Immunotropic Effects of Gonadotropin-Releasing Hormone Analog under Conditions of Emotional Painful Stress

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

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 5, pp. 504-506, May, 2002
Original article submitted March 11, 2002

Surfagon, a synthetic analog of gonadotropin-releasing hormone injected before emotional painful stress dose-dependently changed the number of antibody-producing cells in rats and phagocytic and functional activities of neutrophils in mice. In castrated animals this peptide increased all studied parameters. This suggests that sex steroids are not involved in the realization of these effects.

Key Words: *analog of gonadotropin-releasing hormone; immune response; emotional painful stress; phagocytosis; functional activity of neutrophils*

Interrelationships between the nervous, endocrine, and immune systems attract much recent attention [1,7]. However, the role of the hypothalamic-pituitary-gonadal system in these regulatory interactions is little studied. We previously demonstrated a pronounced effect of surfagon, a highly active gonadotropin-releasing hormone agonist, on animal behavior under conditions of positive [11] and negative emotional reinforcement [9,10,14] and on immune response in rats [2]. Here we studied the effect of surfagon on humoral immune response, immune and endocrine organs, and phagocytic and functional activities of neutrophils under conditions of emotional painful stress.

MATERIALS AND METHODS

Experiments were carried out on adult male Wistar rats (180-200 g) and male CBA mice (22-25 g). The animals were divided into groups (10 animals each). Surfagon (pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-ethylamide) was synthesized at the Cardiology Research Center, Russian Academy of Medical Sciences. For modeling emotional painful stress the animals were placed in pairs in a cage with electrified floor. Electrical current (maximum voltage was 60 V for rats and 40 V for mice) was applied to the floor for 2 min (this period was divided into 2 stimulation series with 1-min interval).

In rats the immune response was evaluated by the number of antibody-producing cells (APC) in the spleen on day 5 after immunization with T-dependent antigen (sheep erythrocytes) [4]. The peptide was injected for 4 days after immunization intraperitoneally in daily doses of 0.1 and 5 μg/kg (in stressed animals 15 min before stress). Controls were injected with an equivalent volume of normal saline. For evaluation of the effects of stress, non-stressed animals were injected with normal saline and their parameters were compared with those of stressed controls.

For evaluation of functional and phagocytic activities of neutrophils, the mice were intraperitoneally injected with the peptide for 4 days (daily doses 0.1 and 5 μg/kg). Neutrophils were isolated from peritoneal exudate induced by intraperitoneal injection of 0.5 ml 10% sterile peptone 2.5 h before cell isolation [4]. Functional activity of neutrophils was evaluated under microscope by the percentage of diformazan-positive cells in spontaneous and stimulated NBT test [3]. Vaccine from *S. marcescens* strain served as the stimulatory factor. Phagocytic activity of neutrophils was evaluated after 10-min incubation with *Staphylococcus albus* for 30 min at 37°C [4]. Phagocytic number (number of bacteria captured by one neutrophil)

Department of Pathological Physiology, Kursk State Medical Institute.
Address for correspondence: big@ksmu.kursknet.ru. Bobyntsev I. I.

0007-4888/02/1335-0436$27.00

and phagocytic index (percentage of neutrophils participating in phagocytosis) were evaluated on Romanowskii-stained smears. Mouse thymus, spleen, and adrenals were weighed. The schedule of peptide injections was similar to that used in studies of the immune response.

The experiments were carried out on intact and castrated animals. Castration was carried out under hexenal narcosis through median scrotal incision [5]. The animals were used in experiment 12 days after intervention.

The significance of differences was evaluated using Student's *t* test.

RESULTS

Comparison of the test parameters in control groups showed that stress significantly (by 65%) decreased the number of APC (Fig. 1). The effect of surfagon on the immune response in intact animals depended on its dose: low dose increased and high dose decreased the number of APC. In castrated animals both doses produced an immunostimulatory effect.

Functional and phagocytic activities of neutrophils decreased in controls exposed to stress compared to unstressed controls (Table 1). The weight of the thymus decreased and that of the spleen and adrenals increased, which is typical of stress reaction. In animals receiving surfagon the weight of the thymus did not differ from that in unstressed animals. Surfagon had no effect on the weights of the spleen and adrenals. Functional and phagocytic activities of neutrophils increased significantly after the lower dose of the peptide and slightly decreased after the higher dose.

Comparison of intact and castrated controls showed a significant increase in the weights of the studied organs after castration and the absence of appreciable differences in the neutrophil activity. Surfagon increased the weight of the thymus in castrated animals (similarly to that in intact mice) and did not change the weight of the spleen. The lower dose of surfagon significantly increased the weight of the adrenals. Phagocytic and functional activity of neutrophils increased after both surfagon doses. The difference between spontaneous and stimulated NBT test reflecting functional reserve of neutrophils decreased by 27% in intact stressed controls and returned to the initial level after injection of surfagon in a dose of 0.1 μg/kg (Table 1). In castrated mice this parameter was virtually the same in the control and experimental animals.

