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Case report

Parathyroid gastrin and parathormone-producing tumour in the Zollinger-Ellison syndrome of MEN 1 origin

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Summary. A case of Zollinger-Ellison syndrome of multiple endocrine neoplasia type 1 (MEN 1) origin with hyperparathyroidism and with a rise in serum gastrin due to an unusual parathyroid "gastrinoma" has been investigated. The patient had multiple endocrine tumours (pituitary and parathyroid), but no evidence of pancreatic or duodenal gastrin-producing neoplasm. Radio-immunoassay, immunohistochemistry and electron microscopy showed gastrin in one parathyroid adenoma. These findings, together with a decrease of gastrinaemia after parathyroidectomy suggest that true gastrin was produced by parathyroid tumour cells and that they themselves may be the origin of the hypergastrinaemia. Our ultrastructural investigation extends these observations and the results are discussed.

Key words: Zollinger-Ellison syndrome – Multiple endocrine neoplasia type 1 – Gastrin – Parathyroid – Hyperparathyroidism

Introduction

Multiple endocrine syndromes are characterized by evidence of various hormonal hyperfunctional states due to hyperplasia or neoplasia of different endocrine glands (Ballard et al. 1964; Wermer 1974). The parathyroids, the pancreatic islets and the anterior pituitary cells are the most commonly involved, followed by the adrenals and the thyroid. Hyperparathyroidism (HPT) appears to be the central feature of these conditions (Cope et al. 1958).

It is well known that primary HPT is often associated with increased gastric acid and high serum gastrin levels (Christiansen and Aagaard 1972; Dent et al. 1972). A relationship between the parathyroids and certain gastrointestinal hormonal functions is further confirmed by the resolution of hypergastrinaemia and gastric symptoms after parathyroidectomy (Dent et al. 1972; Macleod et al. 1987). An extra-gastric source of the hypergastrinaemia has often been suggested in these conditions, but only occasionally documented (Polak et al. 1971; Dent et al. 1972; Stremple and Watson 1974; Cassar et al. 1975; Bolman et al. 1977).

We report the clinico-morphological, immunohistochemical and ultrastructural investigation of a case of unusual mutiple endocrine neoplasia (MEN) showing high gastrin content in the parathyroid adenoma. The localization of gastrin in a surgically resected parathyroid tumour has not been well reported (Stremple and Watson 1974; Cassar et al. 1975; Fabri et al. 1986; Ouchi et al. 1990).

Case report

A 52-year-old woman was admitted in 1988 to the First Surgical Clinic, Policlinico S. Orsola, University of Bologna. The family history was negative. Her past medical history included a gastric resection for duodenal ulcer in another hospital in 1977 and a wider gastric resection for post-anastomotic peptic ulcer in 1979. In 1981, during another period of epigastric discomfort from recurrent anastomotic ulcer, increased serum gastrin (1000 pg/ml) and calcium (12 mg/ml) levels were documented. In November 1981 markedly raised prolactin levels and a ballooned sella turcica on radiography of the skull were observed. A pituitary adenoma secreting prolactin was removed. The patient was well until 1983 when hypergastrinaemia (1000 pg/ml) and an increase in serum calcium (11.60 mg/ml) and parathormone (PTH) (350 pg/ml) levels were found. A diagnosis of MEN type 1 with Zollinger-Ellison (ZE) syndrome was made and an antacid therapy with an H_2 antagonist was given. Intermittent dyspeptic symptoms were noted later and in January 1986 she was readmitted with melaena from a bleeding anastomotic ulcer. Omeprazole therapy (20 mg daily) immediately reduced active bleeding and subsequently an endoscopic examination revealed the complete re-epithelization of the ulcer. Repeated

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investigations showed continued persistent hypergastrinaemia. In 1988 the patient began to complain of diffuse bone pain and recurrent episodes of renal colic. She was still hypercalcaemic (13.4 mg/ml) with increased PTH levels (780 pg/ml). A scintigraphic exploration with thallium revealed uptake in the two left parathyroids, one of them, moreover, showing a cystic appearance on ultrasonography. Computed tomography of the pancreas and adrenals was normal. On this occasion the patient was admitted to our hospital and she underwent parathyroidectomy. At operation the left upper and lower glands were enlarged and totally removed. On exploration of the neck the right parathyroids appeared normal macroscopically. Roentgenograms showed generalized bone changes.

Materials and methods

Two parathyroid tumours were obtained fresh from the operating room and a liquid aspiration from the cystic areas was immediately performed for hormonal radio-immunoassay determination.

