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Case report

Single secretory granules contain both GH and Prolactin in pituitary mixed type of adenoma

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Summary. A mixed type of pituitary adenoma is described consisting of heavily and sparsely granulated cells. It produces GH and prolactin (PRL) and has been examined by immunocytochemistry. The superimposition immunocytochemical procedure reveals that single cells contain both GH and PRL. Furthermore, electron immunocytochemistry using adjacent sections reveals that single secretory granules contain both GH and PRL simultaneously.

Key words: Human pituitary adenoma – Secretory granules – GH-PRL – Immunocytochemistry

A single hormone is usually secreted by pituitary adenomas; however, an adenoma secreting growth hormone (GH) and prolactin (PRL) simultaneously is called a mixed tumor (Corenblum et al. 1976). Morphological evidence suggests that mixed GH and PRL secreting pituitary tumors are composed of distinct populations of somatotrophs (GH-secreting cells) and mammotrophs (PRL-secreting cells) frequently arranged in groups (Corenblum et al. 1976; Guyda et al. 1973). Morphological evidence also suggests that two-cell types with two different granule size classes are present in such adenomas (Guyda et al. 1973; Robert 1973; Yoshida et al. 1975; Horvath and Kovacs 1976). Corenblum et al. (1976) revealed that mixed adenomas, using light microscopical immunocytochemistry, consist of GH or PRL-secreting cells within the tumors. But in 1978, Landolt summarized evidence for production of both hormones by the same cell. In the experimental rat tumor, MtTW15, the majority of immunopositive cells contained only GH or PRL, but a few were observed to contain both hormones (Baskin et al. 1980).

There are no reports of a single granule containing both hormones, or different granules containing different hormones in the same cell which secretes GH and PRL simultaneously. The purpose of this case study is to determine whether single secretory granules of adenoma cells contain both hormones or not.

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Materials and methods

A human pituitary adenoma was removed by surgical hypophysectomy from a 35-year old female patient suffering from typical acromegaly. The patient exhibited local symptoms, including visual disturbance, headaches, and also endocrine abnormalities, such as galactorrhea, infertility, decreased libido and no ovulation. Serum GH and PRL levels were measured by radioimmunoassay (Special Reference Laboratories, Tokyo) with maximum levels of 40 ng and 30 ng/ml respectively.

Pieces of adenoma tissue were fixed immediately for 2 h in 1% glutaraldehyde in 0.1 M phosphate buffer PH 7.4 at 4° C, and then washed several times with 0.1 M phosphate buffer containing 2% sucrose. The pieces were postfixed in 1% osmium tetroxide in Millonig's buffer (Millonig 1962). Then they were dehydrated in a graded series of ethanol using propylene oxide as a transitional solvent and embedded in a mixture of Epon 812 and Araldite 502 (Mollenhauer 1964). Polymerization of the resin was carried out at 40° C for one week. Ultrathin serial sections for electron microscopy and an adjacent thick section (2 μm) for light microscopy were cut using glass knives on the ultramicrotome (LKB 8800 Type).

Prior to immunocytochemical staining, Epon Araldite was removed from the thick sections with a 14% sodium methoxide (Dotait, Wako, Japan) in absolute methanol solution for 10 to 15 min. The thick sections were then bleached by immersing in 2% aqueous solution of hydrogen peroxide for 20 min. These steps were performed at room temperature. The ultrathin sections were stained with uranyl acetate and lead citrate and observed with a JEM 100B electron microscope.

Electron microscopical immunocytochemistry was carried out according to the method of Baskin et al. (1979) with slight modification: 1) sections mounted on the nickel grid were etched with freshly prepared 15% hydrogen peroxide solution for 20 min and washed several times with distilled water, 2) sections were floated on a drop of antiserum against human PRL (Calbiochem, La Jolla, Ca, USA) or mouse PRL (courtesy of Dr. Kohmoto, Tokyo University) both diluted 1:1,000 with PBS (PH 7.4). Adjacent sections were floated on a drop of antiserum against human GH (Bioactive Chemicals Lab., Ltd., Tokyo) diluted 1:125 with PBS (PH 7.4) and incubated 90 min at 30° C. Then the adjacent sections were washed with PBS and floated again on a drop of peroxidase labeled anti-rabbit IgG fraction (Goat, Cappel Lab., Cochranville, PA, USA) at 30° C for 30 min, and 3) the grids were washed with PBS, floated on DAB solution (0.004% 3,3′-diaminobenzidine tetrahydrochloride, 0.003% H_2O_2/0.05 M Tris-HCl, PH 7.4) for 30 min, and then washed with distilled water. The sections were observed with the JEM 100B electron microscope without electron staining.

