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Calcitonin Gene-Related Peptide and Calcitonin in MEN-2 and Sporadic Pheochromocytomas: An Immunohistochemical Study

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Calcitonin Gene-Related Peptide and Calcitonin in MEN-2 and Sporadic Pheochromocytomas: An Immunohistochemical Study

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Ten pheochromocytomas (five from patients with multiple endocrine neoplasia type 2 and five sporadic) were stained immunohistochemically with antibodies to calcitonin (CT) and calcitonin gene-related peptide (CGRP) by means of the peroxidase-antiperoxidase method. CGRP positive cells were found in variable numbers in all of the investigated tumors. No demonstrable difference was noted between the hereditary tumors and the sporadic tumors. Staining with CT antibody also showed cells with a positive reaction in eight of ten tumors. The distribution of the two peptides was similar, and in some cells their coexistence was visualized. Pheochromocytomas thus represent an alternative site to medullary thyroid carcinoma for CT and CGRP production. The possible influence of this on serum peptide levels needs investigation. (Henry Ford Hosp Med J 1987;35:115-7)

Pheochromocytoma and medullary thyroid carcinoma (MTC) are found as sporadic tumors and as part of the multiple endocrine neoplasia type 2 (MEN-2) syndrome. In MTC, immunochemical and immunohistochemical demonstration of calcitonin (CT) and possibly CT gene-related peptide (CGRP) play an important role in diagnosis, follow-up, and even in the distinction between sporadic and hereditary tumors (1-3). Since a genetic link exists between MTC and the pheochromocytoma in MEN-2, we investigated whether or not CT and CGRP were produced in the pheochromocytomas, and if immunohistochemical studies of these peptides would reveal differences between sporadic and hereditary pheochromocytomas.

Materials and Methods

Ten surgically removed pheochromocytomas were studied: five tumors from patients without any known hereditary disposition, and five from four patients with the MEN-2 syndrome (one had a new pheochromocytoma in the opposite gland three years after removal of the initial tumor).

Four to six μm formalin-fixed, paraffin-embedded tissue material sections (initially rehydrated in graded alcohol and rinsed in phosphate-buffered saline at pH 7.1) were stained using the peroxidase-antiperoxidase (PAP) technique (4) with diaminobenzidine (0.05%) for visualization. False-positive staining due to endogenous peroxidase was prevented by treatment with 35% H_2O_2 in methanol for 40 minutes. The three layers in the PAP staining consisted of rabbit antibody against CGRP (polyclonal) (2) or CT (polyclonal from DAKO, Denmark, monoclonal from Nordic), followed by swine-anti-rabbit-Ig, and finally the peroxidase-rabbit-antiperoxidase complex. Fetal calf serum (1%) was added to prevent nonspecific absorption before addition of each layer of antibody in the three-layer technique. Incubation with the primary antiserum (dilution 1/800) was for 18 hours at 4°C, and with swine-anti-rabbit-Ig and the PAP complex for 30 minutes each. Sections from a MTC were used as positive controls.

Thin (2 to 3 μm) serial sections were cut and stained for CGRP and CT to demonstrate more clearly the relationship between the distribution of the two peptides.

Results

Cells with positive reaction with CGRP antibody (Ab) were found in all the tumors investigated. Numbers varied from only a few scattered cells to a more pronounced reaction in a large number of cells. The intensity of the reaction in the cells likewise was variable, ranging from faint to very dark staining cells.

Staining with Ab to CT showed cells with positive granules in eight of ten tumors. Two sporadic tumors, however, showed no positive reaction, and one of the hereditary tumors contained only a few positive cells whereas another tumor showed only faint but widespread positive reaction. In most cases the distribution of CT positive cells was similar to that of the CGRP positive cells, and CT staining showed similar variation from only slight to pronounced reaction, and from a few to numerous cells. Study of adjacent 2 to 3 μm sections showed that some cells contained both CT and CGRP, but that other cells stained for only one peptide.

Comparison of sections of tumor from patients with MEN-2 (Figs 1 and 2) with those from sporadic cases (Fig 3) showed no convincing difference in either content or distribution of the peptides. The density of positive cells was found to be somewhat less in pheochromocytomas than in the MTC used as a control.

