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Case report

Immunoreactive luteinizing hormone in functioning corticotroph adenomas of the pituitary

Immunohistochemical and tissue culture studies of two cases

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Summary. Two pituitary adenomas removed from a 37-year-old woman and a 26-year-old woman with typical Cushing's disease were studied by light and electron microscopy, immunohistochemistry and radioimmunoassay of tissue culture media. Both patients had high plasma levels of cortisol and normal levels of luteinizing hormone (LH). Both tumours were monomorphous, composed of densely granulated corticotrophs; the tumour cells contained periodic acid-Schiff positivity, were arranged in a sinusoidal pattern and, ultrastructurally, contained well-developed cytoplasmic organelles. By immunohistochemistry the majority of tumour cells contained immunoreactive adrenocorticotropin (ACTH); approximately 10% of the tumour cell population contained LH immunoreactivity. The LH-positive cells tended to form clusters scattered widely throughout the tumour tissues. LH immunoreactivity was demonstrated in some ACTH-immunoreactive cells on serial sections. Large amounts of immunoreactive ACTH and smaller quantities of LH, follicle stimulating hormone and α-subunit were released into the culture media and release of the glycoprotein hormones responded in parallel to corticotropin releasing hormone stimulation or inhibition by cortisol. These findings indicate that LH can be simultaneously produced and released by ACTH-producing tumour cells of otherwise typical functioning corticotroph adenomas. The capacity for LH production may be acquired during neoplastic proliferation. This is the first detailed report of concurrent production of LH by pituitary corticotroph adenomas.

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Introduction

Functioning pituitary corticotroph adenomas are the main cause of Cushing's disease and account for approximately 8% of surgically removed pituitary adenomas (Horvath and Kovacs 1988a; Kovacs and Horvath 1986). Their histological, immunohistochemical and ultrastructural features are well established and widely known (Kovacs and Horvath 1986; Saeger et al. 1988).

Recently, several studies have revealed that pituitary adenomas frequently produce multiple hormones simultaneously (Beck-Peccoz et al. 1986; Heitz et al. 1987; Horvath et al. 1983, 1988; Kovacs et al. 1989; Osamura and Watanabe 1987; Scheithauer et al. 1986). The exception appears to be most corticotroph adenomas, which synthesize and release adrenocorticotropin (ACTH) and the related pro-opiomelanocortin-derived peptides almost exclusively (Kovacs and Horvath 1986; Scheithauer et al. 1986). Although there have been some reports of functioning corticotroph adenomas concurrently producing prolactin (PRL) (Bigos et al. 1977; Sherry et al. 1982) and α-subunit (Berg et al., in press), little has been published about the production of β-subunits of glycoprotein hormones by corticotroph adenomas (Heitz et al. 1987; Saeger et al. 1988).

We studied two cases of pituitary adenoma which caused Cushing's disease and produced immunoreactive β-luteinizing hormone (β-LH) as well as ACTH. The

bihormonal and monomorphous nature of these tumours was demonstrated by electron microscopy, immunohistochemistry and tissue culture. This is the first detailed report of LH production by functioning pituitary corticotroph adenomas.

Case reports

Case 1

A 37-year-old woman noted weight gain over 2 years and some non-pigmented striae around the waist. She also noted the appearance of fine hair on the face. Menses had remained regular but were foreshortened from 4- to 2-day periods. She had been known to be hypertensive for about 2 years and had been on treatment for the last year. Hormonal studies revealed that plasma cortisol level in the morning was elevated at 760 nmol/l (normal, 140–600), plasma ACTH level in the morning was inappropriately high at 16 pmol/l (normal, <22), and other pituitary hormone levels including LH were within normal limits. Plasma cortisol was poorly suppressed by low-dose dexamethasone but was completely suppressed by high dose dexamethasone. A computed tomographic (CT) scan showed no definite abnormality in the sella, but magnetic resonance imaging disclosed an area of slow signal intensity anteriorly and on the left. Selective complete trans-sphenoidal removal of a left-sided pituitary adenoma was performed. The postoperative course was uneventful.

