Morphological Changes in Immune and Endocrine Organs of Mice Injected with a Gonadotropin-Releasing Hormone Analog

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Infection of surfagon (gonadotropin-releasing hormone analog) in a dose of 0.1 µg/kg to male CBA mice stimulated lymphocyte migration from splenic B zones, caused moderate thinning of the thymic cortex, thickening of the zona fasciculata of the adrenal cortex with signs of corticocyte activation. After administration of 5 µg/kg surfagon we observed alteration of the splenic T zone, drastic thinning of the thymic cortex, and extension of the zona glomeruloza in the adrenal cortex. These effects were retained after castration, which attests to their steroid-independent nature.

Key Words: *gonadotropin releasing hormone analog; thymus; spleen; adrenals; castration*

Previously we revealed pronounced neurotropic [3,4] and immune [1,2] effects of surfagon, a highly active analog of gonadotropin-releasing hormone (GRH). High biological activity of surfagon towards the regulatory systems of the body prompted investigation of possible structural changes in the immune and endocrine organs after injection of the peptide. One more important aspects necessitating this study is wide use of GRH analogs in clinical practice.

We studied morphological changes in the thymus, spleen, and adrenals of mice after repeated injections of surfagon.

MATERIALS AND METHODS

Experiments were carried out on male CBA mice (20-22 g) divided into groups (10 animals per groups). Surfagon (pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-ethylamide) was synthesized in Cardiology Research Center, Russian Academy of Medical Sciences. The peptide was injected intraperitoneally in doses of 0.1 and 5.0 µg/kg once a day for 4 days. Controls received an equivalent volume of saline.

The mice were sacrificed by ether narcosis and weight indexes of the thymus, spleen, and adrenals (organ/body weight ratio) were calculated. For further studies, the organs were fixed in 10% formalin, embedded in paraffin, and the sections were stained with hematoxylin and eosin. Serial sections of the adrenals and thymus were made in the frontal plane, sections of the spleen through the central part of the pulp tangentially to the surface. In order to make the morphological data more objective, the diameters of splenic follicles, thickness of adrenocortical zones and thymic cortex, and diameters of thymic bodies (Hassal bodies) were measured using a MOB-1-15x screw ocular monometer. At least 30 measurements of each object were made in each experimental series. Morphometry of the adrenals was performed on median sections, splenic follicles were measured only if they contained strictly transverse sections of arteries.

Experiments were carried out on intact and castrated animals. Castration was carried out under hexenal narcosis through the medial incision in the scrotum. The animals were taken into experiment 12 days after surgery.

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

RESULTS

Morphological changes after surfagon injection to intact animals were dose-dependent: shifts in the main morphological parameters of all examined organs were observed after the lower dose and only in the thymus after high dose.

The peptide in a dose of 0.1 μg/kg significantly (by 17%) decreased the diameter of splenic follicles and changed their structure (lymphocyte devastation of the mantle and central zones). Due to this splenic pulp acquired a more uniform structure with well discernible lymphoid accumulations only around arteries (Fig. 1, *a*). Together with the decrease in the organ weight (Table 1), these shifts reflect enhanced migration of lymphoid elements, primarily from B zones.

