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Immunohistochemical colocalization of growth hormone (GH) and α subunit in human GH secreting pituitary adenomas

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Summary. This immunohistochemical study disclosed that 9 of 15 GH secreting pituitary adenomas contained α subunit positive cells. These cases also contained PRL positive adenoma cells, but LHβ was negative. Of these 9 cases, 4 cases showed occasional FSHβ containing cells, 2 of these also contained a few TSHβ positive cells. By mirror section technique, variable numbers of adenoma cells were found to contain both GH and α subunit. Immunoelectron microscopically, both GH and α subunit were localized in secretory granules which suggested their co-release from the tumour cells. The presence of GH and α subunit in rough endoplasmic reticulum indicated their active production in the tumour. In the normal adult anterior pituitary gland, about 10% of GH cells contain FSH α, β and LHβ subunits and had appearances suggesting the co-production of GH and FSH as well as LH. The colocalization of GH and FSH α is considered to be associated with the neoplastic transformation GH cells which possess the intrinsic potentiality of differentiation toward α subunit. However, the mechanism for the lack or deficiency of β subunits in the neoplastic condition remains to be further investigated.

Key words: Pituitary adenoma – Acromegaly – Immunohistochemistry – Growth hormone – α subunit

Introduction

It is well known that functioning pituitary adenomas in man mainly consist of prolactin (PRL) secreting, growth hormone (GH) secreting, and adrenocorticotropic hormone (ACTH) secreting tumours (Horvath et al. 1983; Horvath and Kovacs 1986; Kovacs et al. 1981; Kovacs 1984; Kovacs and Horvath 1986; Mukai 1983; Schatz et al. 1985). Follicle stimulating hormone (FSH) or thyroid stimulating hormone (TSH) secreting tumours have been reported to be rare (Beitins et al. 1977; Borges et al. 1984; Kovacs et al. 1981; Kovacs 1984; Mukai 1983). The GH secreting tumours are well known to produce other hormones including PRL, FSH or LH (Bassetti et al. 1986; Beck-Peccoz et al. 1985; Fukaya et al. 1980; Horvath et al. 1983; Kovacs et al. 1982; Kovacs and Horvath 1986; Lloyd et al. 1983; McComb et al. 1984; Riedel et al. 1985). It has been claimed that in some of GH secreting tumours, GH and PRL are localized not only in the same tumour cells but also in the same secretory granules (Bassetti et al. 1986). Recently, in vitro studies of a GH secreting adenoma have disclosed the coexistance of GH and α-subunit in the same tumour cells as well as in the same secretory granules (Beck-Peccoz et al. 1985).

Our present immunohistochemical studies are aimed at the elucidation of concomitant localization of GH and glycoprotein subunits such as α subunit, FSHβ, LHβ, TSHβ subunits in the GH secreting adenomas as well as in the normal pituitary glands.

Our results indicated the frequent immunohistochemical colocalization of GH and glycoprotein α subunit. Immunoelectron microscopy suggested the concomitant production and secretion of GH and α subunit in the same tumour cells.

Materials and methods

36 functioning adenomas (18 PRL secreting, 15 GH secreting, 3 ACTH secreting) were stained immunohistochemically for the

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peptide and glycoprotein hormones. Three adult pituitary glands without endocrinological problems obtained at autopsy were also studied. The tissue from the pituitary tumours and the normal pituitary glands was fixed in 10% buffered formalin for light microscopy and was fixed in picric acid paraformaldehyde (Zamboni) or periodate lysin paraformaldehyde (PLP) for electron microscopic immunohistochemistry. The formalin fixed tissue was embedded in paraffin. For conventional electron microscopic studies, the tissue was also fixed in 2% glutaraldehyde in 0.1 M sodium phosphate buffer (SPB) and embedded in Quetol. As immunohistochemical technique, peroxidase-labeled antibody method was performed as previously described (Osamura et al. 1986).

The primary antisera utilized in this study included polyclonal antibodies against GH (NIADDK), GH (DAKO), PRL (kindly supplied by Prof. P.K. Nakane, Department of Cell Biology, Tokai University School of Medicine), PRL (DAKO), FSH α (NIADDK), FSH β (NIADDK), LH β (NIADDK), TSH β (NIADDK), and monoclonal antibody against human GH, (supplied by BioGenex Laboratories, California, USA).

