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Chromogranin A and B in parathyroid tissue of cases of primary hyperparathyroidism: an immunohistochemical study

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Summary. Routinely processed parathyroid tissues from 26 cases with primary hyperparathyroidism (19 adenomas, 7 multiglandular hyperplasia) and 8 normal human parathyroid glands were investigated with antibodies against chromogranin A and B and parathyroid hormone (PTH). Normal parathyroids were immunohistochemically positive for PTH and chromogranin A but negative for chromogranin B. Hyperplastic glands showed a focal staining for PTH and chromogranin A without correlation of the staining pattern on serial sections. Adenomas were either uniformly positive for both PTH and chromogranin A or showed a staining pattern similar to that seen in hyperplastic glands. Focal chromogranin B positivity (less than 10% of cells) was found in 3 cases (1 hyperplastic gland and 2 cases of parathyroid adenoma with an immunohistochemical staining pattern similar to hyperplastic glands). Our immunohistochemical results may support previously published findings that most parathyroid adenomas are monoclonal neoplasms whereas hyperplastic glands are of polyclonal origin.

Key words: Parathyroid gland – Primary hyperparathyroidism – Immunohistochemistry – Chromogranin A – Chromogranin B

Introduction

Chromogranin A, a secretory protein of secretory granules found in a widespread distribution in neuroendocrine and neuronal tissues, has been demonstrated in normal human parathyroid chief cells (Lloyd and Wilson 1983; O'Connor et al. 1983; Wilson and Lloyd 1984; Hagn et al. 1986; Rindi et al. 1986; Hearn 1987; Schmid et al. 1989) and in parathyroid adenomas (O'Connor et al. 1983; Lloyd and Wilson 1983; Wilson and Lloyd 1984; Lloyd et al. 1988; Oka et al. 1988; Weiler et al. 1988). Chromogranin B is a protein of secretory granules distinctly different from chromogranin A, though both proteins have many properties in common, suggesting that they are members of one protein class (Rosa et al. 1985). Chromogranin B has been demonstrated immunohistochemically by Lloyd et al. (1988) both in normal chief cells of the parathyroid and in parathyroid adenomas. Our group, however, has so far been unable to confirm their findings, using immunoblotting and immunohistochemistry on a limited series of cases (Hagn et al. 1986; Weiler et al. 1988; Schmid et al. 1989).

Recently Lloyd et al. (1989) analysed the distribution of the messenger ribonucleic acids (mRNAs) for chromogranin A and B on a variety of tissues by in situ hybridisation demonstrating a diffuse hybridisation signal for chromogranin A mRNA in normal parathyroid and parathyroid adenomas. Chromogranin B mRNA was found in lesser amounts than chromogranin A mRNA both in normal parathyroid and parathyroid adenomas. Chromogranin B mRNA showed focal localisation in normal parathyroid tissue.

In the present study we investigated 26 cases of primary hyperparathyroidism and 8 normal parathyroid glands immunohistochemically with antibodies against chromogranin A and B. Additionally the immunohistochemical staining obtained with these two antibodies was compared with the staining found with a monoclonal antibody against parathyroid hormone (PTH).

Materials and methods

Twenty-six cases of primary hyperparathyroidism were retrieved from the files of the Department of Pathology, University of Innsbruck, Austria. Nineteen cases were originally diagnosed as parathyroid adenomas with single gland enlargement. Parathyroid hyperplasia involving at least two glands was found in 7 cases. The sex distribution was 20 females to 6 males; the mean age of patients was 57 (±34) years. No cases of familial multiple endocrine neoplasia type I were included.

Eight normal parathyroid glands removed accidentially during thyroid surgery were also investigated.

Monoclonal chromogranin A antibody (optimal dilution 1:10000) was purchased from BioGenex Diagnostics (San Ramon,

