PARATHYROID GLANDS IN PRIMARY HYPERPARATHYROIDISM: AN ULTRASTRUCTURAL MORPHOMETRIC STUDY OF 25 CASES

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SUMMARY

In parathyroid glands removed from patients with primary hyperparathyroidism (pHPT), hyperplasias and adenomas cannot be distinguished from one another by light microscopy when only one gland is available for examination. When a second gland is available, it is necessary to establish whether it is normal, suppressed, or hyperplastic. This distinction may be difficult, and the main criterion is the amount of cytoplasmic lipid in the parenchymal cells. If the lipid is abundant, the gland is considered normal or suppressed, and if it is scanty, the gland is interpreted as hyperplastic. We have performed a morphometric ultrastructural study to test the reliability of this criterion. Twenty-five adenomatous glands removed from patients with pHPT, when compared with glands of normal size from euparathyroid patients, showed a significant increase in the parameters indicative of metabolic activity, namely the size of the Golgi apparatus, the amount of rough endoplasmic reticulum, and the length of plasmalemmas. In addition, the amount of cytoplasmic lipid was significantly reduced. Furthermore, 25 glands of normal size removed from the same patients with pHPT showed an amount of lipid similar to that of normal glands from euparathyroid patients. However, all the parameters indicative of metabolic activity were significantly higher than those in glands from euparathyroid patients and comparable to those found in adenomatous glands. These results suggest that in pHPT, normal-size glands are as active as adenomatous glands, regardless of a higher lipid content.

KEY WORDS—Parathyroid gland, primary hyperparathyroidism, electron microscopy, morphometry, cell count.

INTRODUCTION

It is widely believed that adenoma of a parathyroid gland is the most frequent cause of primary hyperparathyroidism (pHPT) and that less often, generalized hyperplasia is the cause. Adenomas involve a single gland, the remaining glands being normal or suppressed, whereas hyperplasia affects all glands. It is essential to distinguish between these two conditions because the surgical treatment is different. A diagnosis of adenoma cannot be made solely on the basis of gross enlargement of one parathyroid gland in the presence of three normal-size glands, since 30–50 per cent of patients with hyperplasia show only one enlarged gland ("pseudoadenomatous hyperplasia"). Furthermore, the microscopic differential diagnosis between hyperplasia and adenoma may be difficult in this setting of
single-gland enlargement. It is now customary to examine a second, macroscopically normal-sized gland, and if it has a 'normal' or 'suppressed' histological appearance, the diagnosis of adenoma of the enlarged gland is made. If the normal-size gland shows histological signs of hyperactivity, the diagnosis of hyperplasia is favoured. The main morphological criterion currently used to classify parathyroid glands as 'normal', 'suppressed', or 'activated' is the amount of cytoplasmic lipid in the parenchymal cells. This practice has been widely accepted and encompasses the concept that lipids are scarce during the active stage of chief cell synthesis and secretion, and abundant during the resting stage. This idea is supported by the fact that most normal glands have a considerable amount of lipid (about 80 per cent of chief cells are vacuolated), whereas adenomatous and hyperplastic glands contain scant lipid. In a previous study, we demonstrated that in most patients with pHPT and one enlarged ('adenomatous') gland, the normal-size glands with a lipid content interpreted as normal by light microscopy showed a large majority of chief cells fulfilling the criteria for hormonal hyperactivity when examined by electron microscopy. These criteria were based on the morphology of the rough endoplasmic reticulum (RER), the Golgi apparatus, and the plasmalemma. Specifically, during active hormonal synthesis and secretion, the RER and Golgi complexes are hypertrophic, and the plasmalemmas are elongated and interdigitated. On the basis of these findings, it was suggested that the usual cause of pHPT is hyperplasia rather than adenoma, regardless of the gross and light microscopic appearances of the glands.
This paper presents the results of a morphometric study conducted at the electron microscopic level on parathyroid glands removed from patients with pHPT, in order to define the relationships between intracytoplasmic lipid content and morphological parameters of secretory activity.\textsuperscript{12-19}

**MATERIALS AND METHODS**

**Clinical**

Thirty-five patients were included in the study. Ten patients (three males and seven females, median age 45) with a non-functioning adenoma of the thyroid gland having normal blood levels of calcium and phosphorus underwent thyroidectomy with coincident removal of a normal parathyroid gland (NPG). These parathyroid glands were used as controls. Twenty-five patients had pHPT on the basis of standard clinical and biochemical parameters, and they all had a grossly enlarged (15–80 mm) ‘adenomatous’ parathyroid gland, which was removed at surgery; the other parathyroid glands were all of normal size (4–8 mm), and one of them was also removed for study. Pre-operative serum calcium levels ranged from 10.5 to 19 mg/100 ml, and serum parathormone (PTH) ranged from 3.2 to 27.5 mU/ml (normal values <2.4 mU/ml). Fifteen of these patients were women and ten were men; their ages ranged from 23 to 66 years (median 55 years). Secondary (renal) hyperparathyroidism and multiple endocrine neoplasia syndrome were excluded in all cases.

