METHIMAZOLE-INDUCED HYPOTHYROIDISM IN RATS: EFFECTS ON BODY WEIGHT AND HISTOLOGICAL CHARACTERISTICS OF THYROID GLAND

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Summary: The aim of this study was to examine the effect of methimazole treatment on the body weight and thyroid gland structure in rats. Methimazole given as 0.02% solution in drinking water for three weeks induced significant decline in T_4 and T_3 levels, as determined by radioimmunoassay. The body weight gain was lowered compared to control animals, while thyroid weight was increased. Histological examination of the thyroid gland revealed a pronounced growth activation of the follicular epithelial component with frequent mitoses, accompanied with improved vascularisation. We assumed that the lower body weight gain despite decreased basal metabolic rate and similar food ingestion can be a result of brown adipose tissue activity.

Key words: methimazole, thyroid gland histology, hypothyroidism, body weight

Introduction

Thyroid gland is specialized for production, storage and release of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). They are major regulators of basal metabolic rate and energy expenditure in homeothermic animals (1), and they are required for normal cell growth and development (2). Apart from general metabolic disturbance, impairment of thyroid hormone production causes serious intellectual and behavioural abnormalities that may affect patient's daily functioning and result in additional stress and depression. Therefore, the research on the thyroid gland has not only significant medical but also social implication.

One of the most frequent thyroid disorders in humans is hypothyroidism, defined as a condition when the production of the thyroid hormones decreases below the normal need of the body (3). Hypothyroidism can result from thyroid disfunction, from impediment in mechanisms that control thyroid func-

tion, or may arise as the complication during treatment of hyperthyroidism. Since control of body weight and appetite are disturbed in hypothyroid patients (1), inexplicable body weight gain despite decreased food intake and loss of appetite are among the first, although unspecific signs of hypothyroidism in men.

Numerous animal models of experimental hypothyroidism have been developed in effort to elucidate mechanisms underlying hypothyroidism and to contribute to resolve accompanied problems. Among other results, it has been unexpectedly shown that the body weight gain in rats and mice as commonly used laboratory animals, was reduced, absent or even negative in comparison to euthyroid controls (4–6).

In the present study we artificially induced hypothyroidism in otherwise healthy and metabolically stable rats in order to elucidate the existing controversies related to effects of experimentally induced hypothyroidism on energy storage/dissipation in rats, as well as in attempt to give acceptable explanation for discrepancies which exist in that regard between human and animal models of hypothyroidism. Hypothyroidism was provoked by potent antithyroid drug methimazole (MMI) that is frequently and preferentially used in the treatment of human hyperthyroidism (7). At the same time we used this opportunity to study morpho-functional alterations of the thyroid gland related to abortive hormones production induced by MMI.

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Material and Methods

Male Wistar rats, weighing 130–150 g at the beginning of the experiment were caged individually on 12:12 h light-dark schedule at the room temperature (22 ± 1 °C), and fed with commercial rat food (Subotica, SCG). The rats were divided into two groups: animals from the first group (n=8) were made hypothyroid by drinking 0.02% MMI (Sigma St. Louis, USA) solution in drinking water for three weeks; animals in the other group (n=6) were untreated control.

Body temperature was measured at the beginning and at the end of the experiment. Good health and condition of all animals were noted during the investigation.

On day 22 of the experiment animals were weighed and killed by decapitation. Blood samples for determination of thyroid hormone concentrations in the serum were collected. Total serum T_4 and T_3 concentrations were determined by radioimmunoassay (detection kits provided by INEP, Zemun, SCG). Both thyroid lobes were excised, weighed and fixed in the 3.5% neutral-buffered formalin where they were stored until embedding in Bioplast. Five μm thick sections were dyed with Mayer's hematoxylin, and observed with Leika MPS 60 light microscope. Samples of other tissues, including interscapular brown adipose tissue, were also isolated and fixed for future studies.

Statistical analysis was performed using Student's t-test and data were presented as means ± SE.

Results

The results presented in *Table I* show that MMI treatment strongly affected body and thyroid gland mass, as well as T_4 and T_3 serum levels.