Light microscopically, the indirect peroxidase labelled antibody method was applied to deparaffinized sections. The primary or peroxidase conjugated second antibody (IgG Fab) was reacted on the sections for 15 min in a moist chamber. After thorough washing in 0.1 M phosphate buffered saline (PBS pH 7.2) the peroxidase was colorized in 3,3′ diaminobenzidine 4 HCl (20 mg/100 ml 0.1 M Tris HCl buffer pH 7.2) containing 0.005% hydrogen peroxide for 5 min. The sections were then counterstained with 1% methyl green, dehydrated and mounted. In order to enable a precise observation of colocalization of two different antigens in the same tumour cells, two consecutive mirror sections were used for immunohistochemical staining described above (Osamura et al. 1980). In the normal pituitary glands, a double staining technique was also performed (Nakane 1968) using monoclonal antibody against GH and antibody against FSH α.

Electron microscopically, the tumour was fixed in the overnight, washed in PBS with graded concentration of sucrose and quickly frozen in alcohol dry ice. 4–6 μ cryostat sections were mounted on albuminized slides. A similar indirect immunohistochemical reaction was performed but each antibody was reacted overnight. Then the sections were fixed in 2% glutaraldehyde for 10 min and washed in PBS. The sections were preincubated in 0.02% DAB solution for 1 h, incubated in the DAB solution with 0.005% hydrogen peroxide for 2 to 5 min, post-fixed in 2% osmic acid, dehydrated in graded alcohol and embedded in Quetol by the inverted gelatin capsule method (Osamura et al. 1986).

The specificity of the antibodies was confirmed by immunohistochemical pre-incubation tests using purified antigens. In brief, 50 μg of the above diluted anti GH antibody (DAKO, Biogenex, California, USA) was incubated at 4° C overnight with purified GH (NIADDK, UCB) or FSH α (NIDDK) at a concentration of 10 μg/ml. Anti FSH α antibody was preincubated with GH(NIADDK, UCB) or FSH α(NIDDK) at 4° C overnight at the concentration of 10 μg/ml. After preincubation, similar immunohistochemical staining was performed on the deparaffinized sections of the tumour and on the normal pituitary glands.

Results

All PRL secreting adenomas and ACTH secretory adenomas were negative for glycoprotein subunits. Only one case of PRL secreting adenoma showed scattered GH positive cells. Among 15 cases of GH secreting tumours, 9 cases (60%) showed the tumour cells to be positive immunohistochemically for FSH α (Fig. 2, Table 1). No apparent correlation was noted between serum FSH levels and the immunohistochemical positivity for α subunit (Table 2). Four of these nine cases showed the presence of GH in many tumour cells but the other cases contained scattered GH positive tumour cells (Fig. 1). The specificities and absence of crossreactivity between anti GH antibody and anti α-subunit antibody were confirmed by the preabsorption tests (Fig. 2). In the former cases, both GH and

Table 1. Immunohistochemistry of GH secreting pituitary adenomas

Cases		GH	PRL	α subunit	FSH β	LH β	TSH β	ACTH
1.	39F	+++	+++	++	+	−	−	−
2.	37M	++++	+	++++	+	−	+	−
3.	50M	+++	++	−	−	−	−	−
4.	29F	++++	+++	++	−	−	−	−
5.	47F	++++	++	+	−	−	−	−
6.	32F	+++	−	−	−	−	−	−
7.	51F	+++	++	+++	−	−	−	−
8.	45F	++++	−	−	−	−	−	−
9.	27F	+++	++	−	−	−	−	−
10.	57F	++++	+	+	−	−	−	−
11.	27M	++++	+++	+++	−	−	−	−
12.	44F	++++	+	++++	+	−	+	−
13.	27F	++	++	+	+	−	−	−
14.	40M	++++	−	−	−	−	−	−
15.	39M	++++	+++	−	−	−	−	−
		15/15	12/15	9/15	4/15	0/15	2/15	0/15

Immunohistochemical staining: ++++ indicates diffuse staining in most tumour cells, +++ : many tumour cells, ++ : frequent tumour cells, + : occasional tumour cells, − : No positive tumour cells

