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Cytophysiological Changes in the Follicular Epithelium of the Thyroid Gland after Long-Term Exposure to Low Doses of Dichlorodiphenyltrichloroethane (DDT)

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Exposure to endocrine disruptors is considered as a risk factor thyroid gland diseases. We analyzed cytophysiological changes in rat thyroid follicular epithelium after long-term exposure to low doses of the most widespread disruptor DDT. Analysis of thyroid hormone production and light and electron microscopy of thyroid gland samples revealed cytophysiological changes in thyroid epithelium related to impaired transport through the apical membrane, suppressed Golgi complex activity, and impaired thyrotrophic hormone regulation of the secretory functions of thyroid cells, which led to compensatory transition from merocrine to microapocrine secret release.

Key Words: *thyroid gland; follicular epithelium; cytophysiology; secretion; dichlorodiphenyltrichloroethane*

Growing incidence of thyroid gland (TG) diseases is attributed to the effects of disruptors on organism [5]. Dichlorodiphenyltrichloroethane (DDT) is one of the most widespread disruptors on the planet [7]. Little is known about the compensatory and adaptive changes in the secretory processes in thyrocytes leading to TG dysfunction under the influence of DDT. We have previously demonstrated that chronic exposure to low doses of DDT is followed by a decrease in the production of thyroid hormones in rats [2].

Here we studied cytophysiological changes in rat thyroid follicular epithelium after long-term exposure to low doses of DDT.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats (n=45) weighing 80-100 g. DDT dose was calculated in accordance to the requirements for the estimation of low doses [9] and limits of DDT content in the food products in the Russian Federation [1]. Treatment group animals (n=24) received solutions with o,p-DDT in a dose of 20 μg/liter (Sigma) instead of drinking water. Average daily consumption of DDT was 1.89±0.86 μg/g. Controls (n=21) received tap water. The absence of DDT, its metabolites, and similar chlororganic substances in tap water and food was confirmed by the gas-liquid chromatography.

The animals were sacrificed on weeks 4, 6, and 10 of the experiment by Zoletil overdose. Histological samples of TG were stained with hematoxylin and eosin for light microscopy. For electron microscopy, TG samples were fixed in 1% glutaraldehyde in 0.1 M cacodylate buffer and in 1% OsO_4, dehydrated, and embedded in epon; ultrathin sections were prepared and contrasted with uranyl acetate and lead citrate. The samples were examined under a Libra 120 transmission electron microscope (Carl Zeiss). The serum concentrations of thyrotropin (TSH), total thyroxin (T_4), free thyroxin (fT_4), total triiodothyronine (T_3), and free triiodothyronine (fT_3) were measured by ELISA using commercial kits (Cusabio Biotech, Monobind).

Statistical analysis of the obtained results was performed using Statistica 7.0 software. Comparison of independent groups by the quantitative parameter was

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conducted using the Student's *t* and Mann—Whitney *U* test. The differences were significant at $p<0.01$.

RESULTS

In control group rats, the TG parenchyma had typical structure with large follicles (diameter 92.98± 7.43 μ) in the peripheral areas and smaller (by 2.5 times) follicles in the central areas of the lobe. Electron microscopy of follicular epitheliocytes of the peripheral area of TG lobes showed cubic cells with micovilli (500-800 nm) located on their apical surface. Most microvilli had homogenous transparent content. Epitheliocyte nuclei were located in the center. Granular endoplasmic reticulum (GER) was well developed, particularly in the basal area of the cells, and presented by numerous channels filled with fine granular content of low electron density. Golgi complex was moderately developed and contained flat cisterns, vacuoles, and microvesicles. Multiple mitochondria were located both on apical and basolateral sides of thyrocytes. Swelled mitochondria with clear matrix and destroyed cristae and mitochondria fused with lysosomes and forming autophagolysosomes were found. The cytoplasm contained numerous lysosomes filled with osmophilic content of low, moderate, and high electron density, which reflected the degree of their maturation. Small colloid drops (diameter 0.2-0.5 μ) were seen; some of them were surrounded by lysosomes. Numerous secretory granules was found under the apical plasmalemma (Fig. 1, *a*). Central follicular epitheliocytes had prismatic form. In comparison with lobe periphery, microvilli of the central area were longer (1 μ) and epitheliocyte nuclei were larger with high content of euchromatin lumps. GER was well developed in both the basal and apical parts of the cells. Most mitochondria had significant changes related to matrix swelling, crist destruction, and autophagolysosome formation. Golgi complex was less developed than in cells at the lobe periphery. Lysosomes were less numerous. Few small colloid drops were found in the apical part of the cells.

