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Fine Structure of the Thyroid Parafollicular Cells in Normal, Vitamin D and CaCl_2 -Treated, and CaCl_2 -Treated Mice

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Since COPP et al. (1962) and HIRSCH et al. (1963) described the calcium-lowering factor named calcitonin or thyrocalcitonin, attention has been focused on the parafollicular cells of the thyroid described by BABER (1876) and NONIDEZ (1932) as a possible source of this hormone. Several reports have been published on the fine structure of the parafollicular cells. Though EKHOLM (1964) and SATO et al. (1966) reported on the fine structure of these cells in the mouse, a more detailed description of the cells in this animal seems to be necessary. Papers dealing with the relationship between the fine structural changes of the parafollicular cells and the blood calcium level are very few except for those of MATSUZAWA (1966), PEARSE (1966) and YOUNG et al. (1968). MATSUZAWA (1966) and MATSUZAWA and KUROSUMI (1967) who observed the reaction of the parafollicular cells several hours after a single injection of calcium chloride and suggested that they might secrete thyrocalcitonin.

The authors intended to study the reactions of the parafollicular cells in long-term hypercalcemia. Their fine structural changes after injections of either vitamin D plus CaCl_2 or only CaCl_2 for several days were examined. The abstract of this study was reported at the Japanese Thyroid Society Meeting (Kōjōsen Kenkyū Dōkōkai), Kyoto (Japan), April 8, 1968. A similar report has been published by ERICSON (1968) after the preparation of the present paper.

Materials and Methods

Twenty five male mice were used for this study. Five animals served as controls. The animals were injected subcutaneously with vitamin D (Chocola D, Eisai Co. 40,000 i.u./day) plus CaCl_2 (3mg/day : 0.3 cc of 1 % water solution) for 2 days (5 animals) or 7 days (5 animals). Ten animals were injected subcutaneously with CaCl_2 (3 mg/day) for 15 or 75 days. All these mice were sacrificed two hrs after the last injection. Thyroids were fixed in 3 % glutaraldehyde buffered with cacodylate at pH 7.4 for 1 hr and postfixed in 1 % OsO_4 buffered with cacodylate at pH 7.4 for 1 hr. Some specimens were fixed in a mixture of one part of 4 % OsO_4 , two parts of cacodylate buffer and one part of 10 % glutaraldehyde for 2 hrs. After dehydration by graded concentrations of alcohol, the tissues were embedded in Epon epoxy resin. Sections, cut on a Porter-Blum ultramicrotome and stained with Millonig's lead, were examined in a Hitachi HS-7 type electron microscope.

Fig. 1. Part of a parafollicular cell of a normal rat. Notice numerous small dense granules in the cytoplasm. $\times 10,500$

Fig. 2. Part of two parafollicular cells of the rat treated with vitamin D plus CaCl_2 for two days.
N nucleus, *G* Golgi apparatus. $\times 28,000$

Fig. 3. A part of a parafollicular cell of the rat treated with vitamin D plus CaCl_2 for two days. Notice the rough endoplasmic reticulum (*R*) and the Golgi apparatus (*G*). Relatively large dense granules are gathered near the plasma membrane, and the small granules are in or near the Golgi apparatus. $\times 25,000$

Fig. 4. A parafollicular cell of the rat treated with vitamin D plus CaCl_2 for 7 days. The dense granules are markedly reduced in number. $\times 14,000$

Fig. 5. A parafollicular cell of the rat treated with vitamin D plus CaCl_2 for 7 days. The dense granules are markedly reduced in number. Notice the Golgi apparatus (G) suggesting granule formation. $\times 17,000$

Fig. 6. A part of a parafollicular cell of the rat given vitamin D plus CaCl_2 for 7 days. Notice the relationship between the rough endoplasmic reticulum (*R*) and Golgi apparatus (*G*). Numerous small vesicles and granules are seen in or near the Golgi apparatus, suggesting active granular formation. $\times 25,000$