In order to make sure anti-mouse PRL serum could be used instead of anti-human PRL, two adjacent thin sections were prepared. One section was stained with anti-mouse PRL serum and the next section was stained with anti-human PRL serum. The same secretory granules of both sections contained reacting products with both antibodies. Immunoreacting DAB deposits were stronger when antiserum to mouse PRL was used than with anti-human PRL serum. Therefore, we used anti-mouse PRL serum in this study.

The specificity of the mouse PRL antiserum had been confirmed by the absorption test and radioimmunoassay system (Ishikawa et al. 1983). Antiserum to human GH was absorbed with human GH (Bioactive Chemicals Lab., HH-52, 5 or 50 μg/ml and Eiken GH-01, Tokyo 10 or 50 μg/ml) at 4° C for 2 days and the effect on staining ability at the absorption site examined using paraplast sections of normal human pituitary. Less than 50 μg/ml of human GH in either preparation abolished the staining completely at an antiserum dilution of 1:500.

Results

Two adjacent thick sections and the next thin section were prepared for the following studies. The two thick de-eponized sections were stained with anti-human GH (Fig. 1b) and anti-mouse PRL (Fig. 1a) respectively, and two types of immunopositive cells and immunonegative cells were seen in this adenoma. The adjacent sections revealed one type of cell that strongly

