Morphofunctional Characteristics of the Kidneys in Chronic Endotoxemia against the Background of Hyperthyroidism

S. A. Kalashnikova, G. A. Kovnatskaya, and A. I. Shchogolev*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 6, pp. 709-712, June, 2011
Original article submitted March 21, 2010

Simulation of chronic endotoxemia in rats leads to the development of nephropathy and nephrosclerosis. Kidney involvement was more pronounced in animals with endotoxemia developing against the background of hyperthyroidism. Direct effects of bacterial LPS and its secondary effects mediated through circulating LPO products under conditions of thyroid hormone excess caused damage to the renal tissue. Immunohistochemical studies demonstrated higher expression of apoptosis markers in nephrocytes of the proximal tubules in rats with hyperthyroidism.

Key Words: *apoptosis; hyperthyroidism; kidney; endotoxemia*

Renal dysfunction is an important component of chronic endotoxemia (ET), which is related to disorders in the natural mechanisms of detoxication and their direct impairment (primarily by the toxic metabolites) [4,5]. On the other hand, the direct effects of thyroid hormones on renal function have been proven [1,2,7]. Excess or deficit of thyroid hormones lead to pronounced changes in the diuretic and natriuretic functions of the kidneys, which attests to the involvement of these hormones in the mechanism of excretion regulation [3,8,9]. However, structural and functional transformations in the kidneys in chronic ET under conditions of thyroid imbalance are little studied.

Here we studied morphofunctional changes in the kidneys in chronic ET under conditions of hyperthyroidism.

MATERIALS AND METHODS

The study was carried out on 72 outbred male albino rats. Chronic ET with predominant involvement of the kidneys was simulated in 18 animals by daily intraperitoneal injections of 4% solution of gentamicin (nephrotoxic antibiotic) in a dose of 20 mg/kg. On day 6 this procedure was supplemented by intraperitoneal injection of LPS (0.2 μg/kg). Day 7 was free from manipulations. Morphological changes in the kidneys by day 30 of the experiment were characterized by nephrosclerosis formation and were paralleled by the development of dysmetabolic nephropathy [6]. Control group consisted of 18 animals sacrificed on days 30, 60, and 90 of the experiment by Nembutal overdosage. The thyroid balance under conditions of chronic ET induced by tetrachloromethane and LPS administration was modified by pharmacological stimulation. Hyperthyroidism was induced in 18 rats by injections of L-thyroxin (Berlin-Chemie) in a daily dose of 100 mg/kg for 7 days, after which chronic ET was induced as described above. The reference group consisted of 18 rats with hyperthyroidism without ET; controls were sacrificed on days 30, 60, and 90.

Plasma concentrations of medium-molecular-weight substances (MMWS), oligopeptides, and MDA (free radical lipid oxidation product) were measured as laboratory biochemical indicators of ET. Serum concentrations of thyroid hormones (thyrotropic hormone, TTH, total and free T_3, total and free T_4) were measured by ELISA using Vector-Best and DRG kits. The

Volgograd State Medical University, Ministry of Health and Social Development of the Russian Federation; *V. I. Kulakov Center of Obstetrics, Gynecology, and Perinatology, Ministry of Health and Social Development of the Russian Federation, Moscow, Russia. ***Address for correspondence:*** Kalashnikova-SA@yandex.ru. S. A. Kalashnikova

material for pathomorphological study of the thyroid gland and kidneys was processed routinely. Morphometric analysis included evaluation of the following parameters: volume of connective tissue (%), urinary space index (ratio of Shumlyansky–Bowman capsule area to glomerular capillary area; arb. units), tubular index (ratio of the height of tubular epithelium to outer diameter of the tubule; arb. units), and the percentage of sclerotic glomeruli.

Immunohistological study was carried out using TRAIL (TNF-related apoptosis-inducing ligand, clone 27B12) and caspase-3 (clone JHM62) antibodies (Novocastra Laboratories Ltd). The results were processed by common methods accepted in biomedical studies using Excel 7.0 (Microsoft) and ARCADA (Dialogue-MGU) software.

RESULTS

The development of hyperthyroidism in animals was verified by serum profiles of thyroid hormones: reduced TTH level and elevated thyroxin and triiodothyronine reaching the peak by day 90 of experimental ET.

Changes in the thyroid gland characterizing its hyperfunction formed the morphological substrate of thyroid imbalance. Shrinkage of the follicles was paralleled by an increase of the follicular epithelium height and its weak proliferation. This was paralleled by appearance of a negligible amount of loose intrafollicular colloid with its marginal vacuolation. On the other hand, the content of interfollicular epithelium increased moderately.

Biochemical analysis of the blood showed significantly higher levels of endogenous intoxication markers in animals with underlying hyperthyroidism in comparison with animals with ET without thyroid imbalance (Table 1).

By day 90, the concentration of MMWS in rats with hyperthyroidism was 1.3 times higher than in animals with the basal model and 4.5 times higher than in controls, while MDA levels in this group were 1.3 and 2.6 times higher, respectively ($p<0.05$).

Morphological changes in the kidney corresponded to the picture of dysmetabolic nephropathy, its severity increasing with the progress of experimental disease. By day 90 of ET, the signs of glomerular damage (glomeruli acquired different shapes and structures) were accompanied by significant increase in the connective tissue volume. The renal tubular nephrothelium was presented by cells with signs of vacuolation and few necrotic nephrocytes. The changes were more pronounced in the proximal convoluted and straight tubules than in the distal ones.

