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

Dadan J[1], Zbucki R[2], Andrzejewska A[3], Winnicka MM[2], Puchalski Z[1]

[1]1st Department of General and Endocrinological Surgery, [2] Department of General and Experimental Pathology,
[3] Department of Clinical Pathomorphology, Medical University, Białystok, Poland

Abstract

In the thyroid gland of mammals, except the basic follicular (F) cells, parafollicular (C) cells are detected. They belong to disperse neuroendocrine cells of the APUD system. Co-localisation of F and C cells in the thyroid gland is not accidental. It seems possible that there is an interaction between them, mediated by the peptidergic hormones. Calcitonin (CT) is proposed as an essential indicator of C cells. The role of C cells in the function of the thyroid gland has been not clarified till now, especially in hyperthyroid state. There are only a few data which document the ultrastructure of C cells in the physiological and pathological state. In the present study, the ultrastructure of thyroid C cells in an experimental model of hyperthyroidism was evaluated. Our preliminary study may confirm the functional interaction between follicular and parafollicular cells in the thyroid gland.

Key words: C cells, hyperthyroidism, calcitonin, ultrastructure.

Introduction

The ultrastructure of the parafollicular C cells is quite similar in all the experimental mammalian species and differs from the ultrastructure of the follicular C cells. Its important characters include: numerous, spherical and electron, densely delineated by cell membrane [1]. Normally, the grain size does not exceed 200nm and consists of homogeny and electron dense material. C cells are closely linked to the base of the F cell neighbouring follicle. Neither desmosoms nor any other type of epithelial connections exists between them. The basal membrane is common for the follicle and is linked to its C cells. An exact analysis of the further series of slices shows that, generally, the group of C cells, lying in the loose connective tissue between follicles has a common basal membrane with the neighbouring follicle [2].

Under an electron microscope, the structure of C cells is much more multilateral and irregular, as compared to the same structure, when observed under a light microscope [3]. Multilateral structure of C cells dominates frequently observed finger, like dendrites of its cytoplasm. Slightly longer dendrites are arranged along the basal membrane. These are linked directly to the base of F cells or run toward capillaries [4, 5]. Solitary lying C cells are usually bigger then F cells, having bigger oval or spherical nucleus. In many mammals, a frequent depression in the nuclear membrane can be observed. Chromatin is present as either a minute scattered structure or as a small dense material in the periphery of the nucleus. In the nuclear membrane, numerous nuclear pores are present. The nucleolus is big, electron dense, single and rarely double. Secreting granules, differing in size, accumulate in the region of cells, which are directly linked with capillaries [6, 7]. In spite of the very characteristic and different ultrastructure of the F cells and different hormonal function, there exists much evidence, showing a close structural and functional relationship between F and C cells. According to many authors, the co-operation between F and C cells is realized by the paracrine roots, by the synthesis of regulatory peptides RP, the number of which is constantly increasing. C cells are not only closely linked to the base of the F cells of its follicle but they also lie together with them in the space, surrounded by basal membrane. Some authors even differentiate a special functional group in the thyroid structure, in a form of group of follicles (epitheliomers), lying in appropriate bifurcated spaces, covered by the common basal membrane. Generated

ADDRESS FOR CORRESPONDENCE:
Jacek Dadan
1stDepartment of General and Endocrinological Surgery
Medical University of Białystok
M. Skłodowskiej-Curie 24A, 15-276 Białystok, Poland
e-mail: klchirog@amb.edu.pl

Figure 1. The thyroid gland of a control rat. Electron micrograph, showing parafollicular cell, which contains granules, filled with a homogenous substance of low and high density. x 7000