Table 2. Serum hormone levels in the cases of GH secreting pituitary adenomas

Cases		GH (ng/ml)	PRL (ng/ml)	FSH (IU/ml)	LH (IU/ml)	TSH (IU/ml)	ACTH (ng/ml)
1.	39F	3.59	45.4	3.1	13.2	2.9	181
2.	37M	330	6	4.5	24.2	6.8	142
3.	50M	–	–	–	–	–	–
4.	29F	114	65	9	14	1.3	19
5.	47F	30.2	21	8	6	0.6	90
6.	32F	51.2	7.2	5.3	10.6	1.1	151
7.	51F	1094	1287	–	–	–	–
8.	45F	34.3	25.2	14.4	8.5	5.1	67
9.	27F	18.0	20.1	7.7	16.6	1.2	73
10.	57F	21.6	15.1	105.3	45.3	2.4	77
11.	27M	–	–	–	–	–	–
12.	44F	269	93	7	9	5	–
13.	27F	63	–	–	–	–	–
14.	40M	88	–	–	–	–	–
15.	39M	20	–	–	–	–	–

FSHα were co-localized in many tumour cells (Fig. 3). In the latter group, some tumour cells contained both GH and FSH α but others contained either GH or FSH α (Fig. 4). In four tumours, some of the FSH α positive tumour cells were also positive for FSH β, and two cases among these showed occasional and scattered TSH β positive tumour cells. LH β was negative in all cases examined (Table 1). These 9 cases which were immunohistochemically positive for α subunit also showed scattered PRL positive tumour cells. Thus, if the tumours were positive for the α subunit, they were also positive for PRL. In these tumours, PRL was localized in the different cells from FSH α containing cells. Three other cases showed the presence of PRL beside GH, even though they were negative for α subunit. Therefore, 12 of 15 cases (80%) of GH secreting adenomas contained immunoreactive PRL and the GH which appeared in the different tumour cells, i.e., a mammosomatotroph cell adenoma (Horvath et al. 1983a). Three cases which were submitted to immunoelectron microscopic study showed the presence of GH and FSH in many round dense cored secretory granules in many of the cells. They were also observed in the cisternae of sparse rough endoplasmic reticulum and perinuclear spaces (Fig. 5).

Double staining and mirror sections demonstrated that, in the anterior lobe of the adult pituitary glands, GH and the glyoprotein subunits were mostly localized in the different anterior cells but that occasional cells (approximately one of thirty cells) contained GH and FSH α, FSH β and LH β (Fig. 6). Double staining revealed evenly dispersed granules in the cytoplasm which were positive for GH and these subunits. The localization of TSH β was different from that of GH or other β subunits.

Discussion

It is well known that glycoprotein hormones possess a common α subunit (Borges et al. 1984; Glaser et al. 1986; Kourides 1976). Antibody against the FSH α subunit was considered to represent the localization of α subunit for glycoprotein hormones.

Our present immunohistochemical study clearly showed that GH secreting tumour cells often show the immunohistochemical presence of glycoprotein subunits, especially the α subunit. It should be emphasized that PRL secreting tumours were negative for glycoprotein subunits. Some or many tumour cells, depending upon the individual case, showed the co-existence of GH and α subunit in the same cells and suggested the concomitant production of both GH and glycoprotein α subunit. A few α subunit-containing tumour cells also contained FSH β or TSH β subunits and it was speculated that complete form of FSH or TSH hormones could be produced in a few of the tumour cells. In most cases, tumour cells could apparently produce only α subunits which may explain the absence of correlation between serum FSH levels and the clinical symptoms of gonadotrophic or thyrotrophic activities. Cases of GH secreting tumours which were also positive for PRL were mammosommatotroph cell adenomas which showed GH and PRL in different tumour cells. Our results, including the immunoelectron microscopic localization of GH and α subunit in rough endoplasmic reticulum and perinuclear spaces, indicated production in the tumour cells and suggested that GH secreting tumour cells possess the potentiality of differentiation toward glycoprotein α subunit production (Beck-Peccoz et al. 1985) as