Case 2

A 26-year-old woman complained of central scalp alopecia, weight gain over 2 years and facial hirsutism. Menses had been normal since her single pregnancy 6 years earlier. On physical examination, she had a distinct cushingoid appearance with a large moon face, obesity, hirsutism, central alopecia and a blood pressure of 140/90 mmHg. Laboratory examination revealed that the cortisol level in the morning was elevated at 588 pmol/l and urinary free cortisols were elevated at 469 nmol in 24-h collections (normal, <220). 17-Ketosteroids were 92 μmol in 24 h (normal, <42), androstenedione 12.7 nmol/l (normal, <7.7) and dehydroepiandrosterone 26.4 μmol/l (normal, <7.5). Plasma ACTH level was elevated at 40 pmol/l. Plasma cortisol was incompletely suppressed by low-dose dexamethasone, but was completely suppressed by high-dose dexamethasone. Serum prolactin level was slightly elevated at 28 ng/l (normal, <20), but other pituitary hormone levels were within normal ranges. A CT scan of the sella revealed a sizeable mass filling the left inferior portion of the sella with supra-diaphragmatic extension (stage 2A). The diagnosis of pituitary-dependent Cushing's disease due to an unusually large adenoma was made and the patient underwent selective trans-sphenoidal resection of the tumour. Her postoperative course was uneventful.

Materials and methods

For light microscopy, surgically removed tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections of 5 μm thickness were stained with haematoxylin and eosin (H&E) and the periodic acid-Schiff (PAS) technique.

For electron microscopy, pieces of tumour tissues were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in an Epon-Araldite mixture. Ultrathin sections were stained with uranyl acetate and lead citrate and investigated with a Philips 410LS electron microscope.

The avidin-biotin-perioxidase complex (ABC) method (Hsu et al. 1981) was performed on paraffin- and Epon-embedded tissues for the localization of seven adenohypophysial hormones: primary antisera directed against human antigens included anti-growth hormone (GH) (Dako, Santa Barbara, Calif., USA; 1:1000), anti-PRL (donated by Dr. H. Friesen, University of Manitoba, Winnipeg, Manitoba, Canada; 1:4000), anti-ACTH (1–39) (NIDDK, Bethesda, Md., USA), anti-β-thyrotropin (β-TSH) (supplied by Bio-Rad, Richmond, Calif., USA; 1:5000), anti-β-follicle stimulating hormone (β-FSH) (NIDDK; 1:1000), anti-β-LH (NIDDK; 1:2000) and α-subunit TSH (NIDDK; 1:250). All antisera were raised in rabbits except the antiserum against GH, which was raised in guinea pig. Sections exposed to the primary antisera were incubated overnight at 4° C. The reaction was visualized with an ABC Kit (Vector Laboratories, Burlingame, Calif., USA) and diaminobenzidine. The specificity of immunostains was verified by substituting the primary antisera with antisera preincubated with excess homologous antigens (10 μg/ml). In addition, the antiserum to LH was absorbed with synthetic ACTH (1–39) (Calbiochem, LaJolla, Calif., USA) and the antiserum to ACTH was absorbed with synthetic LH (NIDDK).

For tissue culture, cells from sterile tissues obtained at the time of surgery were dispersed and plated as described elsewhere (Asa et al. 1986). Cells were allowed to attach for 2–3 days; subsequently media were collected every 24 h. After attachment of cells, experimental incubations were performed. Baseline hormone secre-