Observations

1. Parafollicular cells in normal mice

The fine structure of the normal parafollicular cell in the mouse corresponded generally to that in the rat described by EKOLM and ERICSON (1968). The parafollicular cells were located singly or in groups among the follicles or at the basal part of the follicular cells and generally did not face the follicular lumen. They had a round or oval nucleus in the middle of the cell. Numerous small round dense granules of about $150\text{--}400\text{ m}\mu$ (about $200\text{ m}\mu$ in mean value) diameter were distributed throughout the cytoplasm. These granules appeared as empty sacs when the tissue had been fixed only with osmium tetroxide, whereas an internal dense material of the granule became distinct after fixation in glutaraldehyde as MATSUZAWA (1966) pointed out. The rough endoplasmic reticulum was less conspicuous than that of the follicular cells. Its cisternae were not enlarged but flattened in parallel arrays,

Fig. 7. A part of a parafollicular cell of the rat given vitamin D plus CaCl_2 . Notice the rough endoplasmic reticulum (*R*), and numerous small vesicles in or near the Golgi apparatus (*G*). $\times 19,000$

while those of the follicular developed and irregularly dilated. This is one of the characteristic differences between both cells. The parafollicular cells contained a small number of mitochondria as large as those in the follicular cells and with usual lamellar crests. The Golgi apparatus generally occupied a supranuclear region and showed an arched or circular form consisting of lamellae, small vacuoles, and vesicles. One end of the rough endoplasmic reticulum often terminated at the Golgi region where small agranular buds derived from the rough endoplasmic reticulum were seen. Small granules of 100–200 $\text{m}\mu$ diameter were often seen in the Golgi region. A few multivesicular bodies sometimes occurred near or in this region. Free ribosomes making polysomes were distributed throughout the cytoplasmic matrix. A part of the plasma membrane of the cell faced the basement membrane

and connective tissue space. Dense granules were gathered near the plasma membrane and sometimes in contact with it. Besides the small granules a few heterogeneously dense bodies of 0.5–1.5 μ diameter, suggesting lysosomes, were found in the cytoplasm.

2. Parafollicular cells in hypercalcemic mice

No marked changes in fine structure were found in the parafollicular cells of the animals treated with CaCl_2 for 15 or 75 days. When, however, the hypercalcemia was induced by repeated injections of both CaCl_2 and vitamin D the cells conspicuously changed, especially in the number of granules. The total serum calcium level was 5.4 (5–6) mE/l in the normal mice, 6.3 (6–7) mE/l in the mice treated with vitamin D plus CaCl_2 for 2 days and 6.2 (6–7) mE/l in the mice given vitamin D plus CaCl_2 for 7 days. After 2 and 7 days of lasting hypercalcemia induced by vitamin D plus CaCl_2 , the granule number per unit area of cytoplasm was markedly reduced as compared with that of the normal parafollicular cells. In the normal cell cytoplasm, about 5.2 granules per μ^2 were counted, while in 2 day-hypercalcemia 2.1 granules per μ^2 , and in 7 day-hypercalcemia 0.65 granules per μ^2 were counted. The granules tended to gather at the periphery of the cytoplasm in the vitamin D and CaCl_2 treated animals. The Golgi field became enlarged and its vesicles and vacuoles increased in number. After 7 days of lasting hypercalcemia the granules in all the parafollicular cells decreased strikingly in number, and only a few remained in the Golgi field and rarely at the margin of the cell. The rough endoplasmic reticulum and the Golgi apparatus seemed enlarged and became distinct. Golgi vesicles seemed to be increased in number, and small immature granules were often noticed in the Golgi region.

Discussion

The calcium-lowering factor was first discovered by COPP et al. (1962) and was named calcitonin. They considered that this hormone is secreted from the parathyroid. HIRSCH et al. (1963), demonstrating this factor in the thyroid gland, proposed the name thyrocalcitonin. The parafollicular cells discovered by BABER (1876) and NONDEZ (1932) were then related to the possible site of synthesis and release of this hormone. The electron microscopic changes of the parafollicular cells were studied by MATSUZAWA (1966) and PEARSE (1966) who found a marked decrease of their cytoplasmic granules in the experimentally induced hypercalcemia, and by YOUNG et al. (1968) who reported an increase in the number of light vesicles after perfusion of the thyroid gland, isolated in situ, with hypercalcemic blood.