Although at the beginning of the experiment mean body mass in both control and experimental group of animals was practically equal (K: 142.2 ± 3.66 g; MMI: 141.5 ± 2.40 g), at the end of the treatment the rats from the experimental group were significantly lighter (K: 280.0 ± 5.80 g; MMI: 209.0 ± 6.51 g;

Table I. Changes in body and thyroid weights
and serum T_4 and T_3 levels after MMI treatment

	Control	MMI	Statistics
Body weight gain (g)	137.83 ± 4.820	67.50 ± 5.095	p<0.001
Thyroid weight (mg)	32.32 ± 2.284	43.78 ± 3.172	p<0.02
Thyroid weight (mg/100 g b.w.)	12.39 ± 1.126	20.65 ± 1.510	p<0.01
Serum T_4 concentration (nmol/L)	104.34 ± 8.727	30.20 ± 1.108	p<0.001
Serum T_3 concentration (nmol/L)	1.09 ± 0.137	0.65 ± 0.098	p<0.05
b. w. – body weight			

p<0.001), namely they gained approximately 50% less mass than the control animals. As early as during extirpation, it was clearly visible that the thyroids from the experimental rats were enlarged and, judged by color, better vascularised compared to the controls. Absolute thyroid mass was increased for about one-third, which, together with reduced body mass gain resulted in conspicuous rise in the thyroid mass expressed per 100 g of body weight. In all treated animals, MMI administration elicited a significant decrease in T_3 and T_4 serum levels, although they remained above the assays limits of detection. Finally, contrary to the expectations, body temperature was unchanged after three weeks of MMI treatment (data not shown).

Examinations conducted by light microscopy revealed remarkable morphological alterations in the thyroid gland connected with stimulation of its activity and growth. In the control group of animals (*Figure 1A*) thyroid follicles were mainly large, round and filled with pale colloid. Thyrocytes were cuboidal or somewhat flatter, with roundish or oval nucleus and rarely visible nucleolus. Capillaries in the connective tissue among the follicles were narrowed. In the thyroids from MMI treated rats different types of follicles could be recognised. A majority of follicles were irregularly shaped and narrowed due to largely resorbed colloid. Epithelium often formed infoldings toward lumen or, in places, thyrocyte »islands« were observed in the lumen (*Figure 1B*). Thyrocytes were enlarged, more or less cylindric, with a large euchromatic nucleus and prominent nucleolus. Numerous microvilli and occasionally pseudopods were formed at the apical pole of the thyrocytes. In fewer follicles luminal content remained and thyrocytes were cuboidal.

Mitoses within follicular epithelium were very frequent (*Figure 2A*), as well as cells in different stages of apoptosis. Nuclei of the apoptotic cells displayed condensation of chromatin that appeared as small intensely stained spherical masses (*Figure 2B*).

Finally, better vascularisation of the thyroid gland after MMI treatment was manifested by widened elongated capillaries which followed the margins of the follicles. Endothelial cells were in close contact with the base of thyrocytes, sometimes seemingly pushing them toward the follicular lumen. A connective tissue stroma was hypertrophic and mast cells in the vicinity of the capillaries were not uncommon.

