Activity of thyroid parafollicular (C) cells in rats with hyperthyroidism - immunohistochemical investigations

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Abstract

The aim of this study was an evaluation of the activity of parafollicular (C) cells in a rat experimental model of hyperthyroidism, evoked by an intraperitoneal application of L-thyroxine (40 mg/kg daily) over 15 days. For that reason, immunohistochemical investigations and evaluation of calcitonin (CT) plasma concentrations were performed. Differences in the quantity and distribution, together with enhanced CT-immunoreactivity of C cells, were observed in hyperthyroid rats, in comparison to respective values in the control group, accompanied by a significant diminution of plasma TSH and CT levels. Our preliminary study may point to a functional interaction between follicular and parafollicular cells in the thyroid gland.

Key words: C cells, hyperthyroidism, calcitonin, immunohistochemical study.

Introduction

In the thyroid gland of mammals, except for the basic follicular cells, irregularly distributed cells have been described. The most common name for them is parafollicular cells, or C cells (calcitonin cells). According to Pearse [1], they belong to disperse neuroendocrine cells of the APUD system (amine precursor uptake and decarboxylation) [2]. The role of parafollicular cells in the function of the thyroid gland has not been clarified till now. Despite some controversial data, one could presume that co-localisation of the follicular and the parafollicular cells in the thyroid gland is not incidental. It seems possible that there is some interaction between them, mediated by the release of peptidergic hormones. The kind and amount of the released hormones depend on many factors, e.g., age, sex and health status [3, 4, 5]. Calcitonin (CT), a product of CT/CGRP gene expression, is proposed as an essential indicator of C cells activity [6]. The basic action of calcitonin is the diminution of Ca_{ion}, concentratiom by inhibition of the reabsorption activity of octeoclasts and by facilitation of calcium excretion in the kidneys [6]. The finding that, in homozygotic mice devoid of gene coding calcitonin osteopenia is developed, may indicate that this hormone plays a role in bone tissue homeostasis [7]. Thus, calcitonin is applied in hypercalcemia, Paget's disease and osteoporosis [3]. There are few publications, concerning the evaluation of the structure and function of parafollicular cells in the thyroid gland diseases. There are also only several data, dealing with the problem of the mutual relation between parafollicular and follicular cells in physiological and also in pathological conditions and only single observations, concerning the ectopic production of calcitonin in other tissues [3]. In our previous study, we reported a significant diminution of plasma TSH and CT levels, accompanied by enhanced CT-immunoreactivity in C cells of the thyroid glands in rats, chronically treated (over 30 days) with L-thyroxine [8].

In the present study, a distribution and CT-immunoreactivity of thyroid C cells in an experimental model of hyperthyroidism, evoked by the application of L-thyroxine over 15 days, was evaluated.

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Material and methods

Male Wistar rats (n=20), weighing 90 - 100 g each, were used in the experiment and provided with standard laboratory chow and water ad libitum. The animals were housed at 22^0 C and constant humidity, with a 12/12 light/dark cycle. All the pro-

Figure 1. Light micrograph of thyroid gland of control rat. Positive immunohistochemical reaction for calcitonin is observed in most of C cells. x 300.

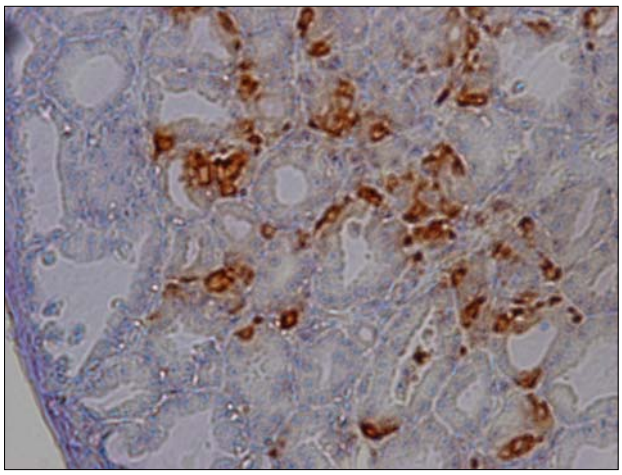
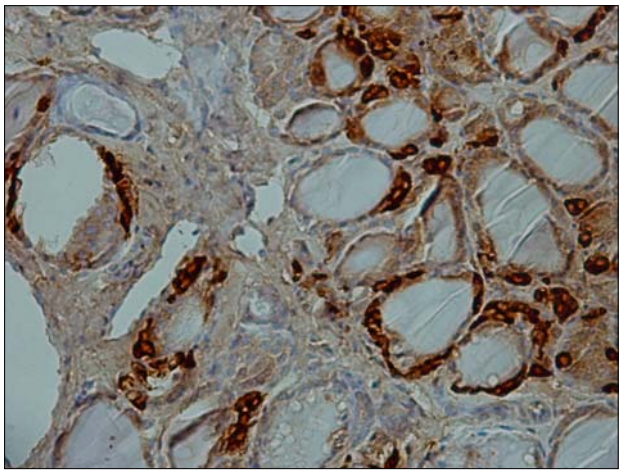