Sections of formalin-fixed and paraffin-embedded parathyroid tumours were stained according to the following methods: haematoxylin and eosin, periodic acid-Schiff (PAS) with and without diastase digestion, alcian blue-PAS, Congo red, Gremelius, Bodian, Masson Fontana and van Gieson elastic. An immunohistochemical investigation was done employing the PAP technique and the following antisera: chromogranin A (monoclonal 1:500, Ortho, Milan, Italy), neurone specific enolase (NSE monoclonal 1:50; Sanbio, Uden, Netherlands), insulin (polyclonal 1:200, Dako, Glostrup, Denmark), glucagon (polyclonal 1:300, Dako), gastrin (polyclonal 1:300, Dako), somatostatin (polyclonal 1:300, Dako), calcitonin (polyclonal 1:200, Dako), bombesin (polyclonal 1:1200, Sera-Lab, London, UK), PP (polyclonal 1:800, Dako). Positive and negative controls were systematically performed. For transmission electron microscopy, significant tissue areas were sampled. Formalin fixed specimens were cut in small fragments of approximately 1 mm^3. The samples were fixed again in 2.5% cacodylate-buffered glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded ethanols and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate and viewed under a Philips 400T transmission electron microscope.

Results

Serum calcium, PTH and gastrin levels detected pre- and post-parathyroidectomy are represented in Fig. 1.

Grossly, the lower and upper gland measured 2.5 cm and 4 cm in maximum diameter, respectively. On section they were encapsulated, soft and orange-brown. The lower gland was completely lobular and solid. The upper also contained large cystic spaces filled with clear, watery fluid. The gastrin and PTH content of the aspirated fluid detected by radio-immunoassay was 900 pg/ml and 450 pg/ml respectively.

Histological sections of each gland showed parathyroid tissue arranged in varying patterns. Solid and nodular islands devoid of interposed adipocytes predominated. Large cystic formations were also evident in the upper gland. The nodules were composed of enlarged chief cells with pale and granular cytoplasm. Individual groups of chief cells presented an intensely clear, vacuolated cytoplasm. Some nodules were entirely composed of oxyphil cells with a brightly eosinophilic granular cytoplasm. The tumour cells were often arranged around a blood vessel in a solid or pseudoglandular formation sometimes forming acini. The lumen of these glandular

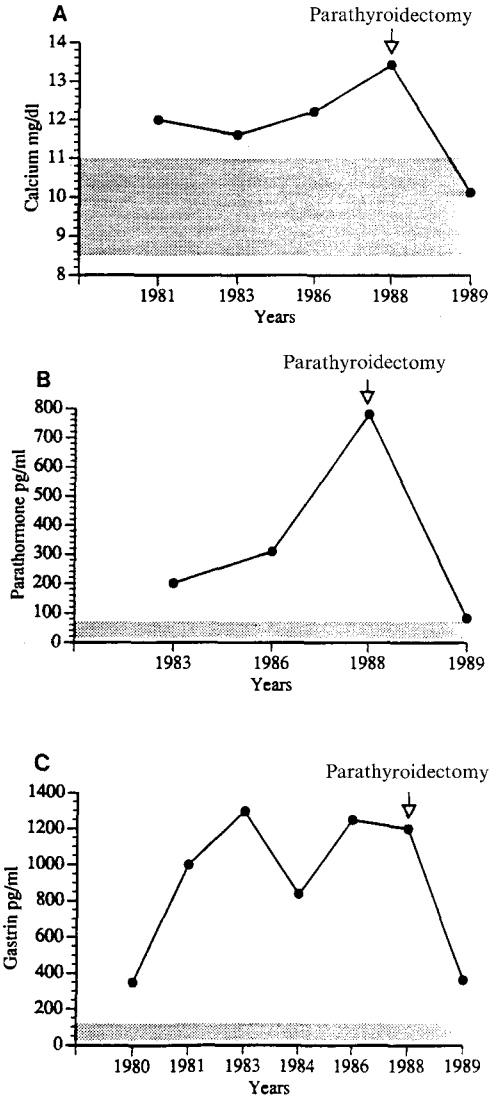

Fig. 1. Histograms of the serum calcium (**A**), parathormone (**B**) and gastrin (**C**) levels

structures was filled with a pink colloid-like material. The tumour elements were minimally pleomorphic with occasional large, hyperchromatic nuclei and rare mitoses. The cyst walls were lined by flat mono- or multistratified chief cells.