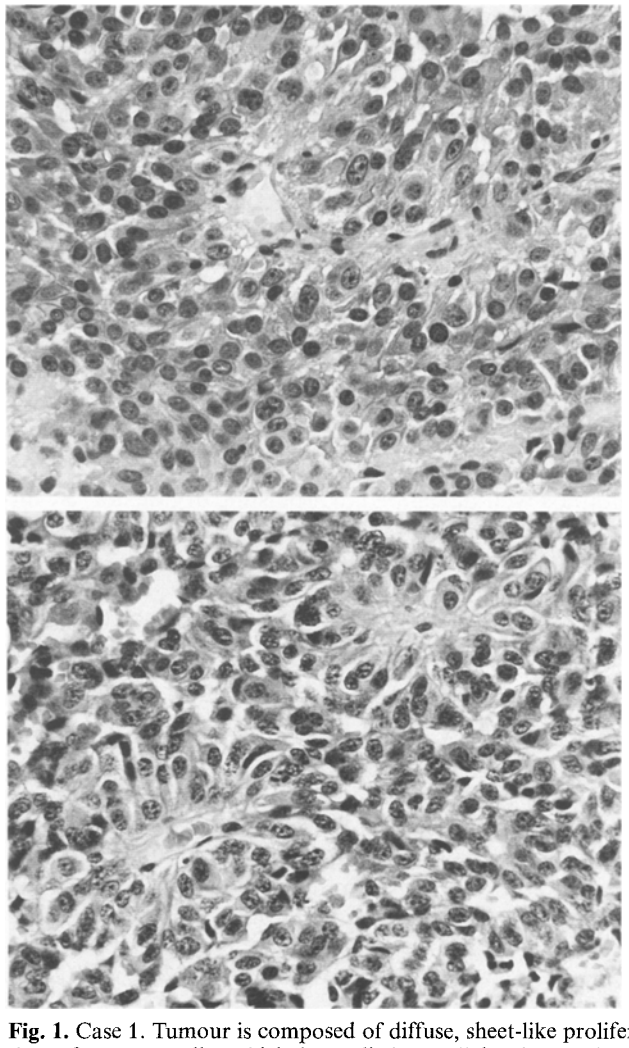

Fig. 1. Case 1. Tumour is composed of diffuse, sheet-like proliferation of tumour cells, which have distinct cell borders and oval to round nuclei. H&E, ×150

Fig. 2. Case 2. Tumour cells are arranged in a sinusoidal pattern and some of them extend their cytoplasmic processes towards blood vessels. H&E, ×150

tion was established for each well in 2-h and 24-h incubations in medium without test substances. Subsequent 2-h and 24-h incubations were performed in medium containing corticotropin-releasing hormone (CRH; 2×10^{-7} mol) or cortisol (2.7×10^{-7} mol). Control wells received vehicle alone. All spent media were stored in polyethylene vials at −20° C, until processed for radioimmunoassay. Culture medium hormone content of pituitary hormones was measured by radioimmunoassay using the standard double antibody technique (Asa et al. 1986). Antisera were directed against GH, PRL, ACTH, TSH, FSH, LH and α-subunit of glycoprotein hormones. Intra-assay coefficiency of variation (CV) was 5–8% and inter-assay CV was 7–10%.

Results

By light microscopy, both specimens contained tumorous and non-tumorous pituitary tissues. In the non-tumorous tissue adjacent to the tumour many corticotrophs exhibited Crooke's hyalinization. Lactotroph hyperplasia was also noted in case 1.

The histological features of the two tumours were similar. They were richly vascularized and had either a diffuse, sheet-like arrangement of tumour cells or a sinusoidal pattern near the blood vessels (Figs. 1, 2). Tumour cells had distinct cell borders and abundant cytoplasm which was moderately to strongly PAS positive. Some tumour cells were polygonal; others were enlongated with cytoplasmic processes extending towards blood vessels. The nuclei were oval to round and mitotic figures were not seen.

By electron microscopy, both adenomas were composed of well-differentiated cells, which were elongated or angular and often associated with long cytoplasmic processes (Figs. 3, 4). The ovoid or somewhat irregular nuclei were euchromatic and harboured prominent nucleoli. In the well-developed cytoplasm of case 1, the rough endoplasmic reticulum (RER) was abundant and showed marked dilation in some adenoma cells (Fig. 3). The less abundant RER of case 2 was composed of