Morphometry of renal tissue revealed increased volume fraction of the connective tissue, more pronounced in rats with elevated concentrations of thyroid hormones in the peripheral blood, and a significant shrinkage of the urinary space and tubular index (Table 2). The number of sclerosed glomeruli was 1.8 times higher in rats with hyperthyroidism in comparison with animals with ET without thyroid imbalance ($p<0.01$).

The unfolding disorders of renal function were paralleled by changes in serum levels of urea and creatinine. By the end of the experiment, urea level in rats with ET without hormonal imbalance and in rats with chronic ET paralleled by hyperthyroidism increased by 1.5 and 1.9 times, respectively, in comparison with the control group. Similar data were obtained for creatinine level, which increased 1.6 times by day 90 in ET without thyroid imbalance and 1.8 times in ET with hyperthyroidism ($p<0.05$).

In order to more precisely evaluate the injuries in various compartments of the nephron under the effect of endogenous toxic compounds, we studied the

TABLE 1. Biochemical Indicators of the Severity of Chronic ET in Rats with Hyperthyroidism ($M\pm m$)

Experiment conditions		Day of experiment		
		30 days	60 days	90 days
MMWS, arb. units (control 0.15±0.02)	LPS+gentamicin	0.41±0.05*	0.47±0.05*	0.53±0.06*
	Hyperthyroidism	0.54±0.09*	0.61±0.11*+	0.68±0.10*+
Oligopeptides, mg/liter (control 115.5±13.8)	LPS+gentamicin	268.5±29.8*	289.4±30.5*	291.0±36.4*
	Hyperthyroidism	275.3±25.9*	311.2±25.7*+	319.1±27.9*+
MDA, mmol/liter (control 5.33±0.43)	LPS+gentamicin	10.91±0.93*	10.51±1.23*	11.07±1.31*
	Hyperthyroidism	12.71±1.03*	13.21±1.31*+	14.07±1.33*+

Note. Here and in Table 2: $p<0.05$ compared to the *control rats, +with the basal model.

TABLE 2. Morphometric Parameters of the Kidneys for Evaluation of the Severity of Chronic ET in Rats with Hyperthyroidism (*M*±*m*)

Experiment conditions		Day of experiment		
		30 days	60 days	90 days
Connective tissue percent volume (control 7.2±0.4)	LPS+gentamicin	9.1±0.9	14.5±1.3*	19.7±4.4*
	Hyperthyroidism	11.3±2.0	17.9±5.1*	23.9±5.5*+
Urinary space, arb. units (control 1.4±0.4)	LPS+gentamicin	1.2±0.1	1.2±0.1	1.1±0.6*
	Hyperthyroidism	1.19±0.2	0.8±0.1*	0.8±0.1*
Tubular index, arb. units (control 0.8±0.1)	LPS+gentamicin	1.6±0.2	0.6±0.1*	0.5±0.1*
	Hyperthyroidism	1.1±0.6	0.4±0.1	0.3±0.1*+
Percent of sclerotic glomeruli (control 0)	LPS+gentamicin	8.0±0.3*	8.9±0.9*	9.3±1.4*
	Hyperthyroidism	9.2±0.4*	10.7±1.3*	17.2±1.4*+

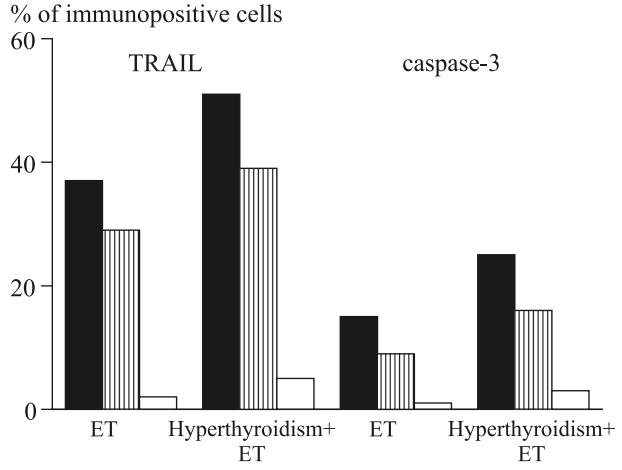

Fig. 1. Increased expression of TNF-dependent apoptosis markers in the renal tubules in chronic ET against the background of thyroid imbalance. Light bars: control; dark bars: proximal tubules; vertically hatched bars: distal tubules.

expression of TNF-dependent apoptosis markers separately in the proximal and distal renal tubules (Fig. 1).

The expression of TRAIL and caspase-3 increased in the following order: distal tubules of animals with classical ET — distal tubules in ET+hyperthyroidism — proximal tubules in classical ET — proximal tubules in ET+hyperthyroidism. The data indicate the contribution of TNF-dependent apoptosis to the mechanism of renal tissue injuries, maximally pronounced in the proximal tubules of animals with ET induced under conditions of thyroid imbalance.

Hence, a complex of pathological structural and functional changes with the formation of dysmetabolic nephropathy and nephrosclerosis develops in the kidneys in chronic ET. These changes are more severe in rats with ET induced under conditions of hyperthyroidism. Renal lesions are caused by the direct destructive effects of bacterial LPS and by secondary factors, toxic metabolites circulating in systemic blood flow under conditions of peripheral thyroid hormone excess. An important component in the pathogenesis of renal disturbances in chronic ET is TNF-dependent apoptosis. More intense expression of apoptosis markers (TRAIL, caspase-3) was found in the proximal tubules of rats with hyperthyroidism, which seems to be responsible for greater involvement of this nephron compartment in the pathological process.

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