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Preliminary immunohistochemical investigations of thyroid C cells in an experimental model of hyperthyroidism

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The role of the parafollicular (C) cells, the second most important cells in the thyroid gland, has not hitherto been clarified. They are considered to be disperse neuroendocrine cells of the APUD system and synthesise and release many of the regulatory peptides. Few publications are concerned with the evaluation of the structure and function of C cells in the thyroid gland or the probable relationship between these cells and the follicular cells in physiological and pathological conditions. For this reason immunohistochemical investigations were carried out into the activity of the C cells in rats in an experimental model of hyperthyroidism caused by chronic thyroxine influence. This C-cell activity was then evaluated. Differences in the quantity, distribution and calcitonin immunoreactivity of C cells were observed in hyperthyroid rats in comparison to the control group, together with a significant diminution of plasma TSH and calcitonin levels. Our preliminary study may indicate a functional interaction between follicular and parafollicular cells in the thyroid gland.

key words: C cells, hyperthyroidism, calcitonin immunoreactivity

INTRODUCTION

In the thyroid gland of mammals a second population of cells, parafocular (C) cells, has been described in addition to the basic follicular cells. According to Pearse [4], they are disperse neuroendocrine cells of the APUD system (amine precursor uptake and decarboxylation). The role of parafollicural cells in the function of the thyroid gland has not hitherto been clarified. Despite some controversial data, it can be assumed that the co-localisation of follicular and parafollicular cells in the thyroid gland is not accidental. It would seem possible that there is an interaction between them, mediated by the releasing of peptidergic hormones [1, 5, 6]. Parafollicular cells synthesise and release many of the regulatory peptides. Calcitonin (CT) has been proposed as the essential indicator of parafollicular cell activity [1, 6].

In the present study the number, distribution and CT immunoreactivity of thyroid C cells was evaluated in an experimental model of hyperthyroidism.

MATERIAL AND METHODS

Male Wistar rats (n = 20) weighting 90–100 g were used in the experiment and were given standard laboratory chow and water *ad libitum*. The animals were housed at 20°C in constant humidity, with a 12/12 light/dark cycle. All procedures were performed in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC)

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and were approved by the Local Ethics Committee in Białystok.

An experimental model of hyperthyroidism was induced by intraperitoneal injection of L-thyroxine (Sigma Chemical Co) at a dose of 40 μg/kg daily over 30 days [3]. A group of 10 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 calcitonin concentration by RIA (radioimmunoassay). Subsequently, the rats were thyreoidectomised. 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 this 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. [2].

Statistical analysis: statistical comparisons were made using the Mann-Whitney test.

RESULTS AND DISCUSSION

After 30 days of L-thyroxine treatment plasma TSH concentration was significantly ($p < 0.001$) reduced as compared to that of the control rats (Fig. 1). The calcitonin plasma level was also significantly ($p<0.05$) reduced in comparison to that of the control group (Fig. 2). Histological differences in the quantity as well as in the distribution of C cells were observed in the hyperthyroid rats in comparison to the control group (Fig. 3). The examined thyroid sections from rats treated with L-thyroxine showed changes in the size of the follicles with a predominance of macrofollicles, full of well-stained, homogenous colloid and enclosed by flattened cuboid epithelium (Fig. 4). These follicles showed the presence of a few C cells, which were more immunoreactive for calcitonin in comparison to those of the control group. In contrast, the smaller follicles, with a higher epithelium were accompanied by a higher number of parafollicular cells, which were less immunoreactive. The periferal parts of the gland were free of C cells.

There have only been a few publications concerned with the activity of parafollicular cells in thyroid gland diseases or the interaction between parafollicular and follicular cells *in vivo.* To the best of our knowledge there is no available data on the activity of C cells in rats with an experimental model of hyperthyroidism. Zabel et al. [7] in their pioneer *in vitro* study have demonstrated that follicular cells enhance the expression of CT mRNA in TT line cell

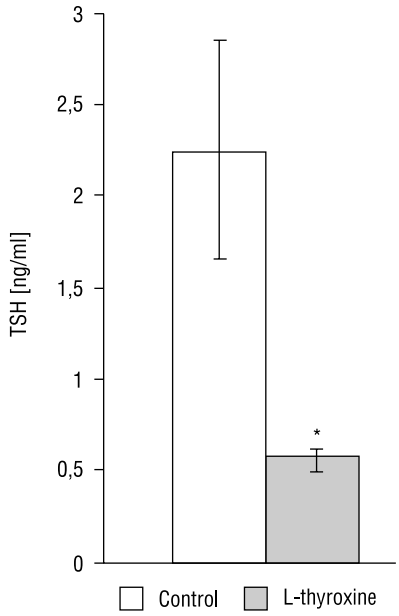

Figure 1. Effect of L-thyroxine given ip. at the dose of 40 μ/kg daily over 30 days on TSH plasma concentration. The columns represent means ± SEM of values obtained from 10 rats; *$p < 0.0003$ (Mann-Whitney test).