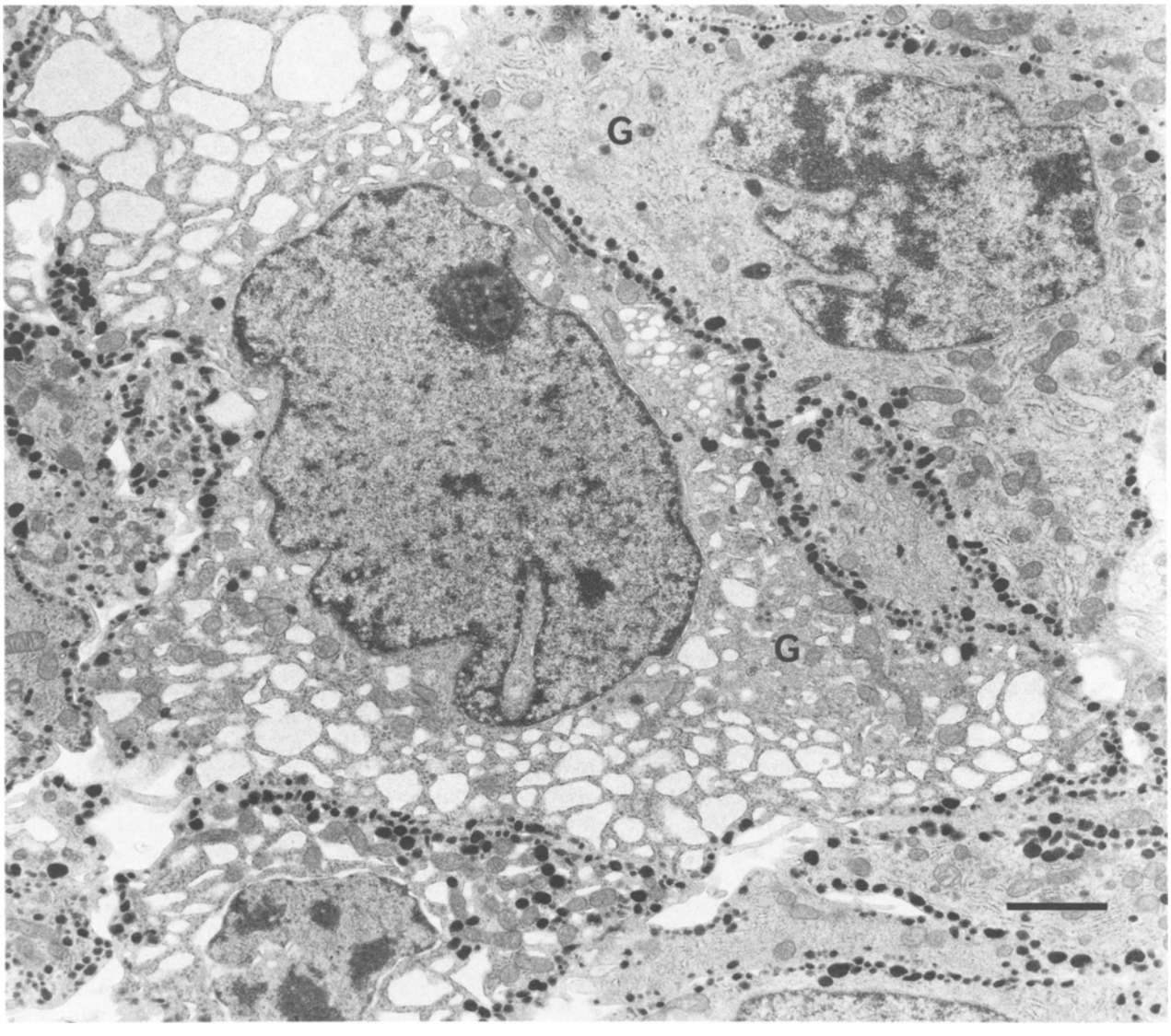

Fig. 3. Case 1. Ultrastructurally, the cytoplasmic organelles are well-developed. The Golgi (*G*) complexes are conspicuous and the abundant rough endoplasmic reticulum is markedly dilated. ×7200. *Bar*, 2 μm

