Disorders in the Secretory Cycle of Follicular Thyrocytes and Their Correction with Thyrotropic Hormone in Experimental Non-Thyroidal Illness Syndrome

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The influence of LPS on the thyroid gland leads to more intensive synthesis and release of thyroglobulin into the follicle lumen and inhibition of its resorption and proteolysis, which reduces the production of thyroxine. Treatment with thyroid-stimulating hormone normalizing the secretory processes in the follicular thyrocytes is a pathogenetically justified method for correction of non-thyroidal illness syndrome in acute endotoxicosis.

Key Words: *thyroid gland; non-thyroidal illness syndrome; endotoxicosis; therapy*

Non-thyroidal illness syndrome, developing in severe infections, including sepsis, is now regarded as one of the lethal outcome risk factors, and hence, the need of its correction is admitted by many specialists. Three main methods for restoration of the patient's thyroid status are used: injection of thyrotropin releasing hormone, of thyroid-stimulating hormone (TSH), or substitute thyroxine and triiodothyronine therapy [2]. The choice of the method for correction of non-thyroidal illness syndrome is a difficult task for clinicians, because its pathogenesis in various diseases is little studied. Studies of the morphofunctional changes and disorders in the thyrocyte secretory cycle in the thyroid gland (TG) in non-thyroidal illness syndrome are extremely rare.

We tried to develop a pathogenetically valid method for correction of non-thyroidal illness syndrome in acute experimental endotoxicosis, based on studies of the thyroid status and morphofunctional characteristics of the secretory process in follicular thyrocytes.

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MATERIALS AND METHODS

Acute endotoxicosis was modeled in male Wistar rats (n=80) by a single intraperitoneal injection of *E. coli* LPS in a dose of 10 mg/kg dissolved in 100 μl saline. After 24 h half of animals received intraperitoneal injection of TSH (0.01 U/kg dissolved in 100 μl saline). Controls (n=20) were injected with the same volume of saline. Experimental and control animals were sacrificed simultaneously by zoletil overdosage 1, 3, 6, 12, 24, and 48 h after TSH injection. Serum concentrations of TSH, thyroxine (T4), and triiodothyronine (T3) were measured by EIA using commercial kits (Monobind). The morphology of TG was studied by light microscopy and computer morphometry using ImageProPlus (Leica Microsystems) software and by transmission electron microscopy (Libra 120 microscope, Carl Zeiss). The follicle section areas, percentage of thyrocyte and colloid areas in follicles, percentage of colloid in follicle lumen, height of follicular thyrocytes, and areas of their nuclei were evaluated separately for the central and peripheral zones of TG lobes constituting one-third and two-thirds of the lobe, respectively.

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Statistical processing was carried out using parametric and nonparametric tests. The differences were considered significant at $p \leq 0.05$.

RESULTS

One day after injection of LPS, the rats developed non-thyroidal illness syndrome characterized by reduced T4 and TSH concentrations in the serum (Table 1). Histological study of TG showed a pronounced uneven plethora of blood vessels with cell stasis and sludge, mild stromal edema, and discomplexation of collagen fibrils. Desquamation of follicular thyrocytes into the follicle lumen was less pronounced than in controls.

A significant reduction (p=0.0061) of the follicle size in comparison with the control was found in the central segments of TG lobes. The percentage of the epithelium in the follicles did not differ from the control, while colloid content in the follicle lumen increased (p=0.010). A significant (p=0.0015) decrease in the follicular thyrocyte height in comparison with the control was found. The percentage of thyrocyte nuclei was the same as in the control.

The follicles in the peripheral zones of TG lobes were larger than in the central zone and in the peripheral zone of controls (p=0.010). The share of the epithelium, content of colloid in the follicles, and height of follicular thyrocytes were similar to those in the control. The follicular thyrocyte nuclei were significantly smaller (p=0.0) than in the control.