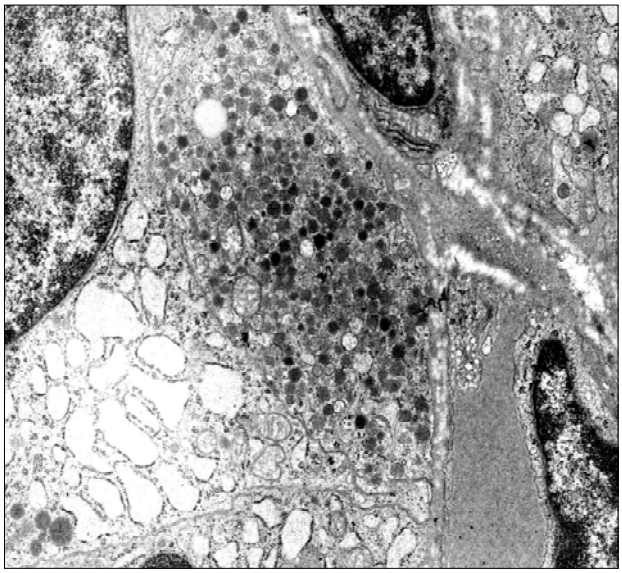
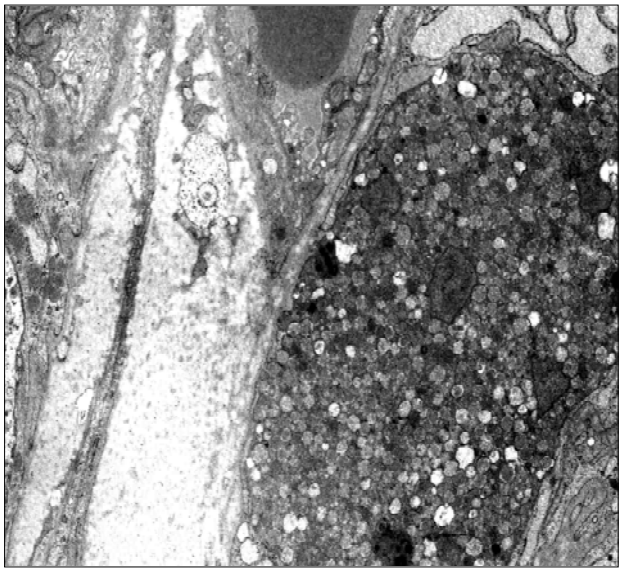


Figure 2. The thyroid gland of a rat with hyperthyroidism. Electron micrograph, showing parafollicular cell, fullfilled with dense granules. x 7000



in this way, a new micro environment for the F and C cells is separated from the capillaries by the basal membrane, through which their endocrine function is realised. Due to such a structure, active biological factors, coming either from direct surrounding or from the distal tissue, can interact with the F and C cells. In this way, this complex structure, consisting of two different types of cells, is integrated with the functions of the whole organism. Simultaneously, the micro environment, formed in this way, enables an effective co-operation between cells by the paracrine root.

Material and methods

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. A group of ten control rats were treated with saline under the same experimental conditions. Male Wistar rats (n=18), weighing 90 - 100 g, were used in the experiment and given standard laboratory chow and water ad libitum. The animals were housed at 20° C and constant humidity, with the 12/12 light/dark cycle. All the procedures were performed in compliance with the European Community Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Local Ethics Committee in Białystok.

At the end of the experiment, under pentobarbital sodium anaesthesia (50 mg/kg), blood was collected from the abdominal aorta of each rat to determine plasma TSH concentrations by radioimmunoassay (RIA). Subsequently, the rats were thyreoidectomized. The tissue, taken for examination, was cut into approximately 1 μm-thick sections and fixed for an hour at room temperature in 2.5% glutaraldehyde solution, prepared in 0.1M phosphate buffer (pH 7.4) and then, for the next 12 hours, in temperature of 4° C. During the subsequent stage, lasting from 17 to 20 hours, the tissue was rinsed in phosphate buffer and fixed for the following 2 hours in 1% OsO_4 solution, prepared in phosphate buffer (pH 7.4).

The material, prepared and fixed according to the routine procedure, was embedded in Epon 812 and sectioned by means of an ultramicrotome. Ultra thin sections of the thyroid glands were contrasted by uranyl acetate and amonium citrate and subsequently examined under TEM (Opton 900 PC type).