After 4-week treatment with low doses of DDT, T_4 concentration in the blood decreased by ~25% comparing to the control level. The size of TG follicles surpassed the control values by 15-20%, follicular epitheliocytes had mostly cubic shape. Electron microscopy of follicular epitheliocytes showed significantly reduced number of microvilli on the apical membrane; their length decreased to 200-300 nm. The areas of GER and secretory vesicles were reduced, especially at the periphery of TG lobes. Very low number of lysosomes was observed, which resulted in accumulation of resorbed colloid (large drops up to 3 μ in diameter) in the cytoplasm (Fig. 1, *b*).

Six weeks after the start of the experiment, a reactive increase in the production of thyroid hormones, especially T_3 (by more than 3 times) was found. In the central area of TG lobes, the appearance of small follicles (by 10-14% in comparison with the control) and intensification of colloid resorption were observed. Regional differences in the structure of TG parenchyma were also manifested in the ultrastructure of follicular epitheliocytes. The cells with numerous lysosomes and electron-dense content, but not secretory vesicles, well developed cisterns of Golgi complex, single colloid drops (diameter 0.2-0.4 μ), moderately developed GER, and abundant microvilli on the apical surface predominated in the central area of TG lobes, which indicates the prevalence of resorption processes and thyroglobulin disintegration over its synthesis and release (Fig. 1, *c*). At the periphery of TG lobes, the cells with widened GER channels, but very low content of secretory vesicles and lysosomes were found, which attested to prevalence of thyroglobulin synthesis over synthesis of lysosomal enzymes and to reduced activity of the Golgi complex (Fig. 1, *d*). The appearance of cells with widened GER channels and cisterns of the Golgi complex, high content of lysosomes and secretory vesicles in the apical part, swelled mitochondrial matrix, and long (up to 1 μ) microvilli reflected activation of both thyroglobulin and lysosomal enzyme synthesis and normalization thyroglobulin cleavage by proteases and its transport via the apical membrane. In the microcirculatory bed, stasis of erythrocytes in capillaries was observed; greater number of capillaries with closed lumens led to plasma flow deceleration and intensification of transendothelial transport of initial substances. These results correspond to the previous data on DDT-induced suppression of Na^+/I^- symporter, which results in reduction of iodide transport into thyrocytes [2].

The thyroid status in rats receiving DDT for 10 weeks was characterized as hypothyroidism; the concentrations of T_3 and TSH decreased by 45 and 25%, respectively. As a result of stimulating effects of TSH, parenchyma restructuring with the formation of microfollicles (diameter 22-25 μ) in both central and peripheral areas of the lobes was observed. Ultrastructurally, follicle epithelium consisted of two types of cells characterized by peculiar mechanisms of secretion. Type one was characterized by intensive synthesis, resorption, and cleavage of thyroglobulin, which was confirmed by large size of cells and their nuclei, widening of GER and Golgi complex, abundant secretory vesicles and lysosomes, elongation of microvilli, and even macropinocytosis of the colloid. These cells were found in microfollicles and were probably a result of epithelium proliferation. The second type of cells was characterized by the absence of microvilli