**Morphology**

All glands were processed for light microscopy and stained with haematoxylin and eosin. In addition, frozen sections were stained with oil red O
to demonstrate stromal (adipose tissue) and cytoplasmic lipid. For electron microscopy, 1 mm fragments of all glands were fixed in 2 per cent glutaraldehyde, stored in Sorensen buffer for 4 h at 4°C, post-fixed in 1 per cent osmium tetroxide, dehydrated in graded ethanol, infiltrated, and embedded in an Epon–Araldite mixture. Thin sections were stained with lead citrate and uranyl acetate, and observed under a Philips 301 electron microscope.

Morphometry

Ten photographs (final magnification × 8500) of the parenchymal area were obtained from each gland according to a systematic randomized protocol. Ten photographs were considered as representative of the whole tissue because in a pilot study of two cases (one normal and one pHPT) no significant difference in the mean values of the measured cell parameters was found when 10 or 50 photographs were used. At the selected magnification (× 8500), all of the parameters to be quantitated were easily identified. Measurement of the cell parameters was performed by means of a semi-automated method which employed a graphics table (on which the organelles were plotted) connected to an appropriately programmed computer (SEM-IPS image analyser, Kontron, Germany). The following parameters were investigated: total cellular, nuclear, Golgi, and lipid areas; and RER and plasmalemmal lengths. Cytoplasmic area was calculated as the difference between total parenchymal and nuclear areas.

The Golgi and lipid regions were expressed as a percentage of the cytoplasmic area; the Golgi area included the vesicular component of the organelle on its trans side. The RER and plasmalemma were
expressed as length densities (nm/µm² of cytoplasmic area). The line extension of RER cisternae sections per µm² of cytoplasm was used as an estimate of the reticulum; this approach was preferred to quantification of the percentage of cytoplasm surface occupied by RER because the reticulum was invariably in the form of flattened cisternae without any measurable content. For each group of patients, the mean ± SE value of all parameters was calculated. Cells poorly preserved from the ultrastructural point of view, as well as those which did not show the nucleus in the section plane, were excluded from analysis. Measurements were made in a blind fashion and reproducibility of the results was tested in repeated measurement of the same section; the data were reproducible within 2 per cent. Statistical comparison of the data was performed with the Student’s t-test. For each pHPT patient studied, parameters from ‘adenomatous’ and ‘normal’ glands were compared.

RESULTS

Morphology

Normal Parathyroid glands from euparathyroid patients (NPGs)—The morphology of NPGs in these patients was consistent with previous findings in normal parathyroid glands. Light microscopy showed chief, oxyphil, and transitional cells arranged in lobules and cords. Stromal and intracytoplasmic lipids were readily visible (Fig. 1, inset). Electron microscopy (Fig. 1) showed chief cells with almost linear plasmalemmas. The nuclei were round and, in some cells, had a well-developed nucleolus. The Golgi apparatus and RER were of variable size, related to the functional stage of the cells. Glycogen was abundant, and mitochondria were small and elongated.

Adenomatous parathyroid glands (APGs)—Light microscopy of APGs showed sparse lipid in the stroma, as well as in the parenchyma (Fig. 2, inset).
Chief cells were the most represented element in the tissue, with a few admixed oxyphil and transitional cells. Dysplastic cytological changes were frequent in the enlarged glands. Electron microscopy (Fig. 2) of the chief cells showed an active Golgi apparatus, hypertrophic RER, and extensive interdigitation of the plasmalemma of adjacent cells. The amount of cytoplasmic lipid varied, but in the majority of the cells only one small droplet was found. Annulate lamellae were found in only one case.

'Normal' parathyroid glands from hyperparathyroid patients (NPG-pHPT)—The light microscopical appearance of NPG-pHPT was very similar to that of NPGs from the ten euparathyroid subjects used as controls (Fig. 3, inset). However, electron microscopy of NPG-pHPT (Fig. 3) showed very active chief cells, similar to those found in APGs. The lipid content varied in different regions of the sections but was roughly comparable to that of NPGs.