Histochemical methods for staining endocrine secretory granules were negative except for the Grimelius stain, which showed weak argyrophilic intracytoplasmatic granules. The colloid-like material within the glandular lumen showed a positive PAS reaction and also reacted positively for amyloid with Congo red staining.

Immunohistochemically, antiserum against gastrin showed a diffuse reactivity in the cytoplasm of the oxyphil elements (Fig. 2) and a rare positivity in the chief cells. Both parathyroid tumours also presented strong and diffuse chromogranin A and NSE immunoreactivity, which appeared less evident in oxyphil areas. Scattered somatostatin-positive cells throughout all the glands were also detected occasionally. All other antisera were negative. Since we performed a retrospective study,

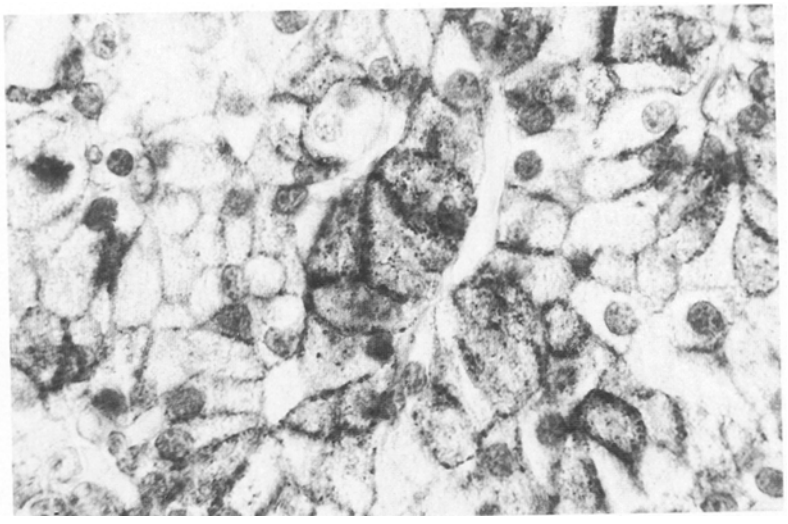

Fig. 2. Gastrin immunoreactivity in oxyphil cells of parathyroid tumour. PAP, ×264

we were not able to determine immunoreactivity for PTH on formalin-fixed, paraffin-embedded tissue.

Ultrastructural examination of oxyphilic nodules revealed typical oncocytes endowed with numerous mitochondria and inconspicuous profiles of rough endoplasmic reticulum. Scattered microfollicles displaying microvilli and a few secretory granules were observed (Fig. 3A). The granules, measuring 260–410 nm in diameter, appeared as electron-lucent vacuoles filled with flocculent material. In addition, large secretory spaces, namely follicles, contained a collection of typical non-branching amyloid fibrils (Fig. 3B). In contrast, chief cells contained parallel arrays of rough endoplasmic reticulum, Golgi complexes, few lipid droplets and focal deposits of glycogen. Microfollicles outlined by punctate desmosomes were found. A discrete number of secretory granules were mainly observed at the periphery of chief cells. The granules showed a great variability in size and

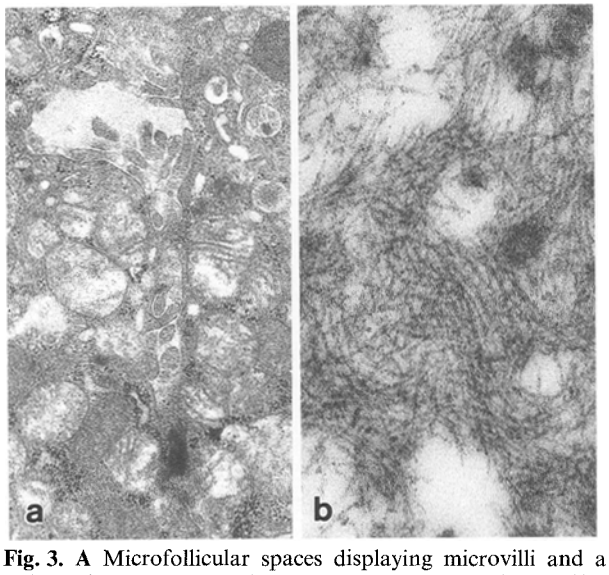

Fig. 3. **A** Microfollicular spaces displaying microvilli and a few polarized secretory granules. TEM, ×15000. **B** High magnification of a follicle containing typical amyloid fibrils. TEM, ×56000