Our experiments demonstrated a pronounced effect of surfagon on the humoral immune response and neutrophil activity under conditions of emotional painful stress. When analyzing the mechanisms of realization of immunotropic effects of surfagon, high endo-

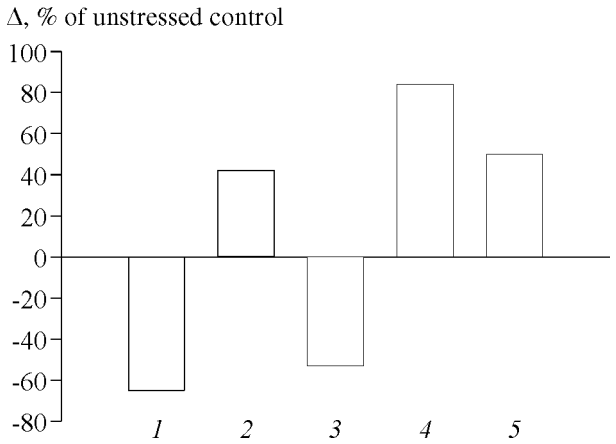

Fig. 1. Effect of surfagon on the number of antibody-producing cells in rats under conditions of emotional painful stress. *1*) stressed controls; *2*, *3*) intact rats; *3*, *4*) castrated rats. Surfagon doses 0.1 (*2*, *4*) and 5 μg/kg (*3*, *5*). All differences from unstressed control are significant.

TABLE 1. Parameters of Neutrophil Activity and Weights of Organs in Stressed Animals Receiving Surfagon (*M*±*m*, *n*=10)

Parameter	Intact				Castrated		
	unstressed control	control	surfagon, μg/kg		control	surfagon, μg/kg	
			0.1	0.5		0.1	0.5
Thymus, mg	23.3±1.5	15.2±1.8^{+}	21.2±2.1*	23.7±2.8*	34.7±1.9$^{?}$	42.0±2.2*	41.0±2.2*
Spleen, mg	74.4±3.3	84.0±2.6^{+}	86.4±2.6*	80.9±2.3	97.2±1.8$^{?}$	93.3±1.9	96.3±2.0
Adrenals, mg	5.1±0.4	7.1±0.6^{+}	7.3±1.2	8.8±1.5	11.9±0.8$^{?}$	8.8±0.8*	11.2±0.7
NBT test, %							
spontaneous	16.2±0.8	10.5±1.1^{+}	14.9±1.7*	8.2±1.0	8.8±1.2	17.3±2.5*	14.4±2.0*
stimulated	30.9±1.4	17.2±1.6^{+}	28.3±3.1*	14.2±1.7	14.5±1.6	27.9±3.6*	21.7±2.9*
Phagocytic index, %	39.8±3.6	24.1±2.7^{+}	36.7±3.9*	18.2±3.0	20.8±2.6	30.4±2.9*	25.4±2.4
Phagocytic number	2.0±0.3	1.5±0.2	1.9±0.3	1.3±0.2	1.4±0.2	2.1±0.3	1.8±0.2

Note. Differences significant in comparison with: *stressed control; $^{+}$unstressed control; ^{o}noncastrated control.

crine [8] and neurotropic activity of the peptide should be taken into consideration. Surfagon modulated pain sensitivity and emotional reinforcement systems in the brain under conditions of emotional painful stress [9, 10,14]. The peptide showed high neurotropic activity in behavioral experiments with positive emotional reinforcement [11]. The fact that surfagon induces functional shifts in the nervous and endocrine systems suggests that this peptide can modulate the state of the immune system [1,7]. Opposite changes in the studied parameters produced by different doses of the peptide in intact animals can be attributed to its different effects on brain functions: predominant activation of the positive reinforcement system after low dose and activation of the negative reinforcement system after high dose. Gonadotropin secretion is also modulated by surfagon: low doses (about 0.1 μg/kg) stimulated secretion of follicle-stimulating hormone, while high dose (5 μg/kg) stimulated secretion of both luteinizing and follicle-stimulating hormones [8]. Moreover, a direct effect of surfagon on the immune system cannot be ruled out [6]. The observed increase in thymus weight in castrated animals is confirmed by published data [12] and is a result of increased cellularity and enhanced secretory activity of the thymus. These changes can be due to the absence of testosterone, which decreases the number of thymocytes [13]. At the same time, the increase in thymus weight after injection of surfagon in both intact and castrated animals can be mediated by other mechanisms directly associated with the effects of gonadotropin-releasing hormone and pituitary gonadotropins [12].

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