Discussion

Giving MMI in drinking water is a conventionally used method for establishing hypothyroidism in experimental animals (5, 8–12). MMI acts as false substrate for thyroid peroxidase, thus blocking the iodination of thyrosine residues within thyroglobulin and the coupling of iodothyrosines into iodothyronines (3, 8). Since the release of hormones is not affected, the onset of effects is somewhat delayed and slowed, until the
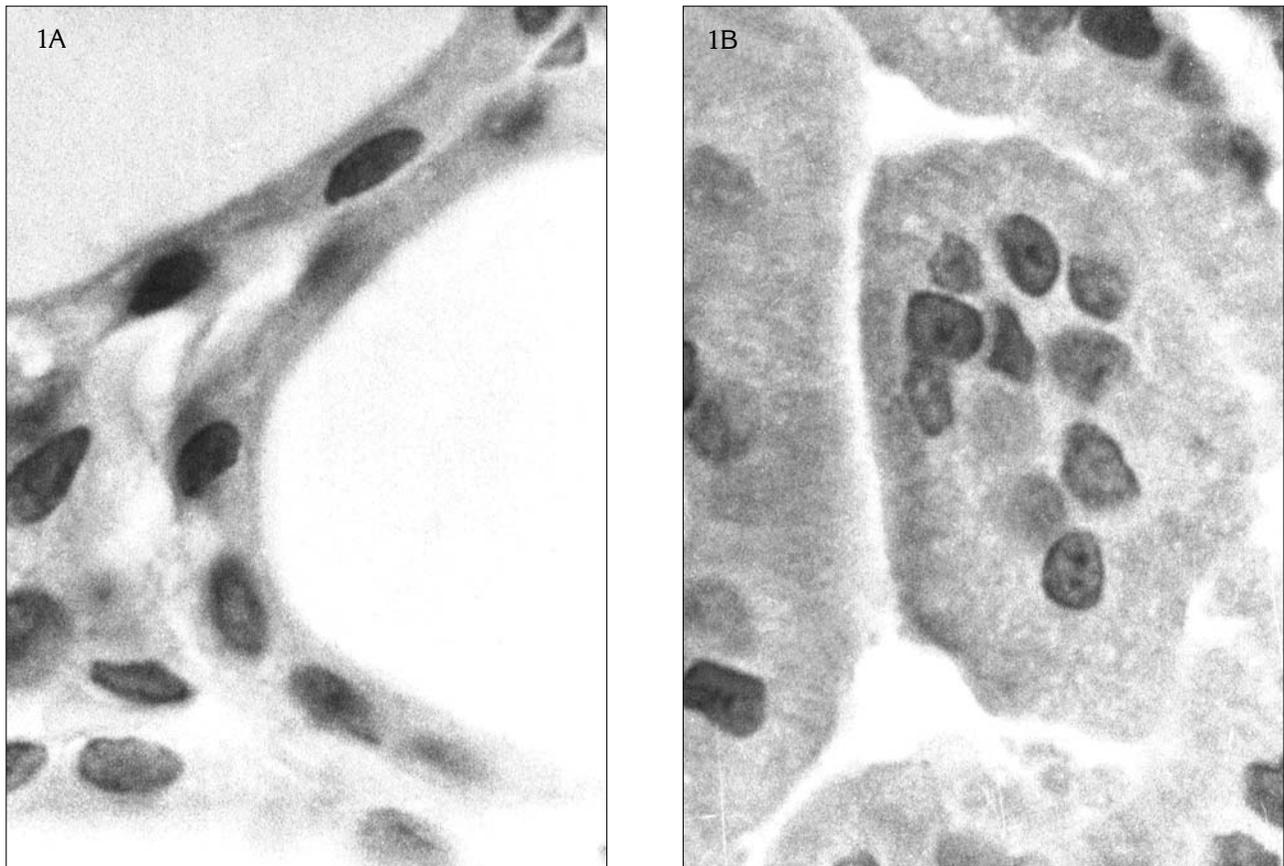
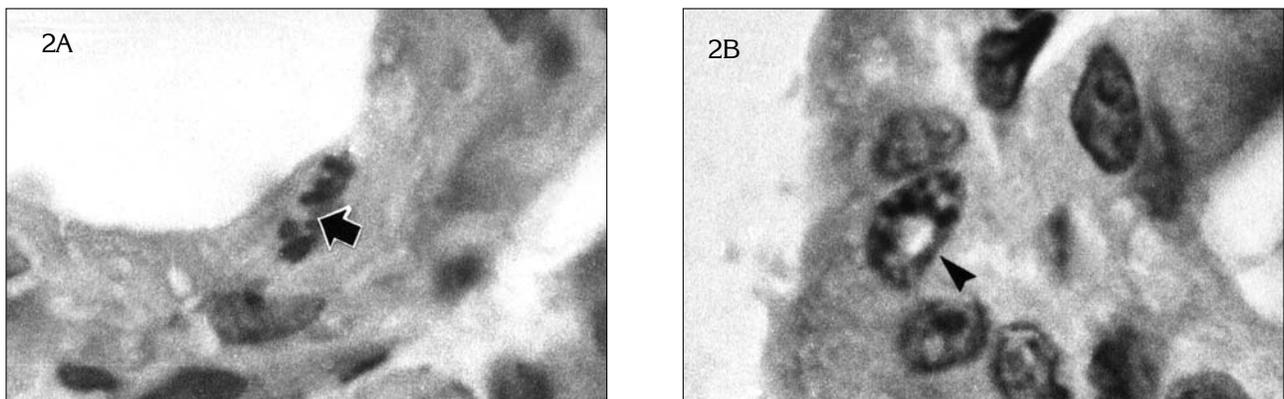Figure 1. Thyroid follicles in rat from the control (A) and experimental group (B). Hematoxylin, × 600.

Figure 2. Mitosis in follicular epithelium (A, arrow). Apoptotic nucleus in follicular
epithelium displaying condensation of chromatin (B, arrowhead). Hematoxylin, × 600.

depletion of the thyroid gland hormone stores. However, the significant decrease in the total T_4 and T_3 serum levels pointed out that administered dose and duration of the treatment were sufficient to induce hypothyroid status in the experimental group of rats.