Electron microscopy of the peripheral and central zones of TG lobes revealed pronounced disorders in the microcirculation: stasis of formed elements, drastic stenosis of the capillary lumens and pericapillary space. Active synthesis of protein was seen in the follicular thyrocytes. Tubules of the granular endoplasmic reticulum and cisterns and vacuoles of the Golgi complex were dilated. Numerous secretory granules and lysosomes with osmiophilic matrix of high electron density were detected (Fig. 1, *a*). The number of lysosomes in the peripheral zone thyrocytes was greater than in the central zone. The number of mitochondria with swelled clarified matrix and almost completely destroyed cristae increased. Dying mitochondria and lysosomes often formed autophagolysosomes. Numerous secretory granules were found under the apical plasmalemma of follicular thyrocyte. Clarification of the microvillous matrix was noted, a morphological sign of fluid resorption from colloid. Solitary colloid droplets of different sizes were found in the thyrocyte cytoplasm. Euchromatin predominated in the follicular thyrocyte nuclei; the nucleoli were hypertrophic and ectopic. Analysis of the follicular thyrocyte ultrastructure in the central and peripheral zones showed that synthetic processes in the central were less active than in the peripheral zone and the formation of lysosomes was more intense than the formation of secretory granules.

These morphological changes indicated heterogeneous reaction of the central and peripheral zones of TG lobes. The follicles were smaller and the synthetic and resorption processes balanced in the central *vs.* peripheral zones. Pronounced imbalance of these processes was found in the peripheral zones. The majority of follicular thyrocytes actively synthesized secretory and lysosomal proteins, despite pronounced energy deficiency because of microcirculatory disorders. No signs of thyroglobulin internalization were detected. The resorption processes were significantly less intensive than thyroglobulin secretion into follicle lumen, which led to stretching of follicles. Hence, TG dysfunction was caused by asynchronization of secretory phases of follicular thyrocytes consisting in stimulation of synthesis and release of thyroglobulin into the follicle lumen and its less intense resorption and cleavage, which led to reduction of serum T4 level. Follicular thyrocytes carry functionally active Toll-like receptors-4 binding LPS [1]. Lipopolysaccharide stimulates the expression of thyroglobulin mRNA in follicular thyrocytes *in vitro* [3]. Our data indicate that *in vivo* LPS stimulates not only thyroglobulin synthesis, but also its deposition in the follicle lumen, thus creating a reserve of the hormone precursor.

Morphological analysis of TG showed that the leading factor in the development of non-thyroidal illness syndrome was direct effect of LPS on TG, but not pituitary dysfunction. Reduction of TSH level augmented TG dysfunction caused by LPS. Direct injury to TG plays the key role in the development of LPS-induced syndrome. This fact is underrated by some scientists, regarding non-thyroidal illness syndrome as a nonspecific reaction caused by dysfunction of the hypothalamic-pituitary complex and reduction of deiodinase-1 activities [4,5].

Hence, use of thyroxine preparations for compensation for T4 deficiency is not pathogenetically justified. Injection of T4 leads to reduction of TG functional activity by the feedback mechanism. The first step of the syndrome correction should be arrest of the thyroglobulin resorption and proteolysis processes in the follicular thyrocytes. TSH is a natural stimulant of these processes. Choosing between TSH and stimulation of the pituitary gland with thyrotropin releasing hormone (TRH) for the syndrome correction, we have chosen TSH, because the observed reduction of the pituitary thyrotropic activity precluded sufficiently high production of TSH in response to TRH.

One hour after TSH injection (0.01 U/kg), serum concentration of T4 increased (p=0.0045), while T3 and TSH concentrations decreased. After 3 h, the

levels of T4 and T3 did not differ from those in the controls. The concentration of T4 did not decrease throughout 48 h after TSH injection, T3 concentration was even higher than in the controls (Table 1).

The size of follicles in the central zone of TG lobes did not change after TSH injection. In the peripheral zones of TG lobes, the follicles were smaller than in rats with acute endotoxicosis. The percentage of the epithelium in follicles did not change. The content of colloid in the follicle lumen reduced in general, but this process was not observed in all sites of TG peripheral zones. The height of follicular thyrocytes did not change in the peripheral zones of the lobes but decreased in the central zone. Thyrocyte nuclei shrank in size. Electron microscopy showed predominating euchromatin in the follicular thyrocyte nuclei of the peripheral zones. The nucleoli were enlarged, often shifted towards the nuclear membrane. Lysosomes and dilated tubules of the granular endoplasmic reticulum filled with floccular osmiophilic contents of moderate electron density were found in the perinuclear zone. Golgi complex cisterns and vacuoles were dilated and filled with osmiophilic material. The mitochondria were swollen, with clear matrix and partially destroyed cristae (Fig. 1, *c*). A moderate number of secretory granules was found at the apical terminals of the cells under the plasmalemma. Microvilli were dilated; signs of colloid endocytosis (peristaltic constrictions, matrix clarification) were seen. The microvilli were elongated, forming peculiar processes, which could be regarded as the initial stage of colloid macropinocytosis (Fig. 1, *b*). Colloid droplets, small and medium sized, were found in the cytoplasm, part of them forming contacts with lysosomes (Fig. 1, *c*). Dying cells with drastic edema and destruction of the granular endoplasmic reticulum, mitochondria, and colloid droplets were found. Similar changes were found in follicular thyrocytes of the central zone, but with some differences. For example, the mitochondrial involvement was less intense. The number of lysosomes was less than in the peripheral thyrocytes.