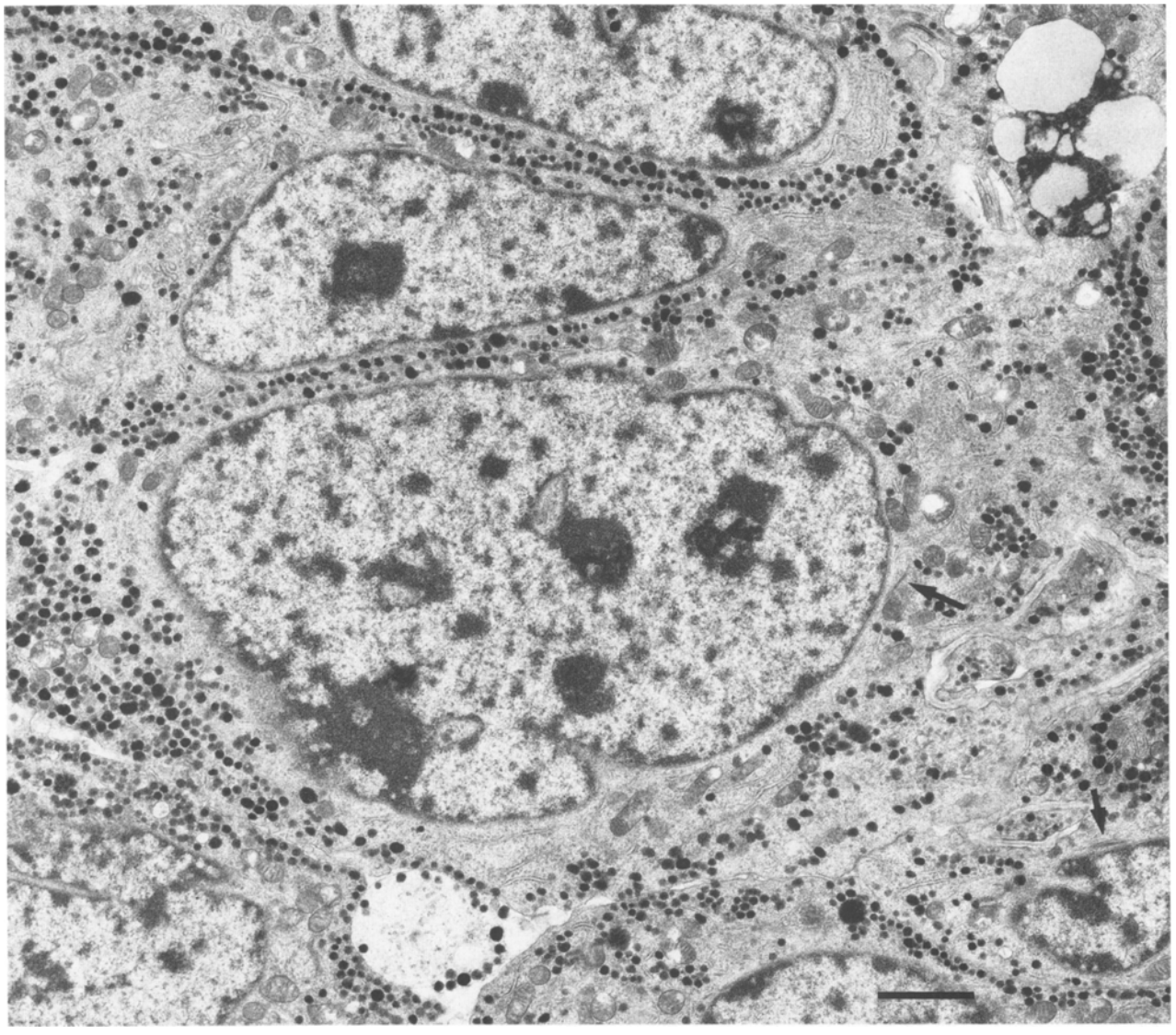

Fig. 4. Case 2. Enlongated tumour cells contain many secretory granules with a diameter of 200–350 nm forming a single row under the plasma membranes. Note type 1 microfilaments near the nucleus (*arrows*). ×7200. *Bar*, 2 μm

slightly dilated short profiles (Fig. 4). The Golgi complexes were conspicuous and harboured several immature secretory granules. The secretory granules, which formed a single row under the plasma membrane, varied in shape and electron density, and measured approximately 200–350 nm in the majority of adenoma cells. A few cells contained secretory granules with diameters as small as 100 nm or as large as 450–500 nm. Type 1 filaments were observed in case 2 (Fig. 4) but not in case 1. These light and electron microscopic features were characteristic of densely granulated functioning corticotroph adenoma (Kovacs and Horvath 1986).