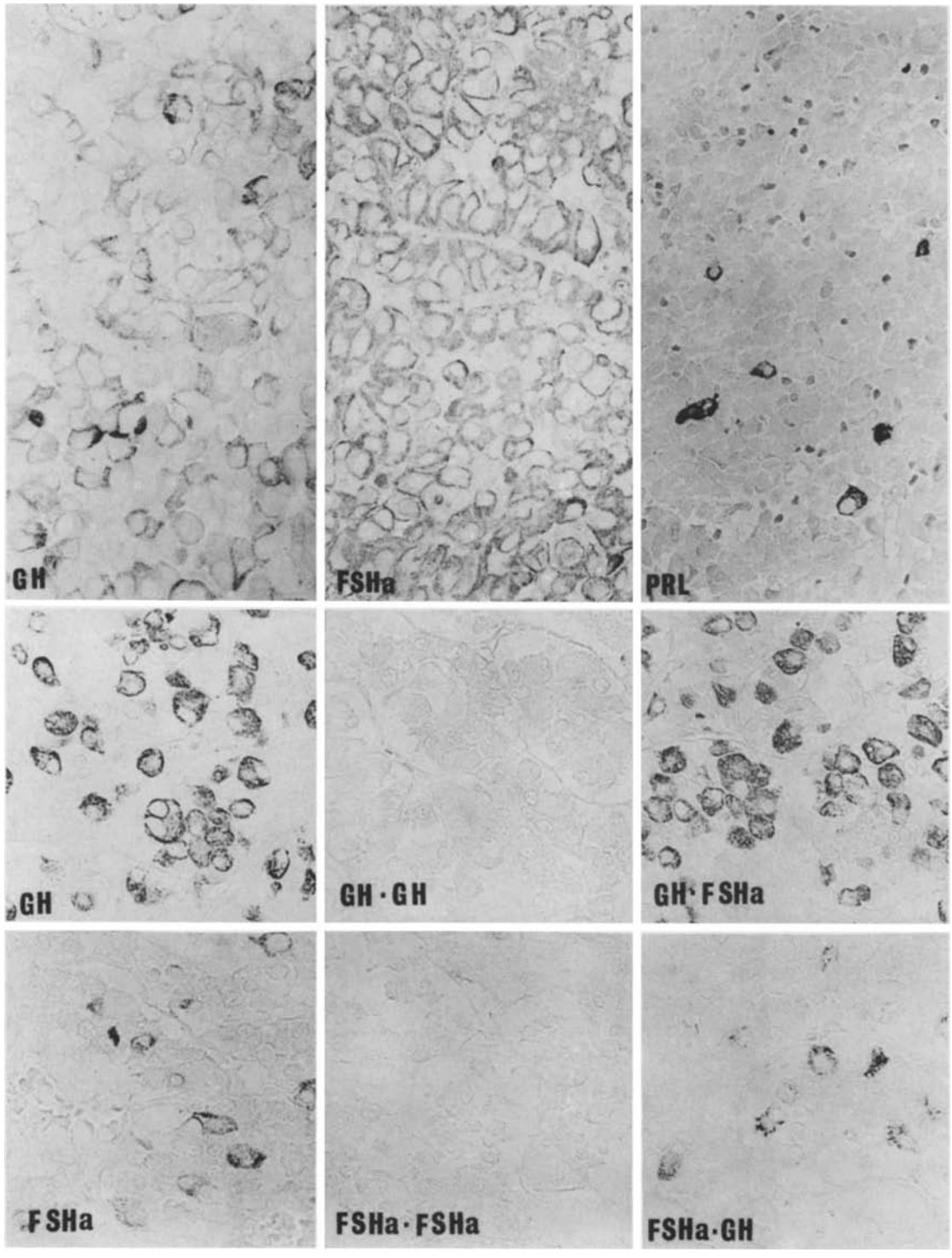
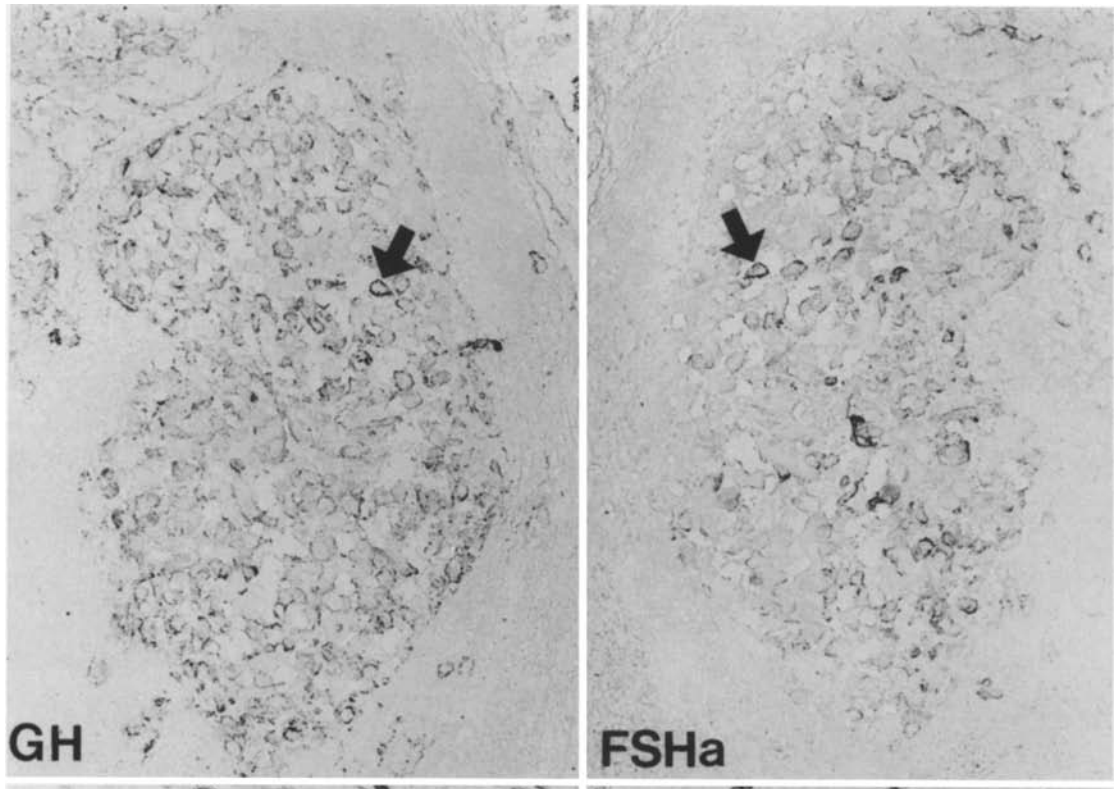