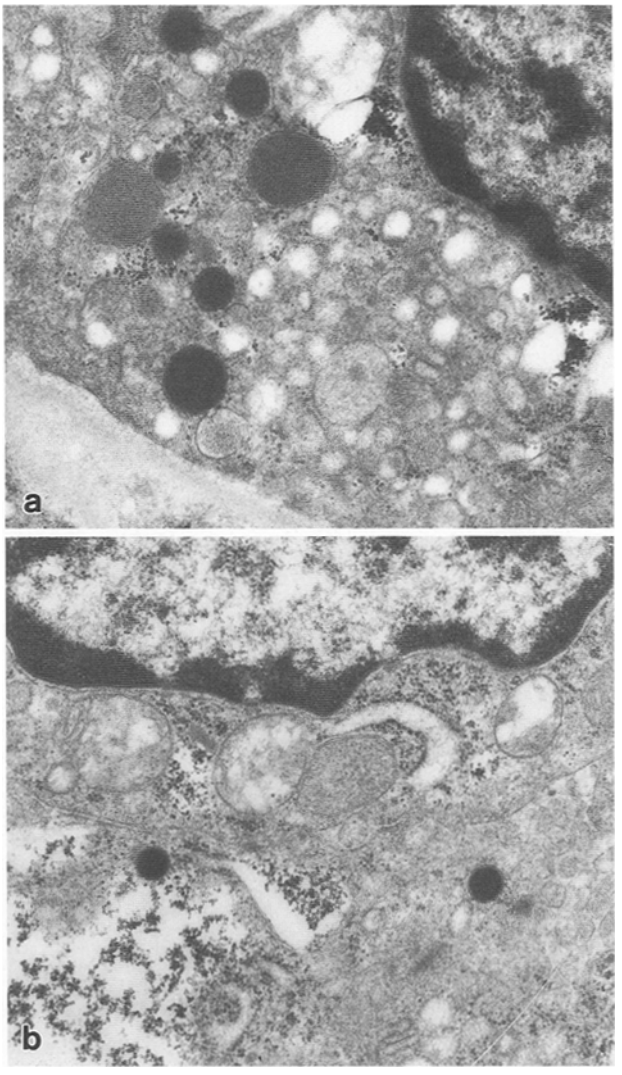

Fig. 4. **A** High-power electron micrograph showing a peripheral collection of pleomorphic G granules with variable diameter and density. Note the transition from punctate or flocculent to dense and compact morphology. TEM, ×25000. **B** High-power view of parathormone granules having dense cores and a slender clear halo. TEM, ×22000

density, thus suggesting a mixed granule population. Most of these measured 185–600 nm in diameter and had a variable density ranging from clear and punctate or flocculent to dense and compact, thus resembling G-cell granules (Fig. 4A). A lesser proportion measuring 180–220 nm appeared as dense haloed PTH granules (Fig. 4B).

Discussion

The evidence of a relationship between parathyroid glands and some gastrointestinal hormones and functions is widely documented (Christiansen and Aagaard 1972; Dent et al. 1972; Berreras 1973; Selking et al. 1981; Stodel et al. 1985). In particular, the kind of changes reported in gastrin, pepsin, calcium and gastric acid levels and the increased incidence of peptic ulcers (Ballard et al. 1964) in patients with HPT suggest the existence of complex connections, some of which are still to be elucidated.

HPT occurs in nearly all patients with MEN type 1 where, interestingly, it is the central feature and generally the primary manifestation of the disorder (Stodel et al. 1985). More than 60% of patients with MEN 1 have either hypergastrinaemia or the ZE syndrome (Ballard et al. 1964). Conversely 20–60% of patients with the ZE syndrome have features of MEN 1 (Friesen 1982; Zollinger 1987). Hypergastrinaemia in these patients is usually thought to be due to a gastrin-producing tumour, most often of the pancreas (Deveney et al. 1983).

The clinical and pathological features of our case are typical of a MEN 1, where exceptionally an unusual parathyroid-secreting gastrinoma was demonstrated immunologically and ultrastructurally.