By immunohistochemistry using the ABC method, immunoreactivities for ACTH, β-LH, β-FSH and β-TSH were demonstrated in both tumours and those for GH and α-subunit in the tumour of case 2. ACTH immunoreactivity was present in the majority of tumour cells and was accentuated along the cell periphery (Figs. 5a, 6a). The second largest cell population was composed of LH-immunoreactive cells; these comprised approximately 10% of the tumour cell populations (Figs. 5b, 6b). They were distributed in either a diffuse or a sinusoidal pattern and tended to form clusters composed of ten or more cells. The immunoreactivity was located either uniformly throughout the cytoplasm or restricted to the cell periphery. Some of the cells had long cytoplasmic processes with intense LH immunoreactivity. Serial sections revealed a considerable number of LH-immunoreactive cells in some areas where almost all tumour cells were immunoreactive for ACTH (Fig. 6a, b). FSH-immunoreactive cells were slightly fewer than LH-immunoreactive cells in case 2 and far fewer in case 1. The distribution of FSH-immunoreactive cells corresponded to that of LH-immunoreactive cells on serial sections, indicating that FSH and LH were co-localized in the same tumour cells. TSH immunoreactivity was observed in approximately 5% of cells in case 2 and only a few cells in case 1; they were scattered

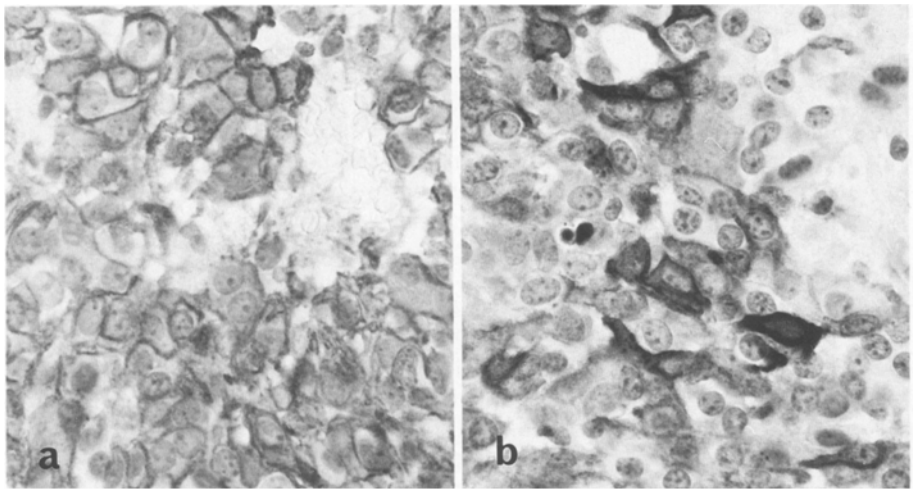

Fig. 5a, b. Case 1. Numerous tumour cells show adrenocorticotropin (ACTH) immunoreactivity accentuated along the cell periphery (**a**). Luteinizing hormone (LH)-immunoreactive cells are seen forming clusters throughout the tumour tissue (**b**). ×480

