Parathyroid Cyst Formation Induced by Dihydrotachysterol and Calcium Acetate. An Electron Microscopic Study

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Synopsis

Parathyroid cysts were produced in rats by excessive doses of dihydrotachysterol (DHT) and calcium acetate. Although overdoses of each DHT, calcium or their combination are believed to suppress the activity of the parathyroid cell, the present electron microscopic observation showed signs of enhanced protein synthesis and secretion of the cells in all stages of cyst formation. It seems reasonable to assume that this cyst formation depends upon two main processes: retention of secretory material in the intercellular spaces and liquefaction necrosis of the parathyroid cells which emerges in rather later stages of cyst formation.

Rats simultaneously treated with excessive amounts of dihydrotachysterol (DHT) and calcium acetate develop cystic parathyroids which closely resemble those which are known to occur spontaneously in various species, including man (Selye et al., 1964a, b and c). These parathyroids exhibit a variety of cavities, ranging from numerous, small fluid-filled follicles to large cysts. However, cyst formations are not accompanied by signs of hormonal derangement. Selye and his associates (1964a, b and c) suggested that these were retention cysts resulting from a blockade of the discharge of parathyroid hormone, without suppression of its production within the gland.

In an attempt to elucidate the pathogenesis of the changes, we examined these experimental parathyroid cysts, in various stages of development, by light and electron microscopy.

Materials and Methods

Thirty female ARS/Sprague-Dawley rats (Madison, Wisconsin, U.S.A.), with a mean initial body weight of 93 g (range 90–95 g) and maintained ad libitum on Purina Laboratory Chow (Ralston Purina Co. of Canada) and tap water, were divided into 5 equal groups. Three groups were used in a preliminary experiment to determine the daily threshold dose of DHT which would be required to produce fully-developed parathyroid cysts. Groups 1, 2 and 3 therefore received 30 µg, 50 µg and 100 µg of DHT (Dr. A. Wander, S. A., Berne, Switzerland) respectively, in 0.5 ml of corn oil, once daily per os. Calcium acetate (Fisher Scientific Co., Fair Lawn, N. J., U.S.A.) was administered at the dose of 1 mM in 2 ml of water, twice daily, also by stomach tube. The rats given 30 µg or 50 µg of DHT daily, with calcium acetate, revealed inadequate cyst formations even after two weeks of treatment. The third group, which received 100 µg of DHT, exhibited severe toxic changes and high mortality as early as the 10th day of the experiment, but parathyroid cysts failed to develop. Therefore, we chose 75 µg of DHT as the daily dose for our principal experiment.

In this experiment, Group 4 was untreated and served as controls. Group 5 was given 75 µg of DHT in 0.5 ml of corn oil once daily and 1 mM of calcium acetate in 2 ml of water twice daily, both by stomach tube. In order to study the early changes in the para-
thyroids, two rats were killed on the 8th day, 24 hr after the last DHT administration, and the remaining four on the 14th day, all by decapitation.

For light microscopic examination, the right parathyroids were fixed in alcohol-formol, embedded in paraffin and stained with hematoxylin-phloxine and by the PAS technique. For electron microscopic examination, the left parathyroids were excised immediately after the rats were sacrificed, fixed in a cold 4% glutaraldehyde solution in 0.1 M phosphate buffer, postfixed in Caulfield’s buffered 1% osmium tetroxide, dehydrated in graded ethanol and embedded in Epoxy resin. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined under a Carl Zeiss EM 9A electron microscope.

Results

Changes in the parathyroids after 7 days of treatment

Macroscopically, no changes were detected in the parathyroids.

Histologically, only a few small cavities were found between the solid rows of parenchymal cells (Fig. 2). The lumens of these cavities frequently contained a pale basophilic fluid and cellular debris.

Electron-microscopically, the cysts exhibited...
round or irregular shapes, which represented dilatation of the intercellular spaces. Their walls were lined with parathyroid cells from the surfaces of which multiple microvilli and hyperplastic papillary or vacuolated nodular protrusions emerged into the lumens (Fig. 6, 7). Diffuse, finely granular matter and numerous vesicles of various sizes were found in the lumens of the cavities. Some of them appeared to be extruded cytoplasmic vesicles. The cytoplasm of a few parathyroid cells in the pericystic region was edematous and contained several irregularly shaped lipid granules (Fig. 5). Their plasma membranes were interdigitated. The parathyroid cells lining the cysts had round or oval nuclei, with several slight indentations in their nuclear membranes. The nuclei revealed light, homogeneous matrices with indistinct nucleoli, as seen in the cells of the control rats. The cytoplasm of the majority of these cells exhibited many amorphous, electron-dense vesicles around the well-developed Golgi complex; possibly, these corresponded to secretory granules (Fig. 6, 7). Numerous free or aggregated ribosomes and paired membranes of the rough-surfaced endoplasmic reticulum were prominent. Many oval or elongated mitochondria, with easily-recognizable cristae, were widely distributed throughout the cytoplasm. However, no increase in the number of lysosomal bodies was evident.

Changes in the parathyroids after 14 days of treatment

Macrosopically, the parathyroid glands were semi-transparent and slightly enlarged.

Histologically, cyst formation further progressed, leading to fully-developed sponge-like cavities, some of which contained a varying amount of solid cords of parathyroid cells (Fig. 3).

Electron-microscopically, the cyst walls revealed a single layer of parathyroid cells with multiple microvilli (Fig. 8, 9). The cytoplasm of these cells was rich in free or membrane-attached ribosomes. The well-developed Golgi complex displayed numerous vesicles. Homogeneous electron-dense granules were found both within and around the Golgi complex (Fig. 9). Large, transparent vesicles were scattered throughout the cytoplasm (Fig. 9, 10), some of which were probably secreted into the lumens of the cysts.

