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ORIGINAL ARTICLE

Histological and ultrastructural alterations of rat thyroid gland after short-term treatment with high doses of thyroid hormones

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Abstract The aim of the present study was to investigate histological alterations of rat thyroid gland after short-term treatment with supraphysiological doses of thyroid hormones. Rats from experimental groups were treated with triiodothyronine (T3) or thyroxine (T4) during five days. In both treated groups, thyrocyte height was reduced and follicular lumens were distended. Progressive involutive changes of the thyroid parenchyma were apparent, including follicular remodeling (fusion) and death of thyrocytes. Morphological changes confirmed by quantitative analysis were more pronounced in the T4-treated group. Our results demonstrate that thyrotoxicosis, whether induced by T3 or T4, leads to different grades of thyroid tissue injury, including some irreversible damages. These changes might be explained at least in part by lack of trophic and cytoprotective effects of the thyroid stimulating hormone. Since the period required for morphophysiological recovery may be unpredictable, findings presented here should be taken into consideration in cases where the thyroid hormones are used as a treatment for thyroid and non-thyroid related conditions.

1. Introduction

Thyroid gland is specialized for production, storage and release of thyroid hormones thyroxine (T4) and triiodothyronine (T3). T4 is a quantitatively dominant hormone released from the thyroid gland, while T3 is biologically more active and originates mainly from peripheral deiodination of T4 (Boelaert and Franklyn, 2005). Thyroid hormones are involved in regulation of metabolic rate and energy expenditure in homeothermic animals (Cavalieri, 1997) and they are necessary for normal cell growth and development (Silva, 1995).

Abbreviations: T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone; TRH, TSH-releasing hormone; PI, propidium iodide

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Biosynthesis and secretion of thyroid hormones are regulated by hypothalamus-pituitary-thyroid axis, with the negative feedback loop at the level of both hypothalamus and pituitary gland (Norman and Litwack, 1987; Williams and Bassett, 2011). Namely, thyroid gland activity is positively regulated by thyroid stimulating hormone (TSH) synthesized and secreted from pituitary thyrotrophs, which activity is in turn controlled by hypothalamic TSH-releasing hormone (TRH). Under physiological conditions, circulating thyroid hormones suppress the release of TSH and TRH thus providing the so-called long negative feedback loop regulating thyroid function. In addition, there exist short and ultra-short feedback loops represented by suppressive activity of TSH on both TRH and TSH release (Prummel et al., 2004). A failure at any step of this complex regulatory mechanism leads to dysregulation of thyroid function, which is manifested as either hyper- or hypoproduction of hormones and can cause serious health problems in humans and companion animals. Normal production of thyroid hormones also depends on an adequate supply of iodine (Kelly, 2000).

Thyroid hormones are frequently used in human and veterinary medicine as replacement therapy for thyroid deficiency (Dixon et al., 2002; Escobar-Morreale et al., 2005; Wiersinga, 2001) and, in doses slightly above physiological, in therapy of differentiated thyroid carcinoma (Brabant, 2008). Supraphysiological doses of thyroid hormones are also in use as supplemental therapy for some diseases and conditions that are not associated with thyroid dysfunction such as prophylaxis-resistant affective disorders (Bauer et al., 2002) or Wilson's temperature syndrome (Friedman et al., 2006). Besides, some promising investigations regarding use of supraphysiological doses of thyroid hormones for heart repair after myocardial infarctation are in progress (Pantos et al., 2009, 2008, 2007).

Data on possible side effects of such therapies in humans are still inconsistent and vary depending on tissue, organ or function examined. Despite the fact that hyperthyroidism is commonly associated with insomnia, high doses of T4 used for treatment of mood disorders did not cause sleep impairment in otherwise healthy patients (Kraemer et al., 2011). Also, it seems that supraphysiological doses of T4 are not necessarily associated with bone mineral density loss even after a very long period of treatment (Ricken et al., 2012; but see also Chen et al., 2004). From the animal studies it is known that experimentally induced thyrotoxicosis causes impairment of some cognitive functions (Taşkin et al., 2011) and activates h ypothalamic–pituitary–adrenal axis thus potentially compromising adrenal function (Johnson et al., 2005).

Given the wide use of thyroid hormones as therapeutics in a number of diseases and conditions and rather good common knowledge on their undesired effects on various organs, there is a surprising paucity of studies dealing with the effects of supraphysiological doses of thyroid hormones on the thyroid gland.

Bearing in mind that the structure of any organ closely reflects the state of its function, the aim of the present study was to investigate the effects of treatment with supraphysiological doses of T3 or T4 on histological and cytological characteristics of thyroid gland in euthyroid animals. The obtained results should contribute to better understanding of the possible side-effects and safety of therapy with high-doses of thyroid hormones.

2. Materials and methods

2.1. Animals

The experiment was performed on a total of 18 male Wistar rats, weighing 180–250 g. Animals were caged individually, at room temperature (22 ± 1 °C), in 12:12 h light–dark cycle and had free access to food (commercial rat food, Subotica, Serbia) and tap water. Animal handling and treatment were carried out in accordance with The Serbian Laboratory Animal Protection Law proposed guidelines and protocols approved by The Ethics Committee of the Faculty of Biology, University of Belgrade.

2.2. Experimental design

The rats were divided into three equal groups and treated once a day, for 5 days, as follows: T3-treated rats received injections of T3 (200 μg/kg b.w.) dissolved in 9 mM NaOH; T4-treated animals received T4 (300 μg/kg b.w.) dissolved in 9 mM NaOH; control (euthyroid) animals were injected with vehicle only (9 mM NaOH, 1 ml/kg b.w.). Body temperature was measured at the beginning and at the end of the experiment. In the course of the experiment, all animals were in good health and condition. After the last injection, body mass was measured and rats were sacrificed by decapitation using a guillotine (Harvard Apparatus, Holliston, MA, USA).

2.3. Determination of thyroid hormones in the circulation

For determination of T3 and T4 concentration in the serum, blood samples were collected from the trunk during sacrificing. Total serum T3 and T4 concentrations were determined by the RIA method, at the laboratory of The Institute for the Application of Nuclear Energy (INEP, Belgrade, Serbia).

2.4. Processing of thyroids for light microscopy

After isolation and weighing, the left lobe of each thyroid gland was routinely processed for light and the right one for electron microscopy. For light microscopy, each thyroid lobe was fixed in 3.7% phosphate-buffered formalin (pH = 7.2), dehydrated through an ethanol series and xylol and embedded in paraffin. For general histological analysis, as well as for stereological measurements, 5 μm thick paraffin sections stained with hematoxylin/eosin method, taken from the anterior, medial and posterior part of the thyroid lobe (five non-serial sections per each chosen part of a lobe), were analyzed on Leica DMLB light microscope (Wetzlar, Germany).

2.5. Detection of cell death

Cell death was demonstrated by propidium iodide (PI) staining method (Markelic et al., 2011; Scaglia et al., 1997). Briefly, deparaffinized and rehydrated 5 μm thick sections were stained in 1% PI solution, for 10 min. Glycerol-mounted sections were examined with a Zeiss Observer.Z1 fluorescent microscope and photographed with AxioCam MR3 camera, using AxioVision Rel4.7 software. The occurrence of cell death was estimated by counting normal and apoptotic nuclei in the follicular wall,

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