Hence, morphological changes in TG 1 h after injection of TSH to rats with acute endotoxicosis attested to intensification of colloid resorption. Thyroglobulin synthesis and resorption processes were pronounced and balanced.

Disorders in TG microcirculation persisted 3 h after TSH injection, but were less pronounced, which was good for the mitochondrial structure. Mitochondrial matrix edema and destruction of the cristae were less pronounced than in rats which received no TSH. Electron microscopy of follicular thyrocytes of the peripheral and central lobular zones showed dilatation of the granular endoplasmic reticulum, hypertrophic elements of Golgi complex, and greater numbers of secretory granules under the apical plasmalemma. Signs of not only thyroglobulin resorption, but also of its cleavage (contacts and fusion of colloid droplets with lysosomes) were seen.

Thyroglobulin synthesis and resorption processes in follicular thyrocytes were pronounced in the lobular central and peripheral zones 24 h after TSH injection. In the peripheral zones these processes were active and balanced. In the central zones synthetic processes predominated over resorption. Synthetic processes developed under conditions of energy deficit caused by microcirculatory disorders. The intensity of synthetic and resorption processes was higher than in rats with endotoxicosis receiving no TSH.

Vascular disorders and stromal edema regressed 48 h after TSH injection. The functioning of follicular thyrocytes in the central and peripheral zones was asynchronous. The synthesis and formation of secretory

TABLE 1. Changes in Serum Levels of Thyroid Hormones and TSH in Rats with Acute Endotoxicosis with and without TSH Treatment

Hormone	Time after injection, h											
	LPS						TSH					
	24	27	30	36	48	72	1	3	6	12	24	48
T4	0.65± 0.04*	0.900± 0.055	0.7± 0.1*	0.70± 0.03*	0.74± 0.052*	1.13± 0.07	0.90± 0.08	0.98± 0.01	1.037± 0.063	0.96± 0.084	1.18± 0.14	1.15± 0.10
T3	0.65± 0.04*	0.9± 0.1	0.70± 0.07*	0.700± 0.036*	0.740± 0.026*	1.13± 0.01	0.770± 0.052*	1.00± 0.06	1.21± 0.14	1.640± 0.146*	1.29± 0.04*	1.180± 0.025*
TSH	0.71± 0.08*	0.73± 0.01*	0.660± 0.033	1.100± 0.029	0.850± 0.053	1.600± 0.004*	0.36± 0.03*	0.42± 0.15*	0.66± 0.07*	0.850± 0.089	1.030± 0.014	0.89± 0.02

Note. Values in the control group were taken for 1 U. *Statistically significant difference from the control group ($p<0.01$).