Fig. 1. Immunohistochemical localization of GH, FSH α, PRL in pituitary adenoma. GH and FSH α are localized in many cells, but PRL positive cells are sparse. (×100)

Fig. 2. Preabsorption test of anti GH and anti FSH α antibody. Upper half: GH: anti GH antibody not preabsorbed, GH·GH: anti GH antibody preabsorbed with GH, GH·FSH α: anti GH antibody preincubated with FSH α. Lower half: FSH α: anti FSH α antibody not preabsorbed, FSH α·FSH α: anti FSH α antibody preabsorbed with FSH α, FSH α·GH: anti FSH α antibody preincubated with GH. (×100)



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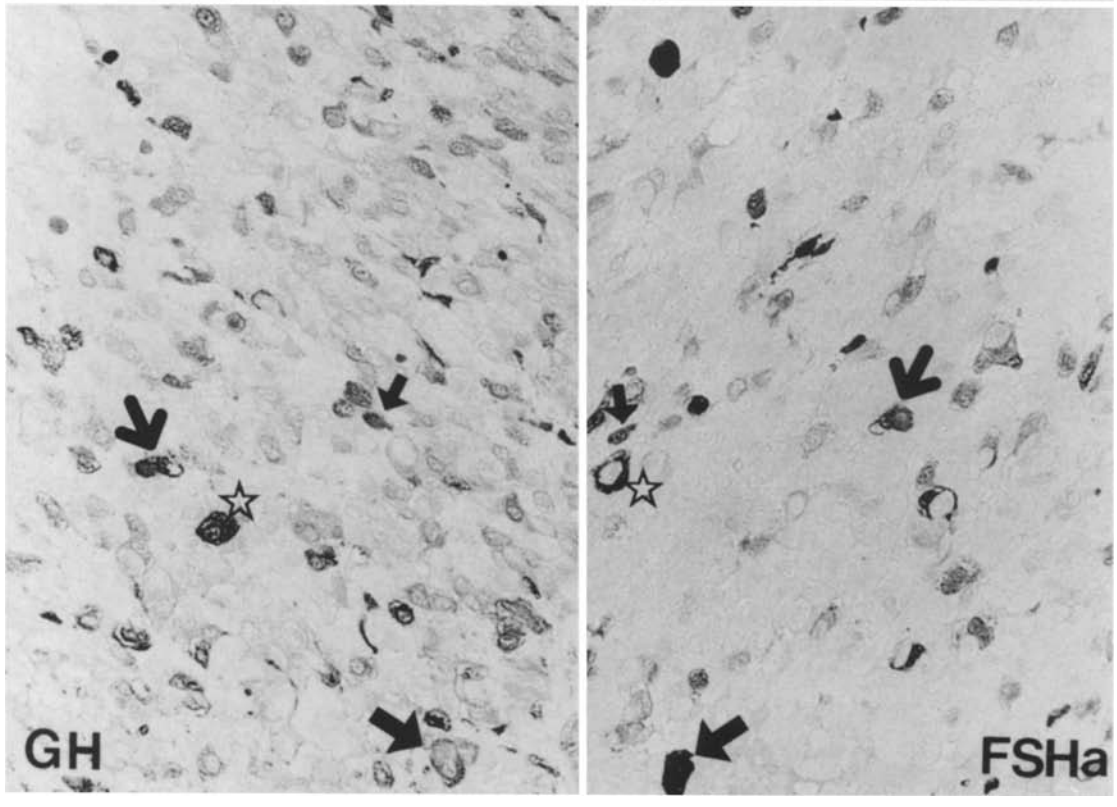