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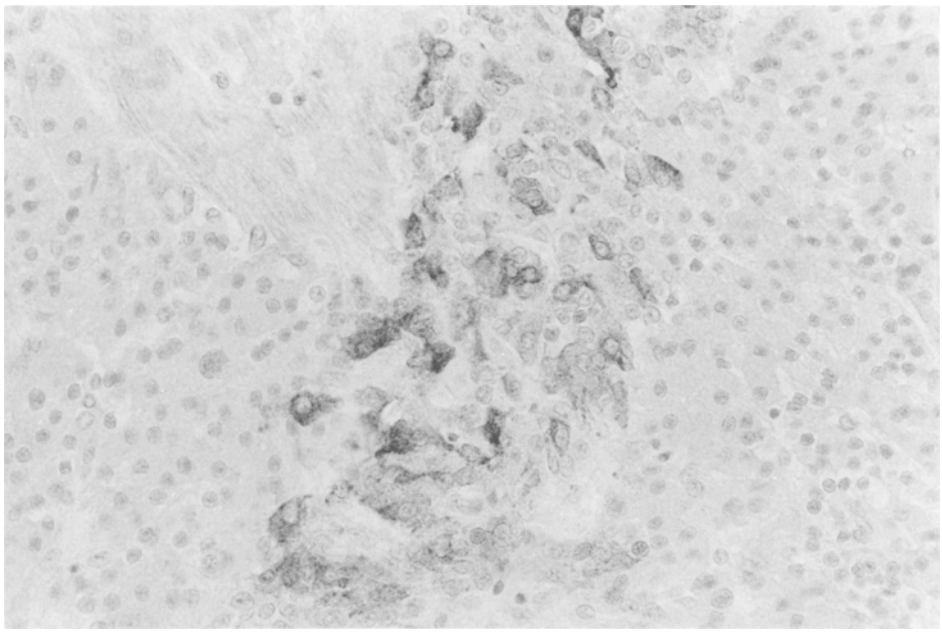

Fig. 1. Focal chromogranin B staining in a case of parathyroid hyperplasia. Indirect immunoperoxidase, haemalum counterstain, ×400