The intercellular spaces between the parathyroid cells were more or less distended, and contained finely granular or dilated vesicles. Some of the parathyroid cells in the vicinity of the cavities showed signs of degeneration and liquefaction of their cytoplasm (Fig. 11). The cytoplasmic matrices were strikingly edematous, the granular endoplasmic reticulum markedly distended, the cell membranes ruptured, and the cellular organelles extruded into the dilated intercellular spaces. The cytoplasms almost completely disappeared. The remaining cytoplasmic organelles were still attached to the nuclei. The intact cells revealed well-developed Golgi areas with numerous vesicles, a large number of ribosomes arranged in clusters or attached to the endoplasmic reticulum membranes, and multiple elongated mitochondria. The secretory granules in these cells Fig. 10 were as numerous as in those which lined the walls of the cysts (Fig. 9).

Discussion

Our findings indicate two processes in the formation of parathyroid cysts: distension of the intercellular spaces caused by retention of secretory fluids, and liquefaction necrosis of the parenchymal cells. In the parathyroid cells, abundant free or membrane-attached ribosomes, well-developed Golgi complex, many amorphous, electron-dense vesicles, and widespread interdigitation of the plasma membranes were noted. Such fine structural characteristics are consistent with those of enhanced synthesizing and secretory activity of the parathyroid cells (Nakagami, 1965; Roth and Raisz, 1966; Zawistowski, 1966; Altenaehr et al., 1969). Therefore, it seems
reasonable to assume that these cells produce large amounts of secretory material which accumulates in the intercellular spaces. This process, together with liquefaction necrosis of the parathyroid cells which appears in a rather later stage, leads to the development of cysts. Our findings are in agreement with previous reports on the pathogenesis of parathyroid cyst formation (Selye et al., 1964a, b and c).

A number of electron-microscopic studies of the parathyroid glands indicate that a high level of plasma calcium is associated with diminished, and a low level with increased, activity of the parathyroid cells (Lever, 1957 and 1958; Roth and Munger, 1962; Munger and Roth, 1963; Roth and Raisz, 1966; Nakagami, 1967; Roth et al., 1968). It has also been demonstrated that high doses of vitamin-D (Capen et al., 1965 and 1968) or a combination of excessive amounts of vitamin-D and calcium (Roth et al., 1968) suppress parathyroid function. On the other hand, our experiments revealed fine structural changes which point to enhanced protein synthesis and secretion by the parathyroid cells, despite DHT and calcium acetate overdosage. However, it is questionable whether the secretory material in the cystic parathyroid is hormonally active and, if so, it still remains to be seen why it is not discharged. It is also difficult to explain why cysts develop, and not parenchymal hyperplasia. Identification of the secretory material by biochemical and immunohistochemical methods as well as measurement of parathyroid hormone content in blood and in cystic parathyroids would provide a better understanding of the pathogenesis and functional significance of parathyroid cyst formation.

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References


Fig. 4. Parathyroid cells of control rat. Multiple elongated mitochondria are scattered throughout the cytoplasms. There are abundant free or membrane-attached ribosomes. Well-developed Golgi complex (G) can be observed. Some of the vesicles contain finely granular material (arrows). One cilium (Cl) is seen. These were rare. ×9,000

Fig. 5. Parathyroid cells after 7 days of treatment. Two small cavities are formed between cells. In the lumen of the cavity on the right, finely granular matter and multiple dilated vesicles are found. ×1,000

Fig. 6. Electron micrograph of cavity wall is shown in Figure 2. Lining cell reveals a large number of small, amorphous vesicles (arrows). Several clear, distended vesicles (V) are found in the nodular protrusion (V). ×9,800

Fig. 7. Parathyroid cells after 7 days of treatment. Papillary hyperplasia of the microvilli in the distended intercellular spaces. Surrounding cells show signs of enhanced protein synthesis and secretion, that is, abundant ribosomes, many scattered round or oval mitochondria, well-developed Golgi complex (G), numerous vesicles (v & arrows) and interdigitated cell borders. ×10,600
Fig. 8. Lining cells of cystic parathyroid after 14 days of treatment. There are numerous microvilli in the cystic lumen and marked interdigitation of the cell borders. Vesicles (v & arrows) with homogeneous contents are assumed to be secretory granules. Abundant free or membrane-attached ribosomes are distributed throughout the cytoplasm. \( \times 10,600 \)

Fig. 9. Many secretory granules (v & arrows) are seen inside and outside the Golgi complex (G) in lining cell of parathyroid cyst. \( \times 19,600 \)

Fig. 10. Parathyroid cells from compact cellular sheets. Complicated excavations of cell surfaces are provided with multiple microvilli. Cytoplasms contain a number of secretory granules with homogeneous electron-dense or less electron-dense material. Some of them are clearly bound by limiting membranes (arrows.) Elongated central cell shows well-developed Golgi complex (G) and multiple vesicles (v) scattered throughout the cytoplasm. Intercellular spaces are distended and contain granular matter and dilated vesicles. \( \times 7,200 \)

Fig. 11. Liquefaction necrosis in lining cells of parathyroid cyst. Severe edema and liquefaction of the cytoplasmic matrix are visible. A part of the cell membrane is raptured and the cell organelles are extruded into the cystic lumen. Upper cell reveals marked dilatation of granular endoplasmic reticulum and a few lipid droplets. \( \times 7,200 \)