Morphological changes in the thymus corresponded to stage 1 of accidental transformation, manifesting in significant (21%) weight loss of the organ and characteristic microscopic signs, such as thinning (by 46%, $p<0.05$) and uneven thickness of the cortex with extensive loose zones, as well as relative increase in the volume of the medulla (Fig. 1, *b*). No appreciable
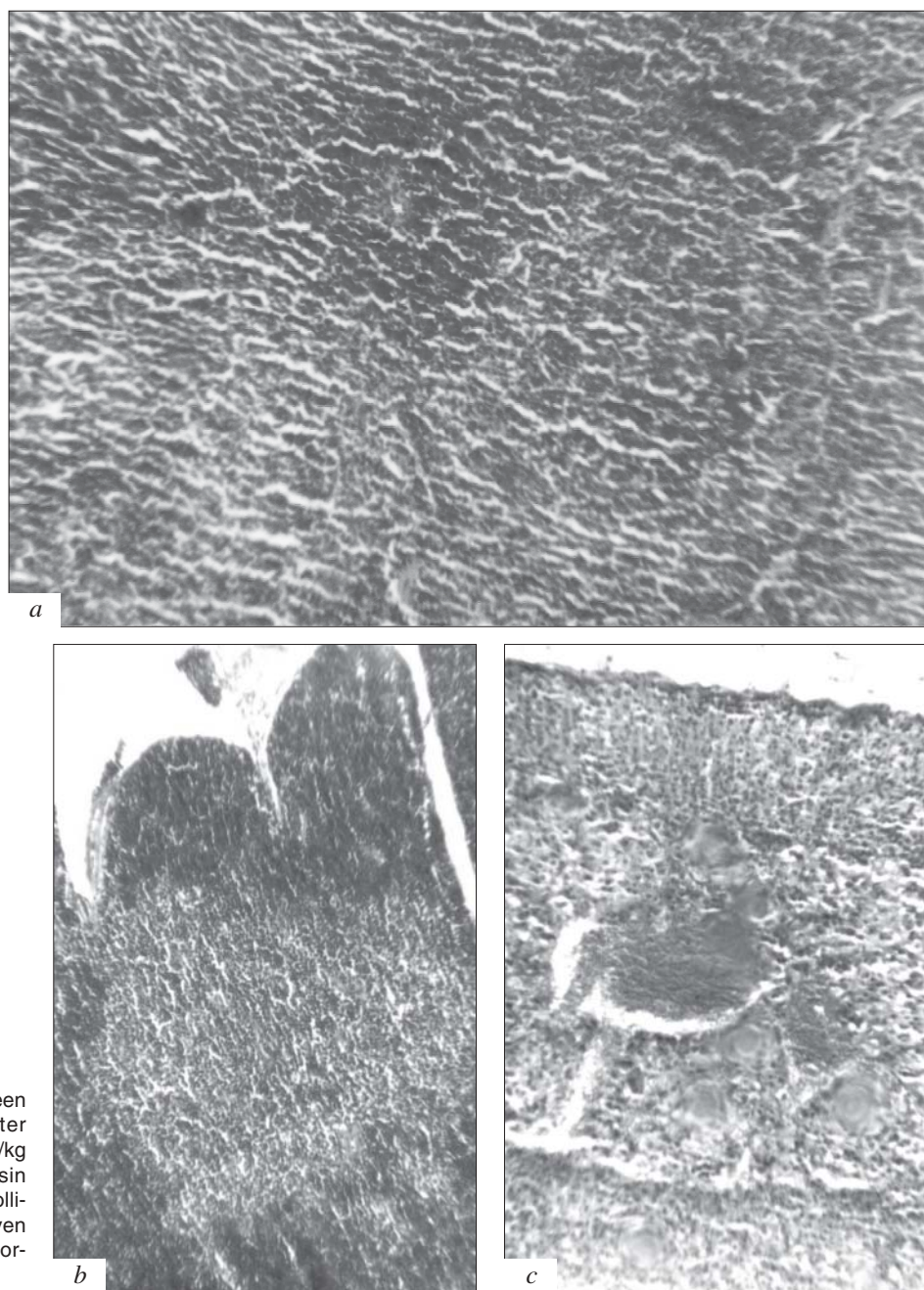