DISCUSSION

The differential diagnosis by light microscopy between parathyroid adenoma and hyperplasia may be difficult. Histologically, the cell types and tissue pattern are the same, and although remnants of
normal parathyroid tissue are often present at the periphery of adenomas, they may also be found around hyperplastic glands. Nevertheless, an accurate diagnosis is essential for choosing the best surgical therapy. Intracytoplasmic lipid content is the histological parameter currently used to distinguish between active and resting parathyroid glands; numerous vacuoles are found in the chief cells of normal or suppressed glands, and few or no vacuoles are seen in adenomatous and hyperplastic glands. However, some authors have found that cytoplasmic lipid has limited usefulness in distinguishing between normal and hyperactive glands. Our previous electron microscopic studies of normal-size glands excised concomitantly with enlarged 'adenomatous' glands in patients with pHPT revealed that the lipid content may be abundant in cells having the ultrastructural features of hyperactivity.

The present work was undertaken with the object of obtaining quantitative data on ultrastructural parameters related to parathyroid cell function and comparing these data with the lipid content in APGs, NPG-pHPT, and NPGs. We measured morphological parameters which had previously been found to be associated with the functional status of chief cells; namely the size of the RER and Golgi apparatuses (organelles required in the synthesis and secretion of parathormone), the degree of interdigitation and, therefore, the length of plasmalemmas (increased subsequent to exocytotic release of the hormone), and the amount of intracytoplasmic lipid.

Some authors have suggested that the morphology of chief cells can change with age or the type of fixation. For this reason, in our study the fixation was performed by the same method for all specimens. Still, the differences between the groups of glands were striking. The chief cells of NPGs had less RER, smaller Golgi apparatuses, less membrane interdigitation, and more lipid than the chief cells of APGs. These findings are in accordance with the hypersecretory activity of the glands in pHPT. A morphometric study conducted by Thyele et al. on eight adenomatous glands from patients with pHPT showed values in agreement with those presented here, but those authors did not study NPGs. Therefore, a full comparison between their data and our data is not possible. A morphometric study comparing APGs and NPGs was conducted by Altenahr et al., and RER was found to be less in APGs than in NPGs, and the mean volume of the Golgi field was the same in APGs and NPGs. In contrast, many other non-quantitative studies have reported abundant RER and hypertrophic Golgi in APGs. Our morphometric findings of enlarged RER and Golgi apparatuses in APGs are also in accordance with results obtained in rats after 'in vivo' stimulation or 'in vitro' incubation of parathyroid glands in a low calcium medium.

An interesting finding of this study originates from the morphometric comparison of NPG-pHPT with APGs and NPGs. The NPG-pHPT have previously been considered inhibited or normosecretory when their lipid content is 'normal' (judged by a comparison with the lipid content of NPGs of euparathyroid subjects). The present work is the first morphometric study at the ultrastructural level on these aspects: it shows that despite a different lipid content, the APGs and NPG-pHPT have a similar development of the secretory apparatus that is hypertrophic with respect to that of NPGs. Therefore, the ultrastructural morphometry does not seem to be in accordance with the hypothesis that the APGs are hypersecretory and the NPG-pHPT suppressed: both types of glands show ultrastructural aspects suggesting hyperactivity. A possible explanation could be that, in most of these cases, the underlying pathology is of hyperplasia with different morphological expression in the different glands; therefore, the diagnosis of parathyroid hyperplasia would seem to be the most appropriate. The different amounts of cytoplasmic lipid in NPG-pHPT and APGs is difficult to explain. In vitro studies showed that parathyroid hormone release, determined by radioimmunoassay, is altered in NPG-pHPT with respect to both NPGs and APGs. Further studies are necessary to evaluate whether NPG-pHPT secrete bioactive parathyroid hormone or inactive fragments, but intracytoplasmic degradation of the hormone cannot be excluded.

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REFERENCES


15. Thiele J. The human parathyroid chief cell—a model for a polypeptide hormone producing endocrine unit as revealed by various functional and pathological conditions. A thin section and freeze–fraction study.


34. King DT, Hiroasa FM. Chief cell intracytoplasmic fat used to evaluate parathyroid disease by frozen section. *Arch Pathol Lab Med* 1979; 103: 609–612.