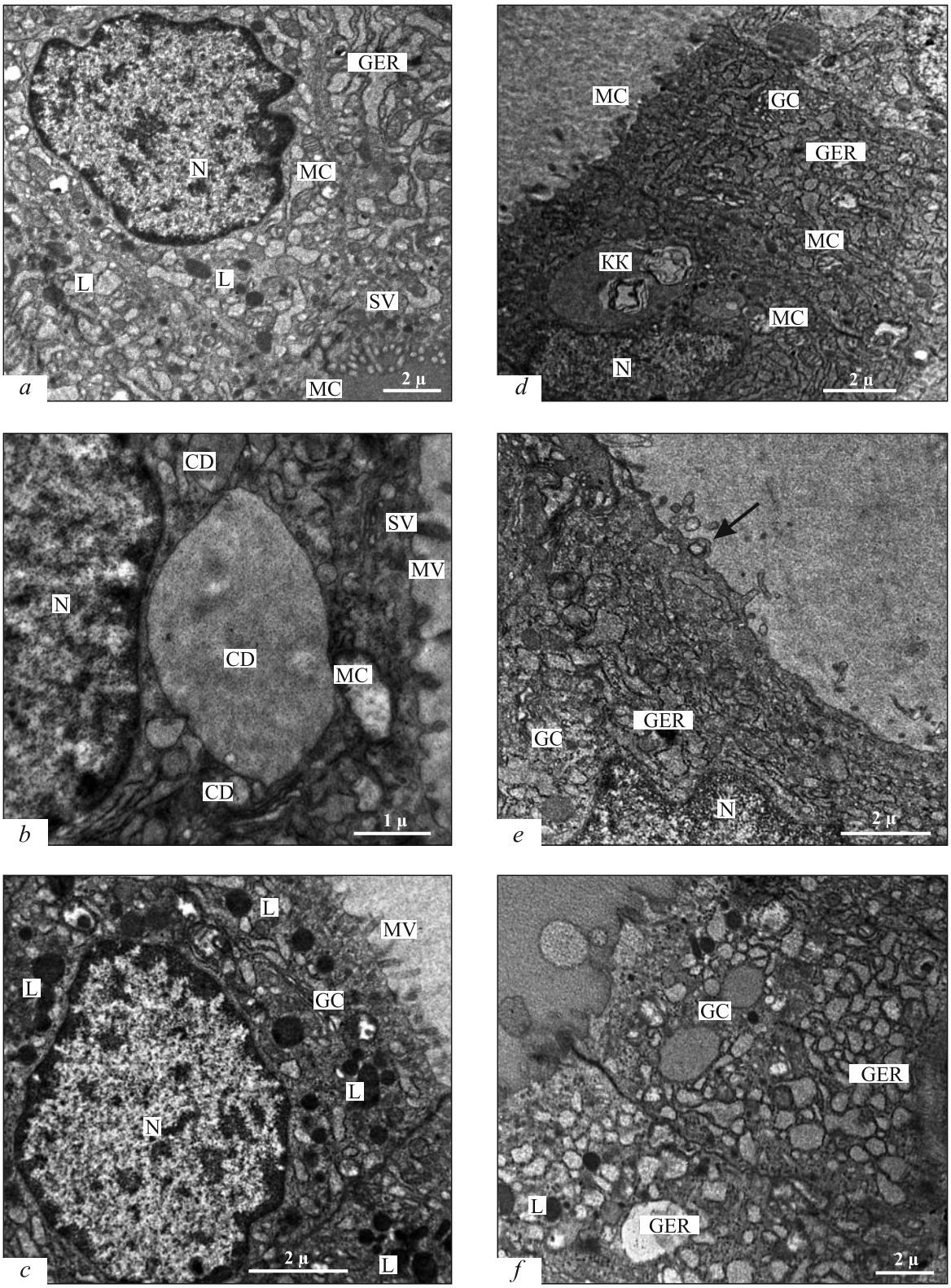

Fig. 1. Ultrastructure of follicular epithelial cells of rat TG after long-term exposure to low doses of DDT. *a*) Follicular epitheliocyte of a control rat, ×5000; *b*) follicular epitheliocyte after 2-week exposure to DDT, ×20,000; *c*) follicular epitheliocyte of the central area of the lobe after 6-week exposure to DDT, ×5000; *d*) follicular epitheliocyte of the peripheral area of the lobe after 6-week exposure to DDT, ×5000; *e*) follicular epitheliocyte of the peripheral area of the lobe after 10-week exposure to DDT, ×8000; *f*) follicular epitheliocyte of the central area of the lobe after 10-week exposure to DDT, ×5000. GC, Golgi complex; CD, colloid drop; L, lysosome; MV, microvillous; MC, mitochondria; SV, secretory vesicles; N, nucleus. Micropinocytosis of the colloid is shown by an arrow.

or reduction of their amount, low content of secretory vesicles in the apical part of cells, and very low number of lysosomes (Fig. 1, *e*). Most likely, these were old cells exposed to disruptor for a long time and weakly responding to TSH stimuli. Thus, the disruptive effects of DDT was to a certain degree similar to the inhibitory effects of thyroglobulin on the secretory function of the follicular epithelium [6] and impaired the regulation of cell functioning by producing together with TSH the suppressive and stimulatory effects on the same processes. For example, in cells with sharply reduced number of microvilli, TSH induced elongation of residual microvilli and colloid macropinocytosis them, but did not stimulate the formation of lysosomes that can perform proteolysis of resorbed thyroglobulin (Fig. 1, *e*). This resulted in compensatory changes in the secretory activity of the follicular epitheliocytes (microapocrine secretion). The follicles contained cells intensively synthesizing proteins, but Golgi complex in these cells was reduced, content of lysosomes was low, secretory follicles were absent on the apical surface, and separation of the apical cytoplasm into the follicle lumen (Fig. 1, *f*). Thus, long-term exposure to DDT induced transition from regulated secretion to non-classical secretion accompanied by the release of synthesized products without generation of secretory granules and by-passing the Golgi complex [3,4]. Under these conditions, not only thyroglobulin, but also protease cathepsin K that usually is not packed in lysosomes [8] can be released into follicle lumen and cleave thyroglobulin directly in follicle lumens.

Therefore, long-term exposure to low doses of endocrine disruptor DDT modified the cytophysiological status of the follicular epithelium of TG and impaired the transport across the apical membrane, suppressed the function of the Golgi apparatus, and disturbed regulation of secretory activity of cells by TSH, which leads to transition from of merocrine to microapocrine release of the secretory product and reverted follicle epitheliocytes to non-traditional evolutionary ancient type of secretion.

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