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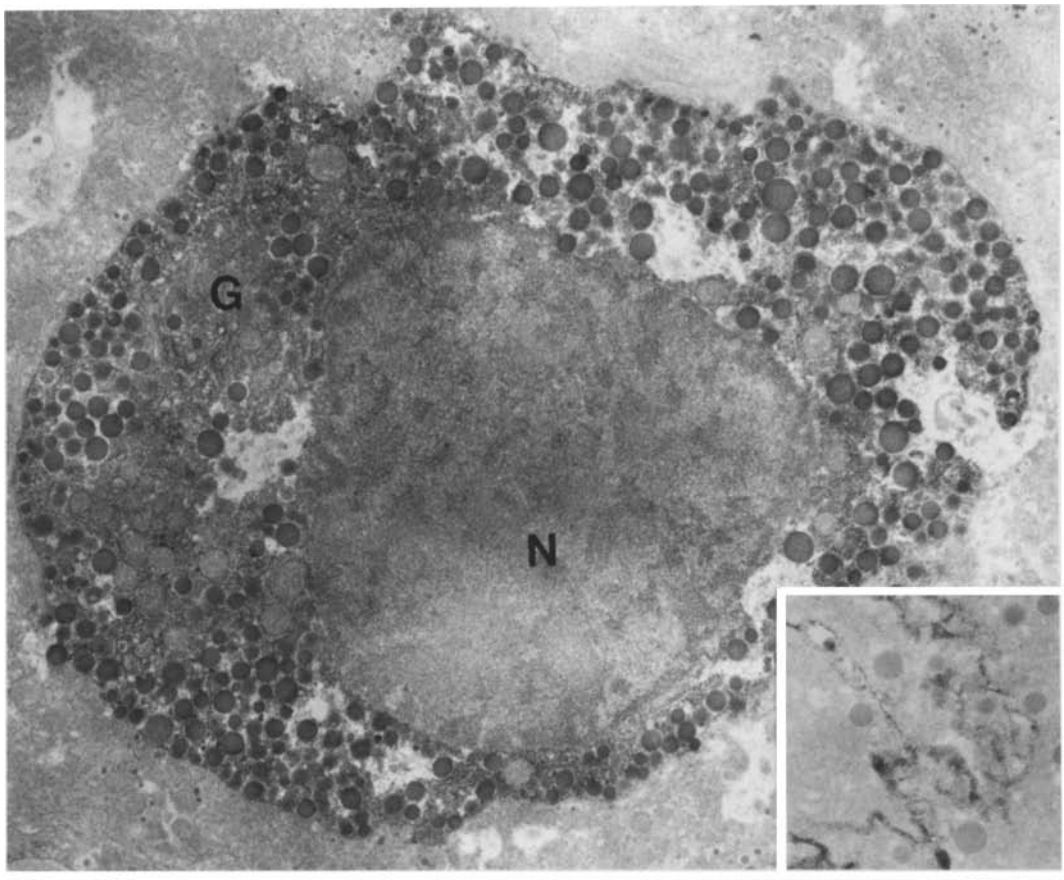
Fig. 3. Co-localization of GH and FSH α in same tumour cells (arrows) (× 150 mirror sections)

Fig. 4. Co-localization of GH and FSH α in the same tumour cells. Pairs of different arrows show the co-localization. (× 150 mirror sections) ☆ indicates the cells which contain either GH or α subunit