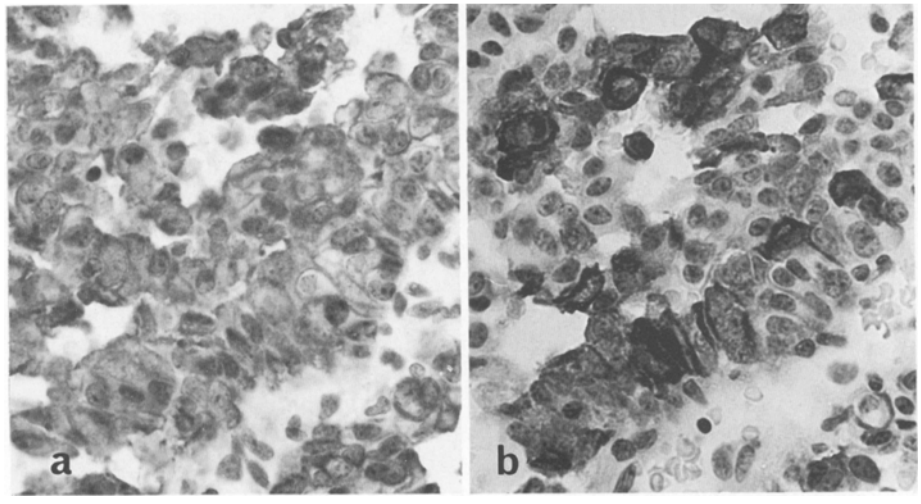

Fig. 6a, b. Case 2. ACTH immunoreactivity is observed in almost all tumour cells (**a**) and intensive LH immunoreactivity is demonstrated in many cells on a serial section (**b**), strongly suggesting the co-localization of ACTH and LH immunoreactivities in the same cells. ×480

throughout the tumour tissue without cluster formation in a different pattern that that of the LH/FSH-immunoreactive cells. α-Subunit and GH immunoreactivities were found in only a few scattered cells of case 2. Absorption tests of the LH immunostain revealed that LH immunoreactivity was abolished by preabsorption of the antiserum with excess LH antigen but not with excess ACTH antigen. No LH immunoreactivity was observed in Epon-embedded tissues.

High concentrations of immunoreactive ACTH were detected in tissue culture media of both tumours. Basal release averaged 1090 pmol/2×10^4 cells/24 h in case 1 and 31200 pmol/2×10^4 cells/24 h in case 2. Low, but significant amounts of immunoreactive LH were measured in culture media of case 1 (118.6 ng/2×10^4 cells/24 h) and case 2 (511.7 ng/2×10^4 cells/24 h). FSH release by case 1 averaged 125 ng/2×10^4 cells/24 h; FSH was not detected in media of case 2. α-Subunit was not detected in media of case 1 but averaged 26.6 ng/2×10^4 cells/24 h in media of case 2. ACTH release by both tumours was increased during incubation with CRH to 120–154% of control, and decreased during incubation with cortisol to 14–52% of control. LH release was similarly increased by CRH (152–250% of control) and inhibited by cortisol (40–73% of control). In case 1, FSH release was decreased to 40% of control during cortisol exposure. α-Subunit release by tumour 2 was increased to more than 300% of control values during incubation in CRH and was decreased to 10–30% of control during incubation in cortisol.

Discussion

The unusual feature of these two pituitary adenomas associated with clinically and biochemically typical Cushing's disease was the concurrent presence of immunoreactive ACTH and β-LH. The specificity of LH immunoreactivity was confirmed by the absorption tests with LH and ACTH antigens. Scattered cells with immunoreactivity for other adenohypophysial hormones within a uniform tumour population may be interpreted as trapped non-tumorous elements. In these two tumours, however, there was conclusive evidence that the β-LH-immunoreactive cells were neoplastic and not intermingled normal cells. Firstly, their presence in clusters

throughout adenoma tissues is different from the distribution of gonadotrophs in the normal pituitary. Secondly, ultrastructurally normal gonadotrophs were not observed in tumour tissue. Thirdly, immunoreactive LH release in tissue culture medium was maintained for the duration of cultures; it is known that contaminating normal adenohypophysial cells disappear or remarkably decrease hormone release after 1 week in culture (Asa et al. 1986). Finally, LH release responded to stimulation by CRH and inhibition by cortisol in parallel with ACTH; this paradoxical behaviour is not characteristic of nontumorous gonadotrophs.

