone can be associated not only with increased synthesis of glucocorticoids, but also with activation of the zona reticularis after repeated stress [6].

Changes in the thymus and spleen of animals receiving Surfagon in both doses manifested in a significant decrease in the volume of lymphoid tissue. These changes were most pronounced after administration of the drug in high dose (by 65 and 23%, respectively). Structural changes in the adrenal glands of mice differed from those in control stressed animals. The diameter of splenic follicles significantly decreased. These follicles had a homogenous structure without reactive sites. The width of the thymic cortex in these mice decreased compared to that in unstressed and stressed animals. The structure of the thymic cortex corresponded to stages 1-2 of accidental transformation. The medullary layer included a considerable number of dilated postcapillary venules that were filled with lymphoid cells. These changes reflected migration of lymphocytes from the thymus. The width of the zona fasciculata in treated mice decreased compared to the control, but remained higher than in unstressed animals. The width of the zona glomerulosa significantly increased. Vacuolation and edema of the cytoplasm and partial decomplexation were observed in cells of the zona glomerulosa. Blood filling was maximum in the adrenal medulla.

The animals castrated after stress exposure and receiving Surfagon in both doses were characterized by specific histological signs of the thymus and adrenal glands.

Morphometric parameters of the spleen in the control group of stressed castrated mice did not differ from those in the control group of stressed intact animals. Structural features of follicles included enlargement of reactive sites and reduction of the lymphoid composition in the red pulp, which contrasted with the general pattern of the parenchyma. The absence of well-formed mantle zone reflected predominance of lymphoid cell proliferation over differentiation and migration in this area.

Structural and quantitative characteristics of the thymus were similar in stressed intact and castrated animals receiving Surfagon in a dose of 5 μg/kg. Quantitative and qualitative characteristics of the adrenal glands in mice corresponded to those in unstressed castrated animals. They included the maximum width of the zona fasciculata in the cortex and plethora, which was consistent with the highest relative weight of the adrenal glands.

Morphological characteristics of organs in castrated animals were studied after injection of Surfagon in low and high doses. Castration and Surfagon treatment prevented stress-induced activation of the adrenal glands and accidental transformation of the thymus and spleen. Structural characteristics of the spleen included the presence of well-formed follicles and reduction of the lymphoid composition in the red pulp. As differentiated from intact con-

TABLE 1. Morphological Changes in the Thymus, Spleen, and Adrenal Glands after Surfagon Administration (*M±m*)

Parameter	Intact				Castrated			
	unstressed	control	Surfagon, 0.1 μg/kg	Surfagon, 5 μg/kg	unstressed	control	Surfagon, 0.1 μg/kg	Surfagon, 5 μg/kg
Body weight index, mg/kg	0.019±0.072	0.662±0.07^{++}	0.936±0.087*	1.024±0.109**	1.734±0.125	1.509±0.066^{+}	1.822±0.077**	1.776±0.074
Width of thymic cortex, μ	296.6±2.6	301.0±1.7	169.2±2.8***	105.8±1.6***	116.3±2.8	185.1±2.5^{+}	250.2±1.7***	295.4±2.6***
Diameter of Hassall's corpuscles, μ	16.8±0.5	41.9±0.7^{+++}	12.8±0.4***	12.7±0.5***	13.5±0.3	16.3±0.5^{+++}	46.4±0.8***	16.7±0.5
Spleen weight index, μ	3.25±0.14	3.67±0.1+	3.84±0.12	3.54±0.12	3.81±0.11	4.27±0.1^{+}	4.36±0.26	4.19±0.08
Diameter of splenic follicles, μ	298.3±2.5	300.7±1.9	266.2±1.9***	232.6±5.6***	292.2±2.4	292.2±2.5	293.5±2.5	294.9±2.5
Adrenal glands weight index, mg/g	0.225±0.016	0.313±0.028^{++}	0.327±0.055	0.380±0.06	0.263±0.02	0.520±0.04^{+++}	0.384±0.033**	0.497±0.28
Width of the zona glomerulosa, μ	34.6±0.4	39.4±0.6^{+++}	40.7±0.7	52.7±0.7***	12.6±0.5	35.0±1.2^{+++}	25.2±0.7	34.8±0.4
Width of the zona fasciculata, μ	129.9±0.9	267.9±1.0^{+++}	268.0±0.7	196.8±0.7***	198.2±1.0	268.6±0.7^{+++}	187.7±2.4***	130.2±0.8***