Figure 2. Light micrograph of thyroid gland of rat treated with L-thyroxine. The enhancement of immunohistochemical staining for calcitonin in C cells is observed. x 300.



cedures were performed in compliance with the European Union Council Directive of 24th November 1986 (86/609/EEC) and were approved by the Local Ethics Committee in Białystok. The experimental model of hyperthyroidism was induced by an intraperitoneal injection of L-thyroxine (Sigma Chemical Co) at the dose of 40 µg/kg daily over 15 days. A group of ten control rats were treated with saline under the same experimental conditions. At the end of the experiment, under pentobarbital sodium anaesthesia (50 mg/kg), blood was taken from the abdominal aorta of each rat to determine plasma TSH and CT concentrations by radioimmunoassay (RIA). Subsequently, the rats were thyreoidectomized. Both thyroid lobes were placed in Bouin's fluid for 24 hours. An immunohistochemical reaction, used for detecting calcitonin in C cells, was conducted on 5 µm-thick paraffin sections, derived from the thyroid glands. In that procedure, specific rabbit antisera against calcitonin, which can be found only in C cells, were used. In the above immunohistochemical study, the ABC (avidin-biotin peroxidase complex) method was applied, according to Hsu et al. [9].

Results and discussion

After 15 days of L-thyroxine treatment, plasma TSH concentration was significantly ($p<0,0004$) reduced (mean 0.52 µIU/ml), as compared to the respective value in the control rats (mean 2.89 µIU/ml). Moreover, CT plasma level was also significantly ($p<0,0004$) attenuated (mean 13.54 pg/ml), in comparison to that in the control group (mean 16.43 pg/ml).

The reduction of plasma levels of both hormones was similar to those, observed in our previous study in rats, treated with L-thyroxine twice longer, i.e., over 30 days. Also the histological pictures of the thyroid glands, derived from the rats, treated with L-thyroxine for 15 days, was very similar to the pictures of the rats, injected with this hormone for 30 days. As we previously observed, the thyroid sections from the rats, treated with L-thyroxine, showed differences in the size of follicles with a predominance of macrofollicles, full of well-stained, homogenous colloid and enclosed by flattened cuboid epithelium (Fig. 2). Those follicles showed the presence of a few C cells, which were more CT-immunoreactive, in comparison to those in the control group (Fig. 1). On the contrary, the smaller follicles, with higher epithelium, were accompanied by a higher number of parafollicular cells, which were less immunoreactive.

There are only few publications, concerning the activity of parafollicular cells in thyroid gland diseases, as well as, the interaction between parafollicular and follicular cells in vivo. The enhancement of CT plasma concentration, observed in 19.2 % of patients with Graves' disease in our earlier study [4], performed on the thyroid glands, taken from patients with simple and hyperthyroid goitre, together with a weak immunoreactivity for CT within C cells, indicating an increase of their secretion potential, may point to an enhancement of the hormonal activity of C cells, evoked by the hyperactive parafollicular cells during Graves' disease. Also Vierhapper et al. [10], in spite of the high prevalence of thyroid C cell hyperplasia in patients with Graves' disease, observed an elevated CT plasma levels also in some patients with hyperthyroid nodular goitre. Till now, there have been no available data about the activity of C cells in the experimental model of hyperthyroidism. In our previous [8], and also in present investigations, the inhibition of parafollicular cells activity, evoked by an application of L-thyroxine, induced the inhibition of C cells activity, expressed by attenuation of CT plasma levels and by enhanced CT-immunoreactivity in C cells. On the contrary, the overactivation of parafaollicular cells observed in thyroid nodules in patients, operated because of Graves' disease [4], caused hyperactivity of neighbouring C cells. Also, Zabel et al. [11] have demonstrated an enhancement of CT mRNA expression by follicular cells in TT line cell cultures, pointing out to a possible interaction between follicular and TT cells, the latter derived from C cells. These data support the possibility that a direct mutual relation between parafollicular and follicular thyroid cells could play an important role in the regulation of the thyroid gland activity.

Acknowledgements

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