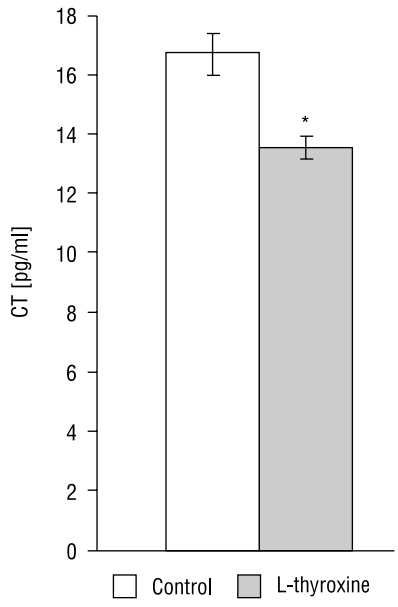

Figure 2. Effect of L-thyroxine given ip. at the dose 40 μ/kg daily over 30 days on calcitonin (CT) plasma concentration. The columns represent means ± SEM of values obtained from 10 rats; *$p < 0.0004$ (Mann-Whitney test).

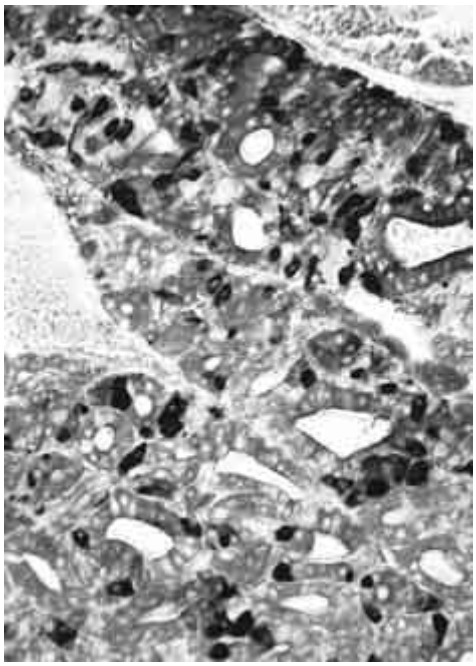

Figure 3. Light micrograph of the thyroid gland of a control rat. A positive immunohistochemical reaction is observed in most C cells (black cells); × 300.

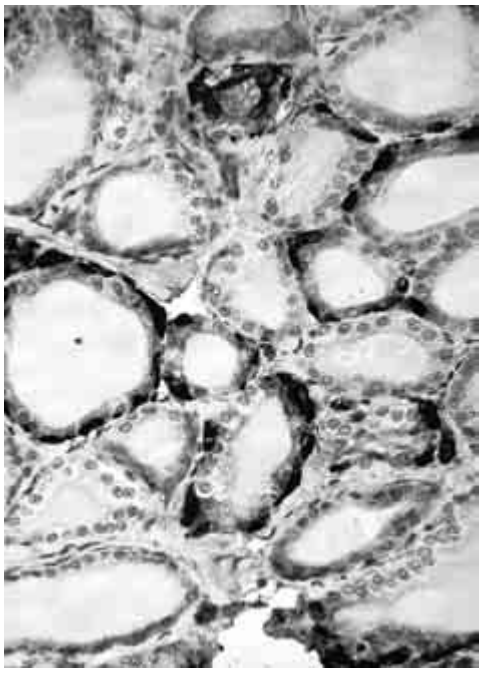

Figure 4. Light micrograph of the thyroid gland of a rat treated with L-thyroxine. Immunohistochemical staining for calcitonin in C cells; × 300.

culture, while the presence of fibroblasts enhance that of CGRP mRNA. These results support the possibility that, besides paracrine factors, direct interactions between parafollicular and follicular thyroid cells could also play an important role in the regulation of thyroid activity.

In the present *in vivo* chronic experimental investigations the relationship was demonstrated between the activity of the thyroid follicular cells and the quantity, distribution and immunoractivity of the parafollicular (C) cells. Moreover, these changes were accompanied by a significant diminution of the plasma TSH and CT levels. The altered immunohistochemical activity of C cells in rats with experimental hyperthyroidism was correlated with the attenuation of CT plasma concentrations. These preliminary observations provide a basis for the assumption that the "cross-talk" between parafollicular and follicular thyroid cells, previously reported by Zabel et al. [7] *in vitro*, is also possible in physiological and pathological conditions. To confirm this hypothesis further investigations are needed.

ACKNOWLEDGEMENTS

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