Note. $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ compared to the corresponding control; $^{+}p<0.05$, $^{++}p<0.01$, and $^{+++}p<0.001$ compared to the corresponding group of unstressed animals.

trols, structures of the B-zone prevailed in the white pulp (reactive sites). We revealed narrow mantle zones and poorly-formed periarterial lymphatic sheaths. Morphometric parameters and total structure of the thymic parenchyma of mice did not differ from those in intact animals. Surfagon in high dose had a stronger effect on structural characteristics of the thymus. Large Hassall's corpuscles were identified after injection of the drug in low dose. The width and lymphoid density of the cortex in these mice were lower compared to intact animals. After injection of Surfagon in high dose Hassall's corpuscles were small and hardly detectable, and structural characteristics of the thymus did not differ from the control. These specific features corresponded to the recovery after early stages of accidental transformation or prevention of stress-induced changes in the thymus due to activation of the reticuloepithelium and compensatory lymphopoiesis in the thymus.

We conclude that repeated administration of GnRH analogue to stressed mice is accompanied by significant changes in organs of the immune and endocrine system. These changes sometimes play a stress-limiting role. Surfagon was most potent in preventing the development of stress-induced changes in the thymus. The thymus has high density of receptors that bind the molecule of GnRH or its analogues [12]. Changes in the spleen were less significant than in the thymus. Morphological changes in the adrenal glands are probably related not only to the stress response, but also to the Surfagon-induced inhibition of stress-realizing endocrine mechanisms. The effects of this peptide persisted after castration and, therefore, could be realized without involvement of sex hormones.

Our results and published data indicate that structural changes in immune organs in the basal state [1] and during stress exposure correspond to variations in immunological reactivity under similar conditions [2,3]. It should be emphasized that the morphogenetic effects of this peptide persist in stressed animals. These data show that the hypothalamic-pituitary-gonadal system plays an important role in the stress response of the organism.

REFERENCES

1. I. I. Bobyntsev, A. A. Dolzhikov, and L. A. Sever'yanova, *Byull. Eksp. Biol. Med.*, **139**, No. 1, 116-120 (2005).
2. I. I. Bobyntsev and L. A. Sever'yanova, *Ibid.*, **133**, No. 5, 504-506 (2002).
3. I. I. Bobyntsev, L. A. Sever'yanova, A. I. Konoplya, *et al.*, *Ros. Fiziol. Zh.*, **83**, No. 9, 1177-1181 (2002).
4. I. I. Bobyntsev, L. A. Sever'yanova, and A. A. Kryukov, *Byull. Eksp. Biol. Med.*, **141**, No. 2, 153-156 (2005).

5. I. I. Bobyntsev, L. A. Sever'yanova, M. Yu. Smakhtin, and A. A. Kryukov, *Ros. Fiziol. Zh*., **91**, No. 10, 1176-1181 (2005).
6. T. A. Obut, *Byull. Eksp. Biol. Med*., **118**, No. 7, 8-10 (1994).
7. O. D. Yagmurov and R. P. Ogurtsov, *Ibid*., **122**, No. 7, 64-67 (1996).
8. N. Azad, N. La Paglia, K. A. Jurgens, *et al*., *Endocrinology*, **133**, No. 1, 215-223 (1993).
9. T. Kadar, G. Telegdy, and A. V. Schally, *Physiol. Behav*., **51**, No. 3, 601-605 (1992).
10. B. Marchetti, F. Gallo, Z. Farinella, *et al*., *Ann. N. Y. Acad. Sci*., **840**, 205-248 (1998).
11. B. Marchetti, F. Gallo, Z. Farinella, *et al*., *Ibid*., **917**, 678-709 (2000).
12. B. Marchetti, V. Guarcello, M. C. Morale, *et al*., *Endocrinology*, **125**, No. 2, 1025-1036 (1989).