Fig. 1. Morphological changes in the spleen (*a*), thymus (*b*), and adrenals (*c*) after injection of surfagon in a dose of 0.1 μg/kg to intact animals. Hematoxylin and eosin staining, ×200. *a*) blurred structure of follicles, narrow periarterial zones; *b*) uneven width, lymphoid devastation of thymic cortex; *c*) plethora of the adrenal medulla.
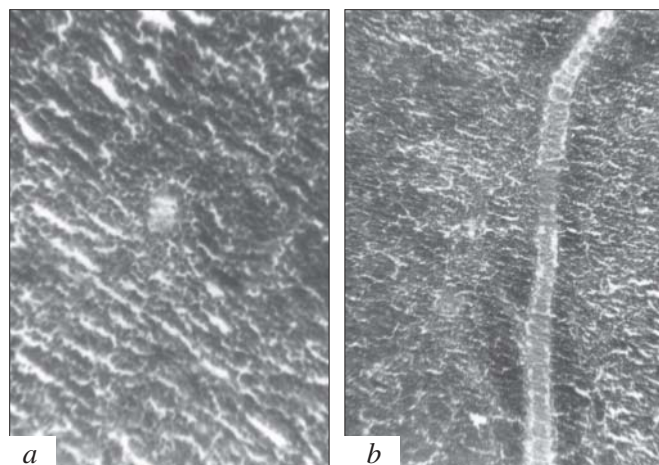
Fig. 2. Morphological changes in the spleen (*a*) and thymus (*b*) after injection of 5 µg/kg surfagon to intact animals. Hematoxylin and eosin staining, ×200.

differences in the reticuloepithelial elements and Hassal's bodies were observed. According to microscopic examination the increase of the adrenal weight (Table 1) was due to nonspecific plethora of the cortex and medulla (Fig. 1, *c*) and to changes in the cortical structure, more significant in the zona fasciculata. These changes consisted in a significant extension of the width of this zone (by 22%), which was associated with signs of corticocyte activation presenting as their enlargement at the expense of abundant intensely stained cytoplasm; in a lesser part of cells it contained small vesicles and was vacuolated.

After injection of high dose of surfagon splenic weight index virtually did not change, the pulp was characterized by diffuse pattern, high cell density, though without formation of clear-cut follicles (Fig. 2, *a*). Periarterial T zones were disperse. Progressive changes in the thymus in this experimental series were justified and corresponded to the picture of accidental transformation of lymphoid organs (Fig. 2, *b*). The mean thickness of the cortex was more than 3-fold

thinned, its structure was characterized by unclear interface, vast foci of lymphocytic devastation, areas with signs of inverted distribution of lymphoid elements, which was intrinsic of stages 2-3 accidental transformation. No formed Hassal's bodies were detected in the medulla. Slight, but significant widening of the zona glomeruloza was seen in the adrenals.

Hence, morphological changes in the studied organs were different in intact animals from different experimental groups. The lower dose of the peptide stimulated lymphocyte migration from the splenic B zones, led to moderate thinning of the thymic cortex, and widening of the bundle zone of the adrenals with signs of corticocyte activation. High dose induced changes in the splenic T zones, a drastic thinning of the thymus, and extension of the adrenal glomerular zone.

Changes in the lymphoid organs and adrenals of castrated animals were characterized by the above mentioned increase in the relative weights and by the relevant morphometrical shifts. No appreciable changes in the follicle diameter were detected in the spleen in comparison with intact controls, but follicular structure was now characterized by compact disposition of lymphoid elements, absence of zonation, and by moderate lymphoid infiltration of the adjacent red pulp zone (migration zones). The red pulp was characterized by predominantly myeloid cellular composition, which, together with the above-described follicular features, resulted in a more definite pattern of the organ parenchyma. The thickness of the thymic cortex decreased more than 2-fold; signs of early stages of accidental transformation were noted. The width of the glomerular zone in the adrenals decreased 2.5 times, while the width of the bundle zone increased, the cortical matter pattern was blurred, and diffuse venous plethora with foci of perivascular edema was observed in the medulla.