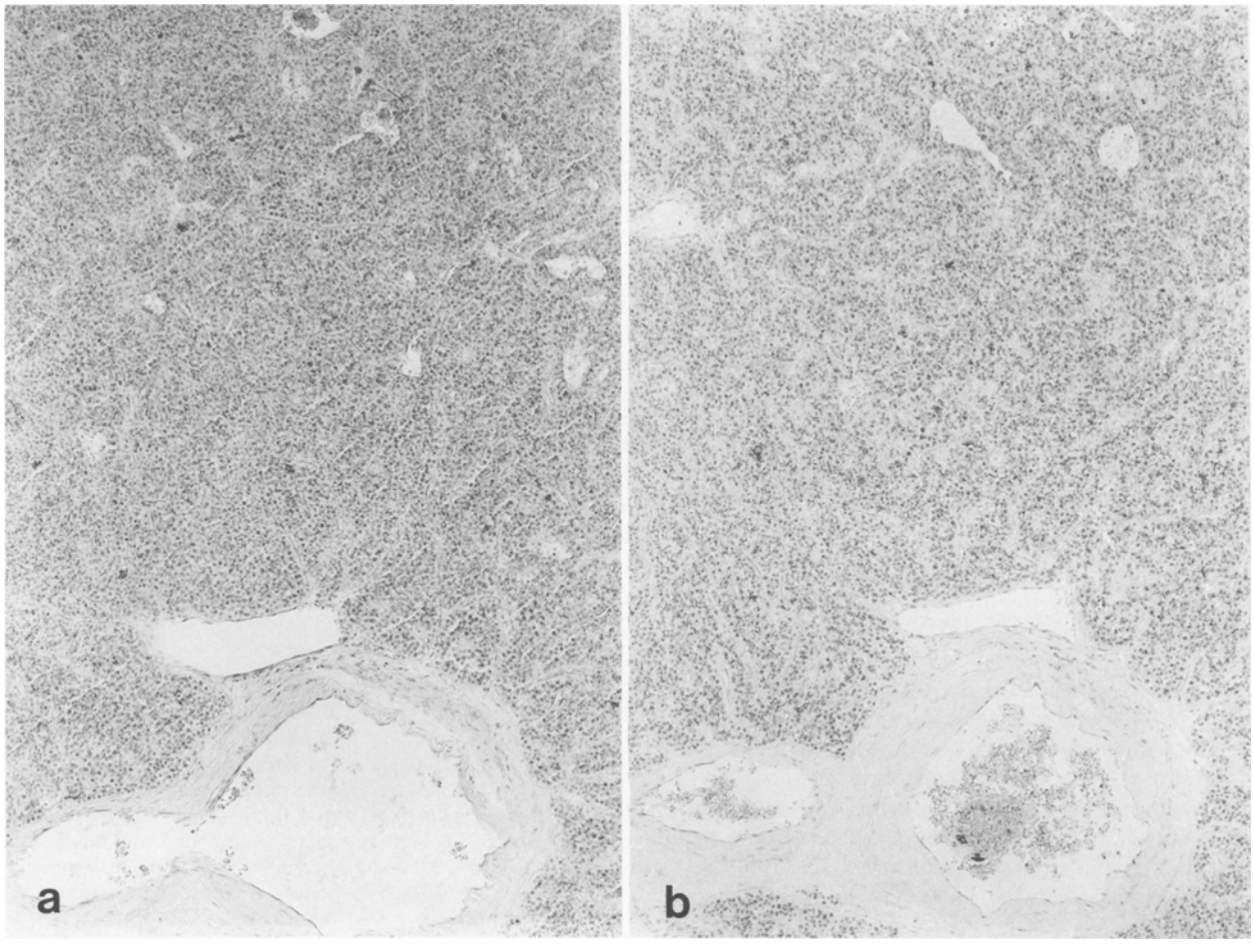

Fig. 2. **a** Diffuse parathyroid hormone (PTH) positivity in a parathyroid adenoma. Indirect immunoperoxidase, haemalum counterstain, ×200. **b** Semi-adjacent section to that shown in **a**. Diffuse chromogranin A staining. Indirect immunoperoxidase, haemalum counterstaining, ×200

Calif., USA). Polyclonal chromogranin B antibody (optimal dilution 1:400) was raised against human chromogranin B isolated from phaechromocytomas by high-performance liquid chromatography as described for rat and bovine chromogranins (Fischer-Colbrie and Schober 1987) but with some modifications (Schober et al. 1987). The monoclonal anti-PTH antibody (optimal dilution 1:4000) was a generous gift from Dr. B. Jasani, University of Wales College of Medicine, Cardiff, UK. Peroxidase conjugated secondary antibodies (sheep anti-mouse antibody, dilution 1:150; swine-anti-rabbit antibody, dilution 1:100) were obtained from Dako (Copenhagen, Denmark).

For immunohistochemistry all tissues were routinely formalin-fixed and paraffin-embedded. Serial sections were cut at a thickness of 4 µm, mounted on chrome-gel coated glass slides, dewaxed, and rehydrated. Endogenous peroxidase was blocked by means of sodium azide, glucose, and glucose-oxidase (Sigma, Munich, FRG) (Andrew and Jasani 1987; Hittmair and Schmid 1989). The primary antibodies were applied at their optimal dilutions overnight at 4° C, followed by the respective peroxidase conjugated secondary antibodies for 45 min at room temperature. Each incubation step was followed by rinsing for 3 × 2 min with phosphate buffered saline (0.01 M, pH 7.2). The enzyme reaction was developed with diaminobenzidine (Sigma) for 5–7 min. Subsequently the sections were counterstained with haemalum, dehydrated, cleared with xylene, and mounted with Entellan (Merck, Heidelberg, FRG).

Normal human adrenal medulla was used as positive controls for chromogranin A and B antibodies. Omission of primary antibodies was used for negative controls.

Results

Normal parathyroid glands showed a focally distributed weak to moderate staining with antibodies against PTH and chromogranin A in the majority of chief cells. A stronger PTH and chromogranin A staining was usually found in a rim of cells located in the periphery of the parathyroids. No chromogranin-B-positive cells could be demonstrated in normal glands.

In the 7 cases of parathyroid hyperplasia all glands investigated showed focal staining with both PTH and chromogranin A. On serial sections, however, there was only a partial correlation of PTH and chromogranin A with regard to the staining intensity and the staining patterns observed. One gland from a case of parathyroid hyperplasia showed focal positivity for chromogranin B (less than 10% of cells; Fig. 1). On serial sections these chromogranin-B-positive areas were only weakly positive for chromogranin A and PTH.

The 19 cases originally diagnosed as parathyroid adenomas could be divided into two groups by means of their different histological features and immunohistochemical staining patterns. Five adenomas were well demarcated, were composed of a single cell type (chief