Fine structural observation disclosed that these tumours were monomorphous, densely granulated corticotroph adenomas (Kovacs and Horvath 1986), suggesting that ACTH-containing and β-LH-containing cells were morphologically indistinguishable. Moreover, co-localization of ACTH and LH in the same tumour cells is strongly suggested by the immunostains on serial sections; a large number of β-LH-immunoreactive cells were noted in some foci where almost all cells were immunoreactive for ACTH. The best way to confirm such co-localization would be the double labelling immunogold technique (Beck-Peccoz et al. 1986; Bendayan 1982), but unfortunately LH immunoreactivity could not be demonstrated by us in Epon-embedded tissues, probably due to loss of antigenicity, which is known to occur more readily in glycoprotein hormone-containing cells than in other adenohypophysial cell types (Horvath and Kovacs 1988b). Moreover, changes in release of LH and other glycoprotein hormones in response to CRH stimulation or hydrocortisone suppression in parallel with ACTH suggest production by the same cells.

Simultaneous production of ACTH and other hormones by functioning corticotroph adenomas has been reported. Hyperprolactinaemia and/or galactorrhoea have been noted in patients with Cushing's disease (Bigos et al. 1977; Levin et al. 1959; Sherry et al. 1982), and prolactin-immunoreactive cells have been demonstrated in approximately 20% of functioning corticotroph adenomas (Heitz et al. 1987; Saeger et al. 1988). Production of α-subunit of glycoproteins by functioning corticotroph adenomas with or without elevated serum levels has been also reported (Berg et al., in press), and immunohistochemical studies have shown that α-subunit-immunoreactive cells are present in 35% of corticotroph adenomas (Heitz et al. 1987). It is noteworthy that co-localization of ACTH and α-subunit in the same secretory granules has been demonstrated in tumour cells of a corticotroph adenoma (Berg et al., in press).

On the other hand, the concurrent production of β-subunit of glycoproteins by functioning corticotroph adenomas has been little known; only the incidence of such phenomenon demonstrated by immunohistochemistry has been reported so far. β-LH was demonstrable in 13–27% of adenomas (Heitz et al. 1987; Saeger et al. 1988). Among 34 functioning corticotroph adenomas examined recently in our laboratory, β-LH-containing cells were noted in 6 cases (18%), but such cells were so few and sparsely scattered in 4 of these adenomas that the possibility of intermingled normal gonadotrophs could not be completely excluded. Only the 2 tumours described here exhibited convincing evidence of bihormonal differentiation.

Multiplicity of hormone production in pituitary adenomas has become well known, especially since the introduction of immunohistochemistry (Kovacs et al. 1989). Distinct tumour types containing GH and prolactin are common, and elaboration of several hormones can be seen frequently in densely granulated GH-cell adenomas, null cell adenomas and silent type 3 adenomas (Asa et al. 1986; Horvath and Kovacs 1988a; Horvath et al. 1988; Kovacs and Horvath 1986; Kovacs et al. 1989; Scheithauer et al. 1986). In addition to these ultrastructurally well-defined adenomas, there are adenomas which are capable of producing two or more hormones, and exhibit unusual ultrastructural features and clinical manifestations due to excess secretion of such hormones (Horvath et al. 1983; Malarkey et al. 1989; McComb et al. 1984; Scheithauer et al. 1986). These tumours are designated as plurihormonal adenomas in the proposed classification (Horvath and Kovacs 1988a; Kovacs and Horvath 1986). Moreover, multiple hormone production may be encountered in some, otherwise typical adenomas, such as lactotroph adenomas with α-subunit immunoreactivity (Scheithauer et al. 1986) and corticotroph adenomas with either prolactin (Bigos et al. 1977) or α-subunit positivity (Berg et al., in press). The present cases belong to the last category. Thus, it appears that almost all types of pituitary adenomas (Kovacs and Horvath 1986) have the potential to produce multiple hormones during the course of neoplastic proliferation.

At least two major explanations can be considered to explain the pathogenesis of plurihormonality in pituitary adenomas (Kovacs et al. 1989; Scheithauer et al. 1986). Firstly, these adenomas may originate from progenitor cells which are capable of multidirectional differentiation. Secondly, adenomas which are composed of a recognized adenohypophysial cell type may acquire a partially different character either by an intrinsic mutational mechanism or due to extrinsic stimulatory factors during proliferation. The former seems to be unlikely, at least in cases reported here, because cells containing ACTH and LH or precursor cells of both corticotrophs and gonadotrophs have not been identified in either the fetal or adult human pituitary.

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