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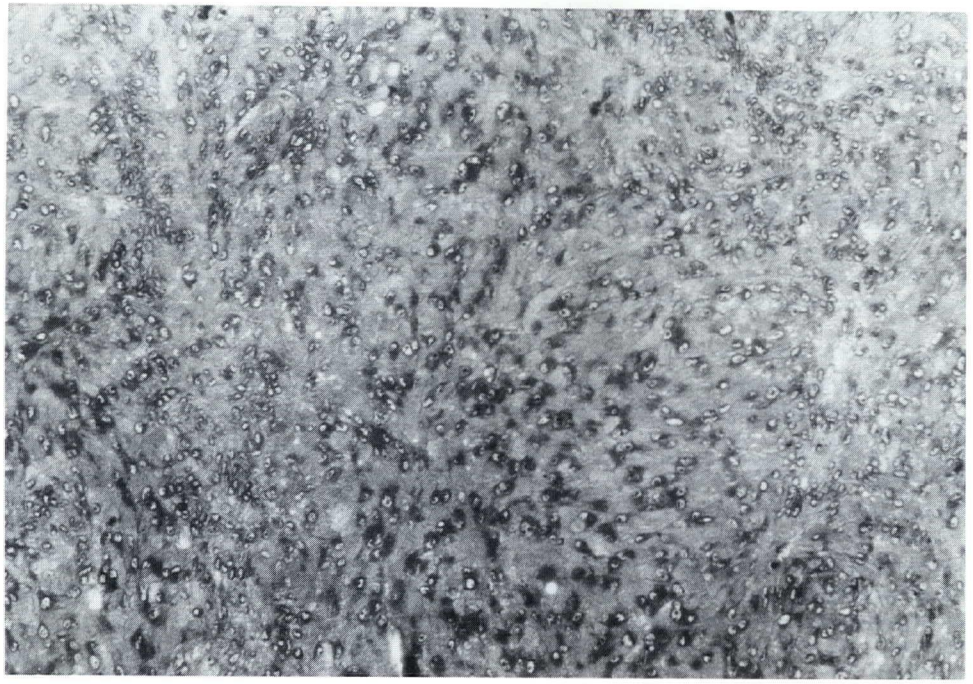

Fig 1—Staining for CGRP in pheochromocytoma from patient with the MEN-2 syndrome. Many positive cells are present. (PAP method) (X 125).

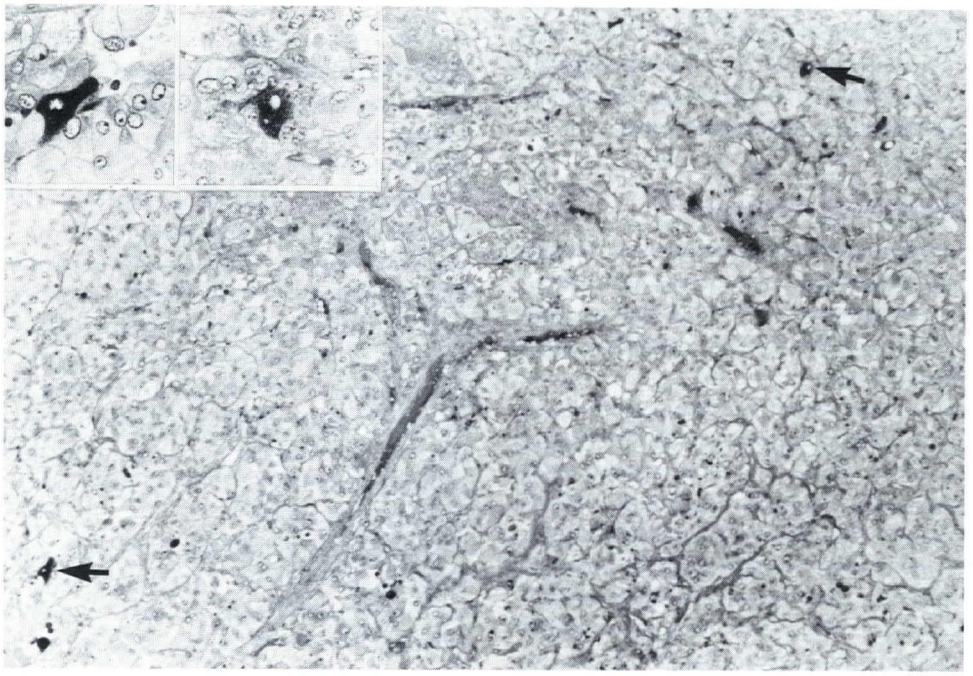

Fig 2—Staining for CGRP in pheochromocytoma from MEN-2 patient. Only a few scattered positive cells are noted. Two positive cells are shown at higher magnification (see arrows) (PAP method) (X 100) (Insets X 1200).