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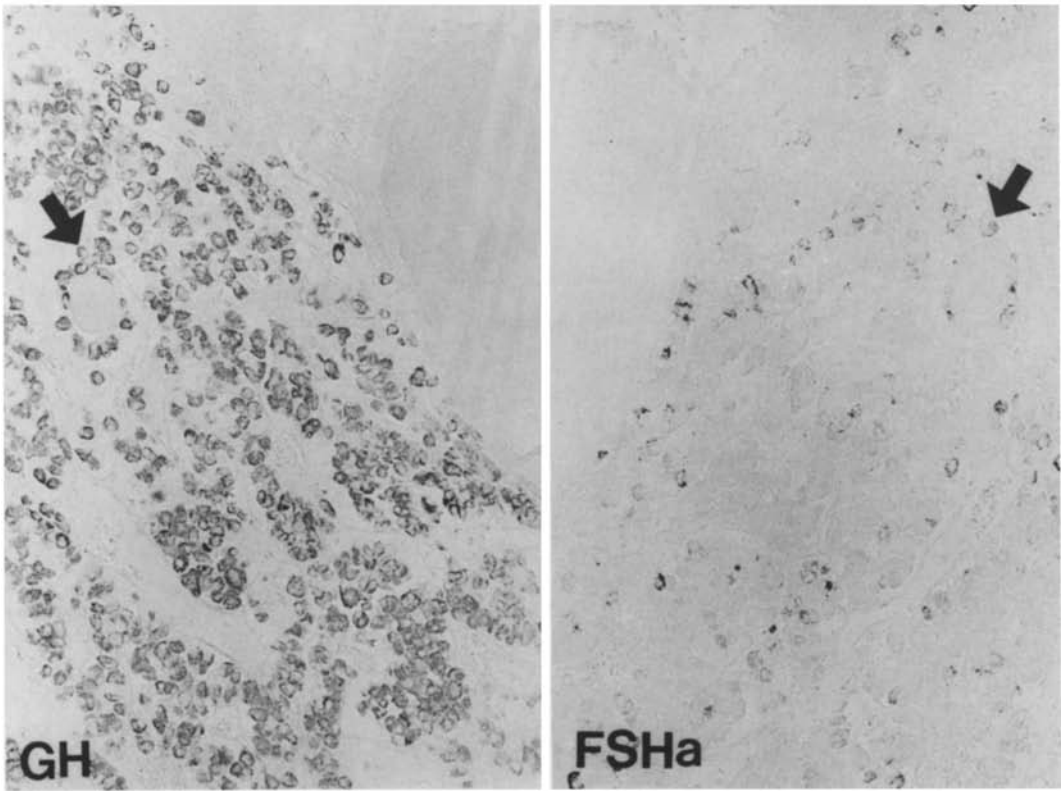

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Fig. 5. Immunoelectron microscopic localization of GH in many secretory granules of the adenoma. Inset: Occasionally, cisternae of rough endoplasmic reticulum contain GH and indicates active production of GH in the tumour cells. (× 5000 Inset: × 14000)

Fig. 6. Co-localization of GH and FSH α in the same cells of the anterior pituitary gland (*arrows*). (× 100 mirror sections)

well as PRL secretion (Bassetti et al. 1986; Horvath et al. 1983; Horvath et al. 1983; Horvath and Kovacs 1986; Kovacs et al. 1982; Kovacs et al. 1981; Kovacs 1984; Mukai 1983). In the normal anterior pituitary gland, GH and the α subunit together with gonadotrophin β subunits were colocalized in some cells. This colocalization in the normal pituitary has not been previously reported. Therefore, the appearance of glycoprotein in GH secreting tumours may be closely related to neoplastic transformation of the normal GH producing cells which possess the capacity for differentiation toward α subunit production. The production of LH β, FAH β subunit has a weak association with the neoplastic transformation of GH cells. The locus for GH production resides in chromosome 11 (Chawla et al. 1983) which is different from that for FSH β occurring on chromosome 17 (Fukaya et al. 1980). It has been also reported that neoplastic condition of the anterior pituitary cells may be associated with only α subunit production but not with β subunits (Capella et al. 1983; Klibanski et al. 1983; Kourides et al. 1976; Landolt and Heits 1986). The question of why neoplastically transformed GH producing cells are more closely associated with α subunit production but only weakly with β subunit production remains to be further investigated.

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