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Alpha-subunit-producing pituitary adenomas

Immunocytochemical and ultrastructural studies

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Summary. Immunohistological techniques demonstrate the alpha-subunit of glycoprotein hormones in the majority of endocrine-inactive, undifferentiated pituitary adenomas and pituitary oncocytomas. In about one-fifth of endocrine-active adenomas, the alpha-subunit is produced in combination with either adrenocorticotropic hormone or prolactin, and it is found in combination with growth hormone in about half of those adenomas causing acromegaly.

Pure alpha-subunit-producing, endocrine-inactive adenomas characteristically have small secretory granules that are destroyed by direct osmium fixation, but are well preserved after prefixation with glutaraldehyde. As only a few atypical prolactinomas show similar secretory granules, and as they display a positive reaction for the alpha-subunit only exceptionally, this ultrastructural feature can serve as a guide to differentiate such adenomas.

Key words: Pituitary neoplasms – Pituitary hormones – Immunocytochemistry – Electron microscopy – Alpha-subunit – Acromegaly

Introduction

Findings from electron microscopic and immunohistological studies in tumour pathology and from radioimmunoassays for determining pituitary hormone levels in the blood dictated a redefinition of the "chromophobe" adenoma. The term is still used by clinicians to describe pituitary adenomas that cause only signs of cranial nerve compression and varying degrees of pituitary insufficiency without signs of hormone hypersecretion. On the basis of results obtained using immunohistochemical, ultrastructural, and cell culture techniques, however, the following classification of pituitary

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adenomas has been proposed for tumours that show no clinical signs of hormone production (Landolt 1978; Landolt et al. 1978). The adenomas are divided into: – (1) *Oncocytomas* which are considered to be unable to synthesize and secrete hormones. (2) *Adenomas that secrete only small amounts of normal hormones*, insufficient to increase blood hormone levels, and therefore not causing typical endocrine syndromes of hormone overproduction. Increased hormone levels can be detected in the fluid from cell cultures of these tumours and by immunohistochemical studies of biopsy specimens (Sasaki et al. 1975). (3) *Adenomas displaying ultrastructural features of hormone synthesis and release, even though no recognized hormones are detected* either in the patient's blood by radioimmunoassay or in the adenoma by immunohistochemical examination. Presumably, such adenomas produce either abnormal substances, hormone fragments not reactive with the currently used antibodies, or as yet unknown hormones.

The pituitary glycoprotein hormones – follicle stimulating hormone [FSH], luteinizing hormone [LH], and thyrotropic hormone [TSH] – consist of two dissimilar, nonconvalently bound subunits. Whereas the beta-subunit confers biological specificity on each of these hormones, the alpha-subunit is species-specific and is virtually identical in all glycoprotein hormones (Pierce 1971; Vaitukaitis and Ross 1974). Elevated serum levels of the alpha-subunit were first reported by Macfarlane et al. (1980), and subsequently by Klibansky et al. (1983) and Ridgway (1983). A total of eight cases were reported. This newly recognized type of pituitary adenoma corresponds to the third category of "endocrine inactive" pituitary adenomas described earlier.

Electron microscopy shows that the small secretory granules of some endocrine-inactive adenomas are destroyed by direct osmium fixation, but remain intact after combined glutaraldehyde-osmium fixation (Landolt 1978). We suggest that this behavior may reflect a morphological characteristic of a separate type of endocrine-inactive adenomas. In this investigation we have focused on the following questions: Are there "pure" alpha-subunit-producing adenomas? Do pituitary adenomas containing the small secretory granules which are disrupted by direct osmium fixation produce the alpha-subunit? Is this ultrastructural sign specific for alpha-subunit-producing adenomas? Can the alpha-subunit also be detected in endocrine-active pituitary adenomas that do not produce one of the the specific beta-subunits of glycoprotein hormones; i.e., the adenomas causing acromegaly, hyperprolactinaemia, Cushing's disease, and Nelson's syndrome?

Materials and methods

Unselected biopsy specimens of pituitary adenomas from 76 patients were studied. Eleven of the patients (8 men, 3 women) showed neither clinical nor endocrinological evidence of endocrine hyperfunction. Sixty-five patients had adenomas causing syndromes of endocrine hypersecretion: 38 prolactinomas (5 men, 33 women), 21 growth hormone (GH)-secreting adenomas (10 men, 11 women), and six adrenocorticotropin (ACTH)-secreting adenomas, of which four were adenomas of Cushing's disease (2 men, 2 women), and two caused Nelson's syndrome (2 women).