Hypergastrinaemia is one of the most common findings in HPT (Dent et al. 1972; Selking et al. 1981; Fabri et al. 1986; Ouchi et al. 1990). It is possible that, directly or indirectly, the PTH or hypercalcaemia might be the cause of the elevated serum gastrin levels per se (Creutzfeld et al. 1971; Selking et al. 1982). However, hypergastrinaemia has been reported in HPT, both in animals (Cowley et al.1975; Delaney et al. 1978) and in man (Creutzfeldt et al. 1971; Polak et al. 1971), due to hyperplasia of G cells with increased gastrin release. However, all these data conflict and have not all been confirmed (Polak et al. 1971; Creutzfeldt et al. 1974). In HPT of MEN origin a pancreatic or a submucosal duodenal gastrinoma, sometimes so small as to escape detection, may be another cause of hypergastrinaemia (Pipeleers-Marichal et al. 1990). It has not been established whether the increase of gastrin levels is always caused by duodenal and/or pancreatic gastrinoma in MEN; however all these observations emphasise the possibility of an unusual cause in any case. The finding of gastrin immunoreactivity in the oxyphil and chief cells of the parathyroid tumours in our patient is interesting and emphasizes this conclusion.

Our case shares some similarities with a few others reported in the literature. Stremple and Watson (1974) described a patient with HPT, hypergastrinaemia, normal parietal gastric cells and no gastric hypersecretion. Gastrin was detected in the chief cells of the parathyroid adenoma by immunofluorescence and radio-immunoassay. Recently, gastrin immunoreactivity was detected in parathyroid tumours of 14 patients with primary HPT (Ouchi et al. 1990). Finally, gastrin positivity was demonstrated by immunofluorescence in the oxyphil cells of the hyperplastic parathyroids of a patient with MEN 1 (Cassar et al. 1975).

In our case the presence of gastrin within parathyroid cells was confirmed by comparative immunohistochemical, radio-immunoassay and ultrastructural investigations. The ultrastructural data exclude the possibility of gastrin absorption by the cells because of the high level of circulating gastrin. Nevertheless, the evidence that gastrin in hyperparathyroid glands is true gastrin and not an artefact has already been confirmed with the detection of G-17 and G-34 gastrin, employing column separation chromatography and immunohistochemistry in patients with primary HPT without MEN (Fabri et al. 1986).

Some of the main ultrastructural features of our case warrant emphasis: the functioning oxyphil cells, the heterogeneity in G granules and the co-expression of G and PTH granules in the same cells. In particular, at the ultrastructural level, the tumour was composed of a mixed population of oxyphil and chief cells. The oxyphil elements were typical hormonally active oncocytes. Oncocytes with a transitional appearance, such as those previously described in isolated cases of functional oxyphilic adenoma (Arnold et al. 1974; Chaudry et al. 1979; Ordonez et al. 1982), were not found. In our case, oncocytes appeared as sparsely granulated cells with G-cell-like granules giving a diffuse reactivity at immunohistochemistry. By an analogy with similar findings observed in other endocrine neoplasms (Ghadially 1985), we therefore hypothesized that oxyphil cells have a reduced capacity to store their secretory products and that gastrin could be released in an uncontrolled, more diffusible manner from the tumour cells. This finding is further supported by the presence in the oxyphil areas of numerous specialized secretory spaces having polarized granules, that is to say, follicles and microfollicles. This leads us to propose a local gastrin regulatory effect within the neoplasm itself. In addition, the detection of a discrete collection of so-called APUD-amyloid filaments within mature-looking follicles seems to reflect a disturbance of the normal intracellular secretory pathway and subsequent product release.

In contrast, the chief cells had a high functional activity as demonstrated by the presence of parallel stacks of rough endoplasmic reticulum, prominent Golgi complexes and numerous pleomorphic secretory granules. Unexpectedly, immunohistochemistry revealed only a weak positivity for gastrin. A pro-hormonal stage of gastrin might explain this apparent discrepancy. Even more intriguing, granules on electron microscopy were heterogeneous with respect to size, shape and electron density and they appeared to be polarized towards the base of the cells inferentially supporting multiple but orderly secretory activity. We therefore suggest that chief

cells contain both typical (clear and punctate granules) and atypical (compact, medium density granules) G-cell granules along with PTH granules (haloed granules with dense core). This feature is in accordance with a previous ultrastructural investigation on ZE syndrome (Creutzfeldt et al. 1975) and further supported by the presence of chief cells having granules with distinct morphology.

These observations clearly demonstrate that the present case is a complex endocrine neoplasm able to produce a variety of secretory substances such as gastrin and somatostatin in addition to the expected and well-known PTH. Moreover, this study suggests that under neoplastic conditions, oncocytes may act as hormonally active cells despite their resting appearance; a local gastrin regulating activity within the parathyroid neoplasm is also hypothesized. Finally, our study confirms the presence of gastrin in parathyroid cells and may represent one of the causes of hypergastrinaemia in patients with HPT with or without MEN 1. The parathyroid should therefore be a site to examine for "gastrinoma", at least in patients with MEN 1.

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