The present study shows a strong reaction of the parafollicular cells to long-term hypercalcemia induced by the vitamin D treatment. The marked reduction of the granules is considered to indicate an increased release of the granules from the cell. The enlarged Golgi apparatus and its increased vesicles suggest the hyperactivity of this structure. The animals treated with CaCl_2 for 15 and 75 days showed no clear change in fine structure of the parafollicular cell, while MATSUZAWA (1966) and MATSUZAWA and KUROSUMI (1967) found a marked degranulation in rats 4 hrs after an intravenous injection of CaCl_2 . This fact may be explained as follows. CaCl_2 induces a temporary hypercalcemia 4 hrs after injection but this state is easily

returned to normal several hours later. So, even if CaCl_2 is injected everyday, the cell structure returns to the normal state several hours after each injection.

The vitamin D, if injected everyday, maintains hypercalcemia. The degranulation of the parafollicular cells in this state suggests that the dense granules in question, containing the blood calcium lowering hormone, thyrocalcitonin, are actively released to counteract the hypercalcemia. This conclusion agrees with that of ERICSON (1968) who recently published an excellent paper dealing with the degranulation of the parafollicular cells of the rat thyroid by vitamin D_2 -induced hypercalcemia. Thyrocalcitonin has been considered to be polypeptide hormone by FOSTER (1968). The present findings on the Golgi apparatus of the parafollicular cells imply that the secretory granules of these cells are synthesized in the rough endoplasmic reticulum and transported to the Golgi region to develop into mature forms as is the case in other protein secreting cells. The enlargement of the Golgi apparatus and the increase of its vesicles in the hypercalcemic animals suggest an activated granular formation in this region of the cytoplasm.

Summary

Electron microscopic studies were made on the parafollicular cells of the thyroids of normal, vitamin D plus CaCl_2 -injected, and CaCl_2 -injected mice.

1. The cytoplasmic granules of $200\text{m}\mu$ diameter are reduced in number in the animals treated with vitamin D plus CaCl_2 everyday for 2 or 7 days. In these animals the Golgi field is enlarged containing numerous small vesicles and newly formed small granules, suggesting an active granule formation there.

2. No marked changes were observed in the parafollicular cells of the animals given CaCl_2 everyday for 15 or 75 days.

3. The parafollicular cells remarkably reacting to the long-term hypercalcemia seem to be related to thyrocalcitonin production. Their granules are thought to be formed in the rough endoplasmic reticulum and Golgi apparatus system as in other protein secreting cells.

正常、ビタミンDと塩化カルシウム投与、塩化カルシウム投与マウスの 甲状腺 旁濾胞細胞の微細構造（内容自抄）

正常マウス、ビタミンDと塩化カルシウム水溶液を注射したマウス、塩化カルシウム水溶液のみを注射したマウスの甲状腺 旁濾胞細胞を電子顕微鏡で観察した。

1. ビタミンD（1日4万単位）と塩化カルシウム（1日3mg）を2日間または7日間注射した動物においては、直径 $200\text{m}\mu$ 位の限界膜をかぶった黒色顆粒数が減少し、ゴルジ野は大きくなり、小胞（vesicles）が著しく増加し、顆粒形成像がよく認められた。これは顆粒形成機能の亢進を思わせる。

2. 塩化カルシウムのみを15日間または75日間 毎日くりかえして注射し、毎日の過性の高カルシウム血症を起こしても、旁濾胞細胞には明らかな変化は認められなかった。このことは 旁濾胞細胞は反応しても すぐに元にもどることを暗示する。

3. ビタミンDと塩化カルシウム投与による長期間持続性の血中カルシウム上昇に反

応した旁濾胞細胞は、サイロカルシトニン (thyrocalcitonin) の生産に密接な関係があると考えられる。また顆粒は一般の蛋白分泌腺のそれと同じように、粗面小胞体-ゴルジ系でつくられると考えられる。

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