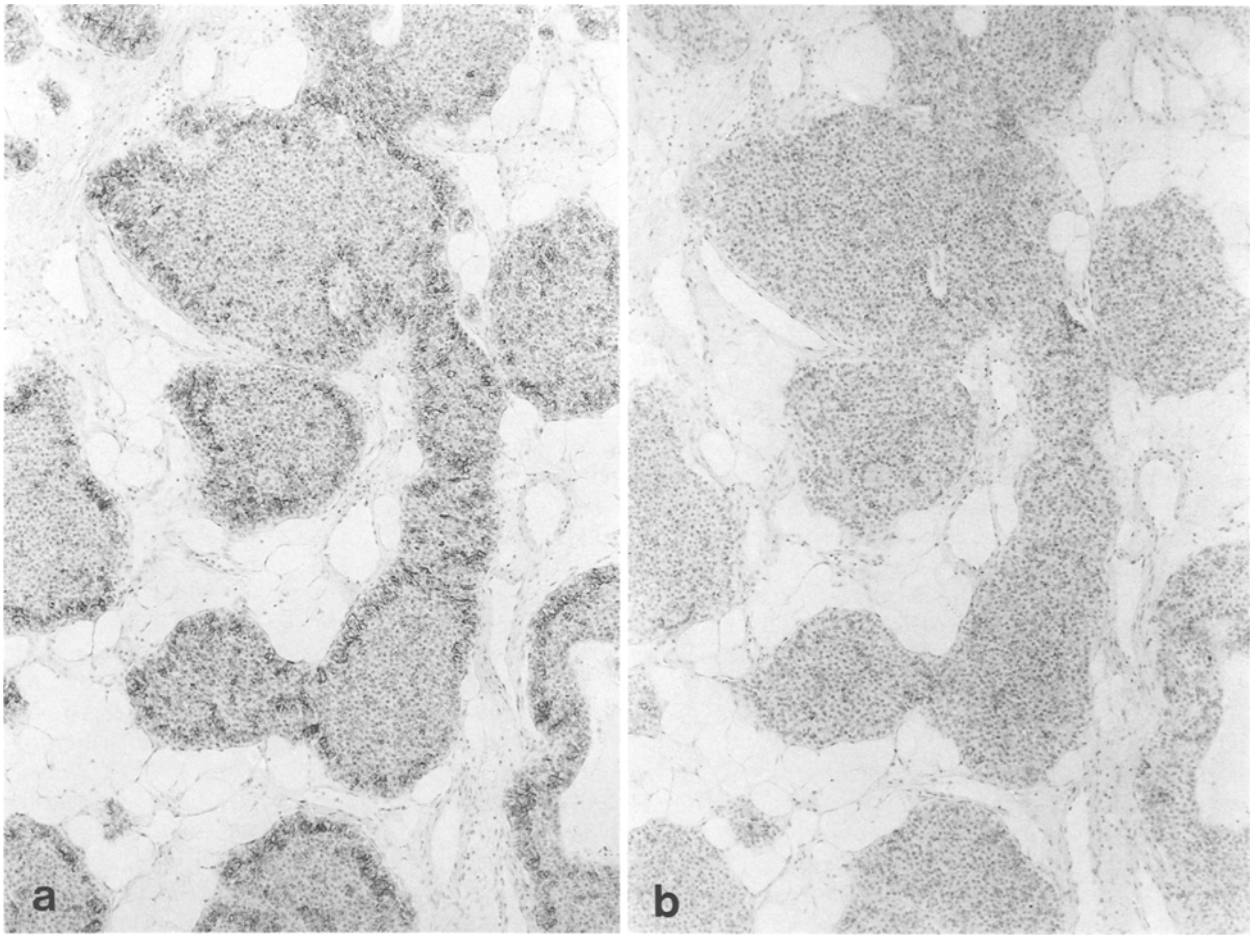

Fig. 3. **a** PTH staining in a case of parathyroid adenoma with focal staining pattern. Note strong peripheral PTH staining. Indirect immunoperoxidase, haemalum counterstain, × 200. **b** Semi-adjacent section to **a** shows an area with a rather diffuse moderate chromogranin A staining. Indirect immunoperoxidase, haemalum counterstain, × 200

cells), lacked fat cells within the tumour completely, and showed no lobular pattern. Immunohistochemically these adenomas were uniformly moderately or strongly stained with antibodies against PTH and chromogranin A (Fig. 2a, b). None of these 5 cases showed chromogranin B positivity.

The second group comprised of 14 cases with a single parathyroid gland enlargement, thus leading originally to the diagnosis of parathyroid adenomas. All enlarged glands contained fat cells within the lesion; 11 glands showed a lobular pattern. In none of the 14 cases could a clearly defined tumour capsule be demonstrated. Immunohistochemically the enlarged glands showed a focal staining pattern for PTH and chromogranin A (Fig. 3a, b) similar to the pattern found in cases of multiglandular hyperplasia. Two cases showed small foci consisting of chromogranin-B-positive cells. On serial sections there was neither a strict correlation of PTH and chromogranin A staining patterns nor of their staining intensity. Chromogranin-B-positive areas showed a weak to moderate positivity for PTH and chromogranin A.