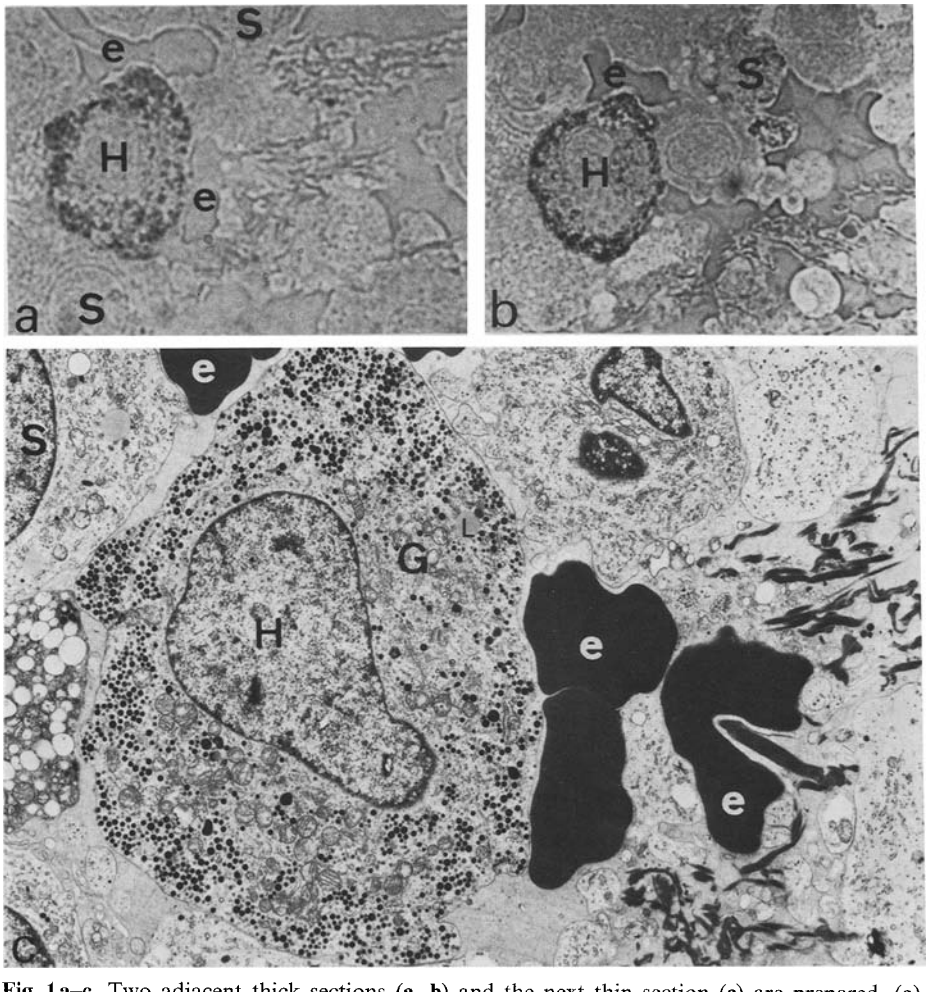

Fig. 1a–c. Two adjacent thick sections (**a, b**) and the next thin section (**c**) are prepared. (**a**) is stained with anti-mouse PRL. Cytoplasm of the heavily granulated cell (*H*) reacts strongly to the antibody; however, the sparsely granulated cells (*S*) react weakly. *e:* erythrocyte ×1,040, **b** is the adjacent thick section of **a** immunostained with anti-human GH. A few immunoreactive deposits in the Golgi area are observed, and the sparsely granulated cell (*S*) is also immunostained. *e:* erythrocyte ×1,040, **c** shows a heavily granulated cell containing a number of secretory granules (250–330 nm in diameter). A well-developed Golgi apparatus (*G*) containing a few secretory granules is frequently observed in heavily granulated cells. The cells usually have ovoid and spherical mitochondria and lysosomes; however, lipid droplets (*L*) are rarely observed. *S:* sparsely granulated cell, *H:* heavily granulated cell, *e:* erythrocyte. ×4,150

stained with both antibodies simultaneously. Some granules in the Golgi area of this type of cell may have reacted with the antibodies, because the Golgi area was weakly stained in these cells. The other type of immunopositive cell stained weakly with the same antibodies. Stainability of these two types of cells with anti-PRL was weaker than that with anti-GH. Electron microscopical observations with the adjacent thin section revealed that