Injection of surfagon to castrated animals most significantly modified the structure of the thymus and spleen. The time course of changes in the morpho-

TABLE 1. Morphological Changes in the Thymus, Spleen, and Adrenals after Injection of Surfagon ($M \pm m$, $n=10$)

Parameter	Castrated			Intact		
	control	0.1 µg/kg	5 µg/kg	control	0.1 µg/kg	5 µg/kg
Thymus weight index, mg/g	1.019±0.072	0.803±0.051*	0.633±0.105**	1.734±0.125+++	1.307±0.157*	1.594±0.099
Thymic cortex width, µ	296.6±2. 6	160.3±1.5***	94.3±1.1***	116.3±2.8+++	295.2±2.6***	187.6±2.7**
Splenic weight index, mg/kg	3.25±0.14	3.06±0.11	3.26±0.05	3.81±0.11++	3.57±0.15	3.28±0.16*
Splenic follicle diameter, µ	298.3±2.5	246.1±2.0**	30 2.1±1.8	299.2±2.4	254.9±1.4*	265.9±2.2*
Adrenal weight index, mg/kg	0.225±0.016	0.280±0.02*	0.281±0.017*	0.263±0.02	0.367±0.013***	0.357±0.018**
Width of zona glomerulosa, µ	34.6±0.4	34.9±0.5	37.2±0.3*	12.6±0.5+++	13.8±0.4	34.8±1.1***
Width of zona fasciculata, µ	129.9±0.9	158.6±1.4*	131.6±0.8	198.2±1.0+++	196.8±3.1	186.0±2.4*

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to the control; +$p<0.05$, ++$p<0.01$, +++$p<0.001$ compared to intact animals.

metric parameters of lymphoid structures of these organs was dose-dependent and opposite: in the spleen the follicle diameters were close to those in intact animals after a high dose of the peptide, while the parameters of thymic lymphoid tissue were similar to the values in intact animals after administration of the lower dose. In the adrenals the complex of nonspecific changes was in general the same, but after surfagon dose of 5 µg/kg the zonal structure of the cortex was better restored and the width of the zona glomerulosa corresponded to that in intact controls. The width of the zone fasciculata decreased significantly, while that of the zona glomerulosa increased 2.5 times. The same picture of surfagon effects in the studied organs after castration indicated that these effects were presumably realized without participation of sex steroids.

Hence, repeated injections of surfagon, a highly active GRH analog, caused appreciable morphological changes in the thymus, spleen, and adrenals. Pronounced structural shifts in the thymus after injection of surfagon and castration confirmed a close relationship between this organ and the hypothalamic-pituitary-gonadal system (HPGS) [10,11,13]. For example, specific binding to receptors in the thymus was detected for the natural GRH molecule and for its highly active analogs. Long-term treatment with GRH agonists restored age-associated thymic weight loss, number of GRH receptors, and proliferative activity of T lymphocytes in rats [7]. GRH and its analogs modulated peptide factor production by thymocytes [6] and stimulated their proliferative response to Con A mitogen [8]. Rat thymocytes produce GRH identical to the hypothalamic peptide by amino acid composition, biological activity, and antigenic characteristics [9].

The changes detected in the spleen were less pronounced than in the thymus. Injection of the peptide caused a significant decrease in the organ weight and width of the follicle diameter in intact and castrated animals. These shifts can result from various effects of HPGS on lymphoid organs [5], specifically, on the formation and migration of B cells [10,14].

Our results can be explained by not only changes in the HPGS function, but also other endocrine mechanisms. This assumption arises from findings detected in different adrenal zones.

In general, morphological shifts in immune organs are in line with our previous findings, when the peptide appreciably modulated the immune response parameters under similar conditions of treatment [1,2]. Hence, surfagon induces functional and appreciable morphological changes in immune organs. High polyfunctional biological activity of this GRH analog towards the regulatory systems of the body (nervous, endocrine, and immune) seems to underlie its effects. Our results confirm the important role of regulatory peptides in the interactions between these systems.

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