Discussion

The discovery that chromogranin A, originally characterised in the adrenal medulla (Helle 1966; Smith and Winkler 1967; Smith and Kirschner 1967; Fischer-Colbrie and Frischenschlager 1985), was similar to secretory protein I of the parathyroid gland (Cohn et al. 1982) has led to the demonstration of its widespread occurrence in neuroendocrine cells and tumours (Wiedenmann and Huttner 1989). In agreement with the literature (Lloyd and Wilson 1983; O'Connor et al. 1983; Wilson and Lloyd 1984; Hagn et al. 1986; Rindi et al. 1986; Hearn 1987; Schmid et al. 1989) chromogranin A was found in the present study in normal parathyroid glands and parathyroid adenomas. In parathyroid hyperplasia distinct focal chromogranin A staining was demonstrated. Focal chromogranin B positivity occurred in our series in only 3 of 26 cases of primary hyperparathyroidism and in none of 8 normal parthyroid glands. However, Lloyd et al. (1988) found a focal positive staining in all parathyroid tissues investigated (2 normal glands and 3 adenomas) using an anti-rat chromogranin B antibody. Subsequently Lloyd et al. (1989) showed a focal chromogranin B mRNA localisation on a small number of cases (1 normal gland and 2 adenomas). Presumably these cells or cell clones unable to synthesise chromogranin B may account for the occurrence of chromogranin-B-negative parathyroid adenomas and hyperplastic glands.

The cases originally diagnosed as parathyroid adenomas showed two different immunohistochemical staining patterns. A uniformly moderate or strong staining with PTH and chromogranin A throughout the lesion was found exclusively in cases which showed a fibrous capsule and were composed of a single cell type. The second staining pattern was similar to that seen in cases of multiglandular hyperplasia. The two different immunohistochemical staining patterns found in the cases originally diagnosed as parathyroid adenomas were both associated with distinct morphological features (fat cells, lobularity, mergence with surrounding tissue) which were previously suggested by Ghandur-Mnaymneh and Kimura (1984) to distinguish parathyroid adenoma from focal hyperplasia. They proposed that focal hyperplasia with a single gland enlargement may be the most common cause for primary hyperparathyroidism. Although the paper published by Ghandur-Mnaymneh and Kimura is regarded as somewhat controversial (Mendelsohn 1988), further studies on this subject seem to be justified to distinguish adenomas from focal hyperplasia. Perhaps investigations of chromogranin A and B with their obviously different cellular distribution may help to answer these questions, leading to a better understanding of the pathogenesis of primary hyperparathyroidism.

Arnold et al. (1988) have recently shown that some parathyroid adenomas are of monoclonal origin. Our finding of an immunohistochemical uniform PTH and chromogranin A staining in 5 parathyroid adenomas may indicate monoclonalty in these cases as well. However, the focal staining observed in hyperplastic glands and 14 cases with a single gland enlargement may be associated with polyclonal origin. Lloyd et al. (1989) described a focal immunohistochemical staining in paraythroid adenomas with two of the antibodies used in their study (LK2H10, anti-bovine chromogranin A) whereas the chromogranin A mRNA was localised by in situ hybridisation in a diffuse distribution. It seems, therefore, difficult to use immunohistochemical stains as criteria to draw definite conclusions about certain cell properties.

We have previously shown, on a variety of endocrine tissues and their tumours, that they contain almost equal amounts of chromogranin A and B (Hagn et al. 1986; Schober et al. 1987; Schmid et al. 1987; Weiler et al. 1987, 1988). In parathyroid tissues, however, chromogranin B can apparently be found only in minute amounts. Further studies may elucidate if there is a different regulation of chromogranin A and B in the parathyroid gland, as already suggested for the adrenal glands (Fischer-Colbrie et al. 1988).

The function of chromogranins remains poorly defined (Winkler et al. 1986). It has been shown that peptides derived from chromogranins have the ability to inhibit the secretion of various hormones with which they are stored and secreted (Efendic et al. 1987; Iacangelo et al. 1988; Simon et al. 1988). An interesting finding is that both the staining pattern and the staining intensity obtained with the three antibodies showed only a partial correlation in most of the cases investigated. In cultured parathyroid tissue secretion of PTH and chromogranin A is stimulated by a low calcium medium and suppressed by a high one (MacGregor et al. 1988). High calcium serum levels as found in primary hyperparathyroidism may therefore suppress chromogranin A only in the parathyroid, which points to different secretion mechanisms for PTH and chromogranin A (Nanes et al. 1989). Plasma chromogranin A levels are not generally elevated in cases of primary hyperparathyroidism

(Nanes et al. 1989). Normal serum chromogranin B levels have also been reported (Sekiya et al. 1989) in cases of primary hyperparathyroidism. We may speculate that these observations reflect one of the failures in the regulatory mechanism of PTH secretion in primary hyperparathyroidism.

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