Results and discussion

Hyperthyroidism is a pathological syndrome in which tissue is exposed to excessive amounts of circulating thyroid hormone. The diagnosis of hyperthyroidism is generally straightforward, with raised serum thyroid hormones and suppressed serum thyrotropin. After 30 days of L-thyroxine treatment, plasma TSH concentration was significantly ($p<0.001$) reduced (mean 0.52 ng/ml), as compared to respective values in the control rats (mean 2.34 ng/ml). Calcitonin plasma level was also significantly ($p<0.05$) reduced (mean 13.34 pg/ml) in comparison to that in the control group (mean 16.69 g/ml). Clear changes in the construction of the ultrastructure existed among the experimental groups of rats. In rats with a low concentration of TSH, a tendency towards low activity of F and C cells was observed, particularly in comparison to respective values in the control group. Microvilli which penetrate within colloid were lower and less numerous. In the cytoplasm near microvilli less number of reabsorbing vacuoles and secreting granules were found. Apart from this, a decreased activity of the rough endoplasmic reticulum, its expansion, less regular shape and less dense ribosomes on the external cytoplasmic surface of its membrane were observed. Mitochondria had much spherical and less comb shape. C cells, situated in the direct neighbourhood of the F cells and surrounded by the common basal membrane, showed the presence of numerous, dark secreting granules, and an irregular cell nucleus, different from that in the control group of rats.

Ultrastructural diagnostic results, obtained from the control group, confirmed the characteristic feature of C cell structure and its colocation with F cell (Fig. 1). In order to examine the activity of the cells, certain points were taken into consideration, mainly the epithelial appearance, microcosmic, rough endoplasmic reticulum, cell nucleus, reabsorbing vacuoles and secreting granules. Ultrastructure of thyroid obtained from rats treated with L-thyroxine showed decreased of activity of endocrine cells, both F and C cells, mainly less numerous, low microvilli near which in the cytoplasm were found less number of reabsorbing vacuoles. Apart from this, a decreased activity of rough endoplasmic reticulum was observed. C cells, situated in the neighbourhood of F cells and together with them, surrounded by the common basal membrane, showed the presence of numerous, dark secretary granules and and irregular nucleus, different from that in the control group (Fig. 2).

Ultrastructural diagnostic results coincide with the previously proved immunoactivity of C cells, defined, using the immunohistochemical technique [8]. In the control group of rats, in C cell, dark and light secretary granules always existed, particularly in the perivascular area. In the cytoplasm of the rats, which received thyroxine, a higher number of secretary grains were observed, which may indirectly indicate an increased accumulation of the regulatory peptides. Cell nuclei in this group of rats were smaller and, some of them, were irregular, as compared to those in the control group.

Preliminary results of these experiments and ultrastructural diagnosis are very promising, forming an ideal supplement of the immunohistochemical diagnosis, proving the theory of close cooperation between C and F cells in the thyroid gland, both in physiological and pathological conditions. According to the authors, there is a justified need for the continuation of these experiments by taking molecular biology technique into account.

Acknowlegments

The study was supported by KBN grant No 1-40-065.

References

1. Ekholm R, Ericson LE. The ultrastructur of the parafollicular cells of the thyroid gland in the rat. J Ultrastruct Res, 1968; 23: 378-402.

2. Lupulescu A, Petrovici A. Ultrastructure of thyroid gland. Buchdruckerei Hutter AG, Reinach BL. 1968.

3. Teitelbaum SL, Moore KE, Shieber W. Parafollicular cells in the normal human thyroid. Nature, London, 1971; 230: 334-5.

4. Alumets J, Hakanson R, Lundqvist G, Sundler F, Thorell J. Ontogeny and ultrastructure of somatostatin and calcitonin cells in the thyroid gland of the rat. Cell Tissue Res, 1980; 206: 193-201.

5. Welsch U, Flitney E, Pearse AGE. Comparative studies on the ultrastructure of the thyroid parafollicular C cells. J Microsc, 1969; 89: 83-94.

6. Atack CV, Ericson LE, Melander A. Intracellular distribution of amines and calcitonin in the sheep thyroid gland. J Ultrastruct Res, 1972; 41: 484-98.

7. Fortney JA. Light and electron microscopic observations of thyroid parafollicular cells of opossum. Gen. Comp. Endocrinol, 1978; 36: 119-32.

8. Dadan J, Zbucki R, Sawicki B, Bialuk I, Zawadzka A, Winnicka MM, Puchalski Z. Preliminary immunohistochemical investigations of thyroid C cells in an experimental model of hyperthyroidism. Folia Morphol, 2003; 62: 319-21.