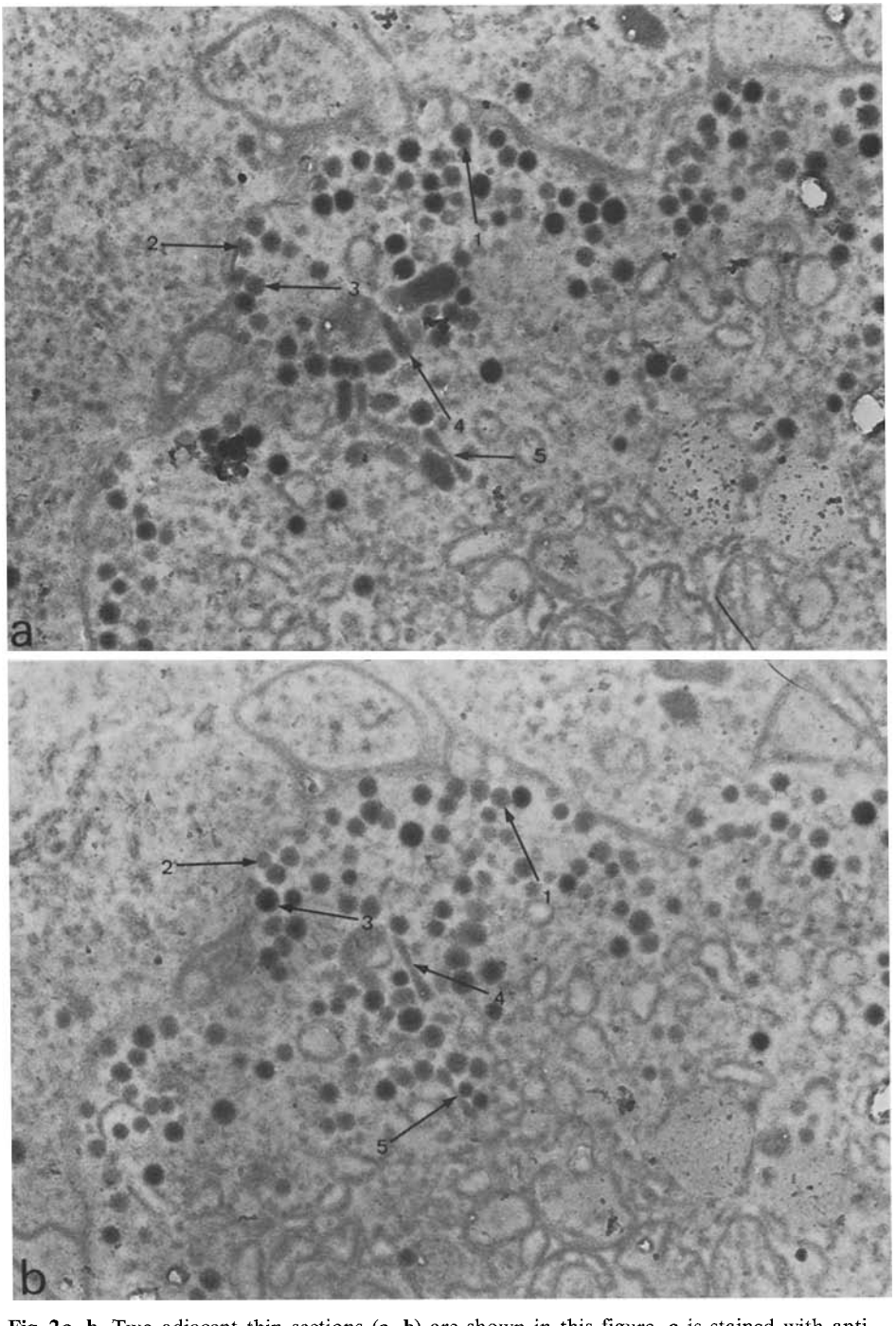

Fig. 2a, b. Two adjacent thin sections (**a, b**) are shown in this figure. **a** is stained with anti-human GH and **b** with anti-mouse PRL. Secretory granules, both spherical and pleomorphic (700–800 nm in diameter), in heavily granulated cell react with both anti-GH and -PRL sera. Furthermore, the same granules react with both anti-GH and -PRL simultaneously (*numbered arrows*). × 21,400

the strongly stained cell type was heavily granulated and the weakly stained cells were sparsely granulated, a few secretory granules being scattered diffusely in the cytoplasm. The average size of secretory granules in the heavily granulated cells measured 250–330 nm in diameter (Fig. 1c). The majority of cells in this tumor were the immunonegative cells which have few secretory granules.

Two adjacent thin sections were prepared. One was stained with anti-human GH (Fig. 2a) and the other was stained with anti-mouse PRL (Fig. 2b). These adjacent immunostained sections revealed that reactive sites for each antibody were observed within the same secretory granules.

Discussion

The pituitary tumor in this study was pathologically designated as a mixed-type adenoma, that is, the vast majority of the cells were chromophobic and a few cells were eosinophilic. Electron microscopical observations allowed us to assume that these eosinophilic cells contained a number of secretory granules in the cytoplasm and that the chromophobic cells contained a few secretory granules. Two varieties of somatotropic adenomas are recognized by electron microscopy: a heavily granulated tumor and a sparsely granulated variant (Horvath and Kovacs 1976; Kovacs et al. 1977). The two subtypes occur with equal frequency (Kovacs et al. 1977; Robert and Hardy 1975); however, sparsely granulated adenomas, which are "chromophobic" by light microscopy, are usually the more common prolactin producing tumor (Kovacs et al. 1977; Lewis and Van Noorden 1974).

Using the superimposition technique the majority of cells in this tumor were the sparsely granulated cells and were observed to be immunonegative. These results clearly suggest that immunonegative properties for light microscopy were caused by the small number of secretory granules, since the electron microscope revealed reacting products with antibody in the secretory granules. In this study, the adenoma secreted GH and PRL and these hormones could be found in single heavily granulated cells in light microscopic immunocytochemistry. However, according to electron microscopical immunocytochemistry, all of the secretory granules in heavily or sparsely granulated tumor cells reacted simultaneously with anti-GH and anti-PRL. It is still open to question whether GH and PRL are found in different molecules in single secretory granules or in one large molecule containing both GH and PRL immunoreactivities. Usually, GH-positive and PRL-positive cells seem to be different types in the GH-PRL secreting tumor, in view of the immunocytochemical study by the mirror section method performed in 24 cases (Fukaya et al. 1980).

This is the first paper to reveal that a single secretory granule contains both GH and PRL. However, it is necessary to ascertain whether the GH and PRL released from the adenomatous hypophysis are "true" GH and PRL.

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