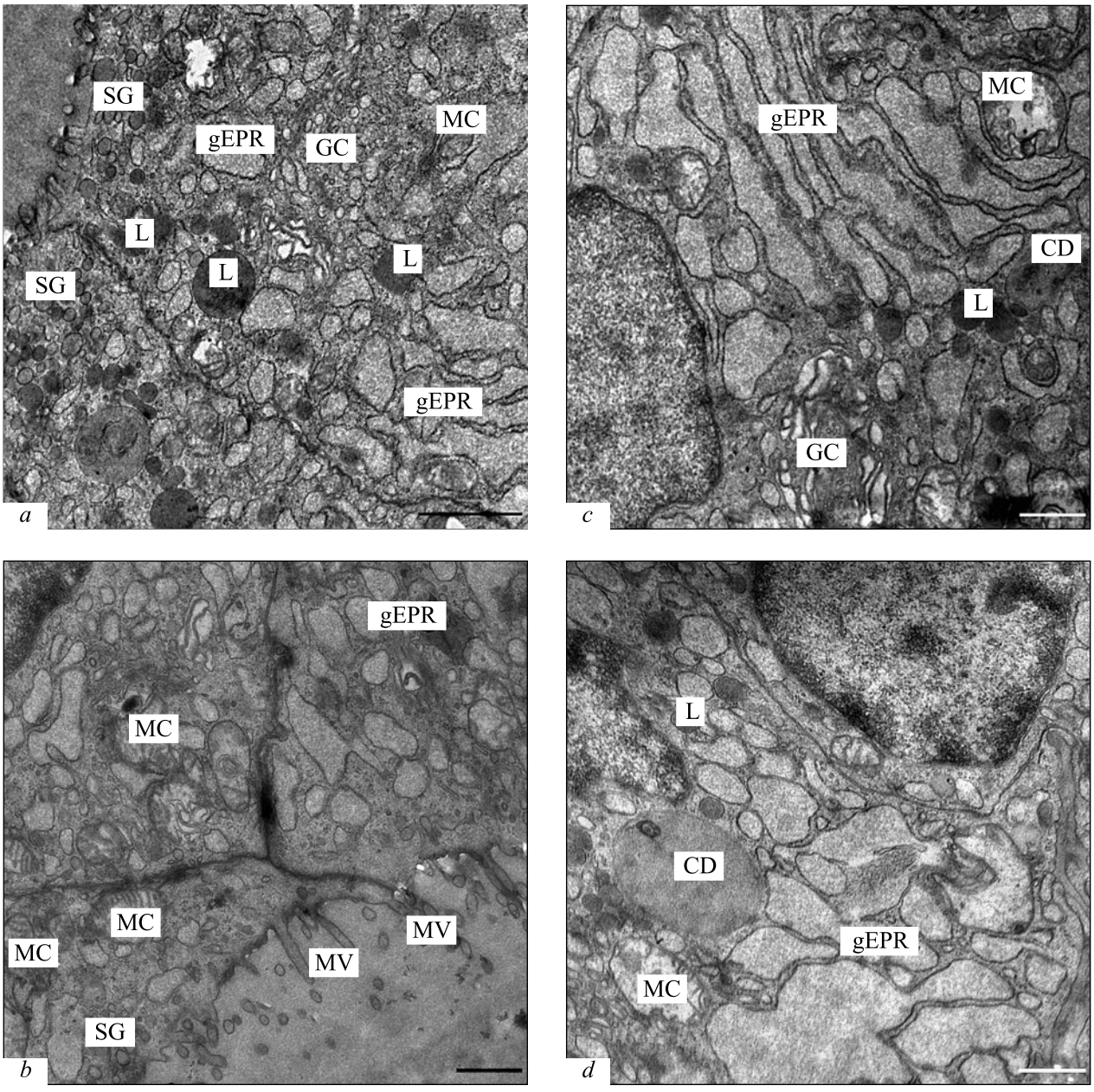

Fig. 1. Ultrastructure of follicular thyrocytes in acute endotoxicosis. *a*) 24 h after LPS injection, ×5000; *b*, *c*) 1 h after TSH injection, ×4000, 5000; *d*) 48 h after TSH injection, ×5000. gEPR: granular endoplasmic reticulum; GC: Golgi complexes; CD: colloid droplets; L: lysosomes; MV: microvilli; MC: mitochondria; SG: secretory granules.

granules and proteolysis of absorbed thyroglobulin were less intense in many cells of the peripheral zone. The synthetic processes, as well as resorption, were more active in the central zone (Fig. 1, *d*). Resorption was realized by micro- and macropinocytosis. Intense transendothelial transport of substances was observed. TG parenchyma differed by structure from TG of rats with acute endotoxicosis which received no TSH. The central zone was presented by larger follicles, lined by lower thyrocytes with more basophilic cytoplasm and smaller nuclei. The follicle lumen contained more colloid. The follicles in the peripheral zone were smaller, thyrocytes were lower, and nuclei were smaller, while the content of colloid in the follicles was greater in comparison with endotoxicosis group without TSH treatment. The synthetic processes were more intense in rats with acute endotoxicosis, while thyroglobulin resorption and proteolysis were more active in rats treated with TSH. No reduction of the follicular thyrocyte functional activity was noted 48 h after TSH injection.

Hence, injection of TSH is a pathogenetically justified and effective method for correction of non-thyroidal illness syndrome in acute endotoxicosis. Treatment with TSH led to recovery of resorption and cleavage of

thyroglobulin accumulated in the follicles, normalizing the secretory activity of follicular thyrocytes.

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