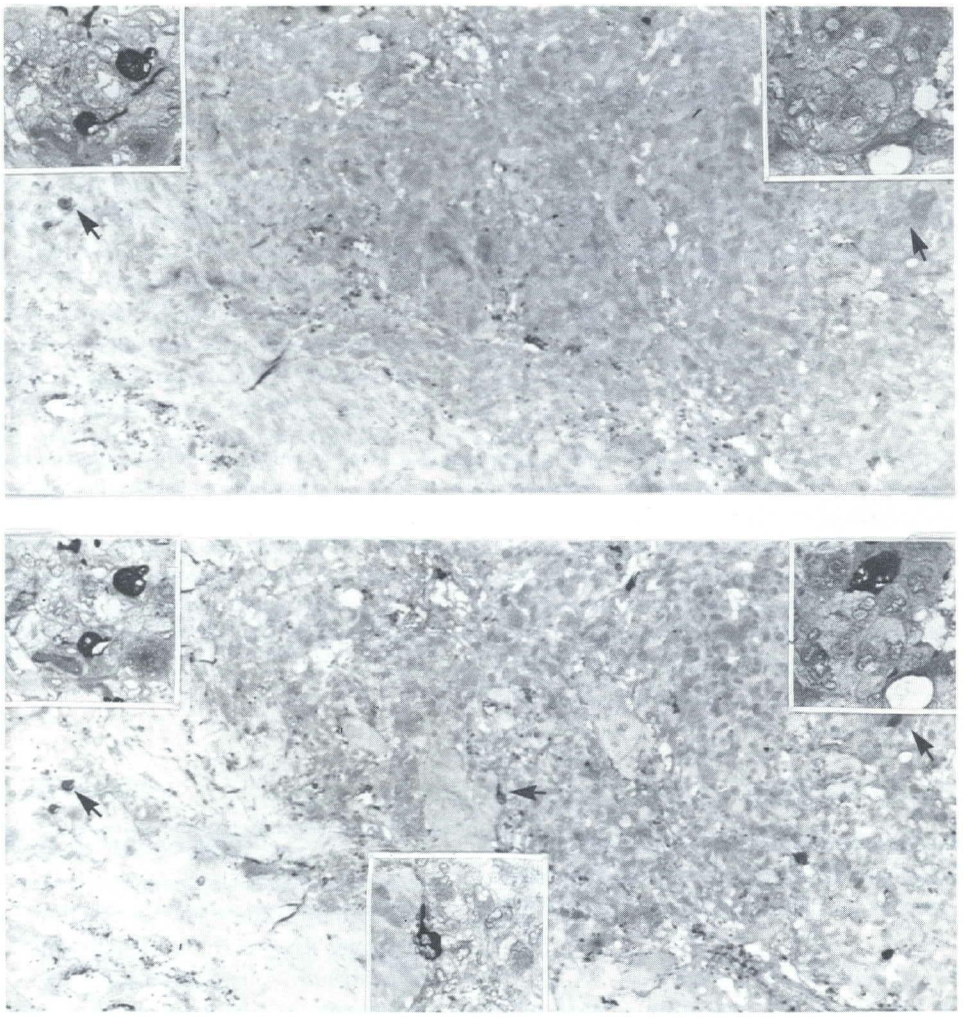

Fig 3—Sporadic pheochromocytoma. Serial sections, 2-3 μm. A) Staining for CT. B) Staining for CGRP. Cells positive for both peptides (arrow at left), as well as cells positive for only CGRP (arrows at center and right) are seen. Some of the CGRP positive cells show slender cytoplasmic extensions (bottom inset) (PAP method) (X 100) (Insets X 960 and 1,200).

Discussion

CT has been demonstrated previously in pheochromocytomas (5). The present study confirms this and extends the observations to include both sporadic and hereditary (MEN-2) tumors. The distribution of CGRP-containing cells was similar to that of CT-containing cells, and coexistence of the peptides in some cells was observed as might be expected considering the close genetic relationship of the two peptides (6,7).

The positive reaction for CGRP and CT in these tumors suggests a possible diagnostic value of serum determinations in patients with suspected pheochromocytomas. In MEN-2, the raised levels of plasma CGRP and plasma CT might not be exclusively related to production by MTC, since pheochromocytomas constitute an alternative site of production of these peptides. Further investigations are indicated to clarify the influence of pheochromocytomas on serum CGRP and CT.

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