The thyroid gland activity is regulated by hypothalamic-pituitary-thyroid axis, including negative feedback loop. Insufficiency of the thyroid hormones in cir-

culation stimulates pituitary to secrete thyroid-stimulating hormone (TSH), which has a critical role in the thyroid growth and activity (13). Under TSH stimulation, the thyroid gland undergoes enlargement, hyperplasia, neovascularisation and morphological alterations of the thyrocytes related to their engagement in production, processing and releasing of thyroid hormones. All these alterations were strongly expressed in

the thyroid gland of MMI treated rats in our experiment, but an increase in hormone levels in circulation was absent due to obstructive effects of MMI.

After MMI treatment, together with numerous mitoses of thyrocytes, cells in different stages of apoptosis were frequently seen. This finding could be a little confusing considering evident thyroid hyperplasia but it becomes clear if we bear in mind that homeostasis in the thyroid gland mass is maintained by balanced cell proliferation and death (14). In hyperplastic thyroid, the number of mitoses was increased, so the same had to go for an absolute number of apoptoses, which made them both easily visible. Distinct hyperplasia of the gland means that proliferative activity was highly favoured in relation to cell death.

It has been previously reported that thyroid hyperplasia induced by antithyroid drug such as MMI was associated with the blood capillary enlargement and neovascularisation (15, 16). Angiogenesis was accompanied with increase in fibroblast growth factor-2 and transforming growth factor $\beta 1$, and decrease in thrombospondin 1, which probably interacted in an autocrine/paracrine relationship (16). Mast cells presented in an increased number near capillaries, were closely associated with connective tissue remodeling and angiogenesis (17, 18).

As we mentioned above, the body mass change was among the most conflicting findings derived from the experiments with animal models of hypothyroidism. In our experiment, MMI treated rats gained less body weigt than animals from the control group and thus, our data were in accordance with those previously published by LeGrow et al. (4). There may be several possible explanations for this retarded growth. Since thyroid hormones generally stimulate growth, slowed weight gain could be simply the result of their insufficiency, associated with diminished food intake. On the other hand, Curcio et al. (19) showed that hypothyroidism induced by thyroidectomy did not influence energy intake and food absorption. Since we did not observe considerable difference in quantity of ingested food in control vs. experimental group of animals, we believe that the possibility that MMI itself negatively affected food ingestion should be rejected. Considering this, the question is what has happened with energy ingested but not built into the body mass. The only logical explanation is that it was released. Since it is well known that in hypothyroidism the cell basal metabolic rate and oxygen consumption are decreased, there must be an alternative pathway for energy dissipation. Taking into account our still unpublished data on increased interscapular brown adipose tissue (BAT) relative mass in hypothyroid group of rats, we assume that BAT activity was the mechanism for energy dissipation responsible for the reduction of body weight gain in hypothyroid rats. The absence of an expected fall in the body temperature due to the lowered metabolic rate offered further support for this idea. The fact that the functionally active BAT is present in rodents but not in adult humans, might explain difference in weight gain in hypothyroidism that exists between rodents and humans.

HIPOTIROIDIZAM KOD PACOVA INDUKOVAN METIMAZOLOM: UTICAJ NA TELESNU TEŽINU I HISTOLOŠKE ODLIKE TIROIDNE ŽLEZDE

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Kratak sadržaj: Cilj ovoga rada bio je ispitivanje uticaja metimazola na telesnu težinu i strukturu tiroidne žlezde kod pacova. Metimazol, davan u vodi za piće u vidu 0,02% rastvora tokom tri nedelje, doveo je do značajnog sniženja nivoa T_4 i T_3 u serumu, što je utvrđeno radioimunoesejom. Prinos telesne težine bio je manji nego u kontrolnoj grupi, dok je težina tiroidee povećana. Histološkim ispitivanjem tiroidne žlezde utvrđena je aktivacija folikularnog epitela, uz povećan broj mitoza i poboljšanu prokrvljenost. Verujemo da je sniženi prinos telesne mase kod hipotiroidnih životinja do kog dolazi uprkos nižoj stopi bazalnog metabolizma i približno jednakoj količini unete hrane, rezultat odavanja energije aktivnošću mrkog masnog tkiva.

Ključne reči: metimazol, histologija štitne žlezde, hipotiroidizam, telesna težina

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