For light microscopy the surgical specimens were fixed in buffered liquid 5% formaldehyde immediately after removal and then were embedded in paraffin. Deparaffinized sections (5 μm) were stained with haematoxylin-eosin, Herlant's tetrachrome, and periodic acid-Schiff-orange G.

For electron microscopy biopsy specimens (1–2 mm in diameter) were fixed in 2% osmium tetroxide (direct osmium fixation) or in 3% glutaraldehyde followed by 2% osmium-tetroxide (glutaraldehyde-osmium fixation) according to the technique of Landolt (1975). The thin sections (interference color gold) were stained with uranyl acetate and lead citrate.

The diameter of 1,000 unselected secretory granules was measured on random electron micrographs of glutaraldehyde-osmium fixed tissue with a semiautomatic particle size analyzer.[1] The average granule diameter was calculated.

All immunocytochemical reactions were performed on deparaffinized sections (5 μm) or semithin Epon sections. The Epon was removed from the semithin sections by a saturated solution of sodium hydroxide in absolute ethanol (Erlandsen et al. 1979; Lane and Europa 1965). The unlabeled antibody enzyme method (Sternberger 1979) or the avidin-biotin complex (Guesdon et al. 1979) were used. The primary antisera were: anti-human-ACTH[2] (dilution, 1:500), anti-human-GH[2] (1:500), anti-human-PRL[3] (1:750), anti-human-beta TSH[3] (1:20,000), anti-human-beta-FSH[3] (1:2,000), and anti-human-beta-LH[3] (1:4,000). The antisera to the subunits of human choriogonadotropin[4] (hCG) were generated and tested for specificity as described previously (Heitz et al. 1983; Vaitukaitis et al. 1971). These antisera were used at dilutions of 1:800 (anti-alpha) and 1:1,600 (anti-beta).

The antisera have been controlled by their extensive use in previous studies of more than 300 pituitary tumours; the immunocytochemical results obtained were in good agreement with the endocrinological results of clinical and radioimmunoassay studies. The specificity of immunostaining was determined by liquid phase absorption (24 h at +4° C) of each diluted antiserum with the appropriate antigen at a final concentration of 10^{-6} M. Antisera to the alpha- and beta-subunits of hCG were absorbed with both alpha- and beta-subunits of hCG and with GH (10^{-6} M). Additional control studies included: (1) preabsorption of diluted alpha-hCG-antibody with beta-subunits of TSH, FSH, and LH (10^{-6} M); (2) nonimmune sera as first layer; (3) rabbit IgG as first or third layer, sheep IgG as second layer; (4) omission of diaminobenzidine (DAB) or hydrogen peroxide from the incubation medium for the peroxidase reaction.

Fixation and processing were identical for both the control tissues and the pituitary tumours. The following human tissues were used as control specimens: normal pituitary and lung obtained at autopsy 3–12 hours postmortem; surgical biopsy specimens of normal pancreas and liver, fresh placenta, and hydatidiform moles; and testicular choriocarcinomas obtained at surgery which are known to produce and secrete gonadotropins (serum beta-hCG levels up to 26.5 ng/ml; normal, <1.0 ng/ml).

Results

Endocrine-inactive adenomas

Light microscopy showed solid pituitary adenomas consisting of isomorphous cells (Fig. 1). Four tumours (Tables 1 and 2, Cases 1–4), which showed dispersed, fine, acidophilic granular material in most adenoma cells, were subsequently identified as oncocytomas by electron microscopy. The

[1] TGZ 3, Zeiss, Oberkochen, FRG

[2] Antisera to ACTH and GH are products of Wellcome Diagnostics, Dartford, England

[3] Antisera to PRL and the beta-subunits of TSH, FSH, and LH, alpha- and beta-subunits of hCG, as well as the beta-subunits of THS, FSH, and LH, were kindly provided by the Hormone Distribution Officer of the National Pituitary Agency, Bethesda, Maryland, USA

[4] Antisera to the alpha- and beta-subunits of hCG were the generous gift of Prof. J.L. Vaitukaitis, Boston, Massachusetts, USA

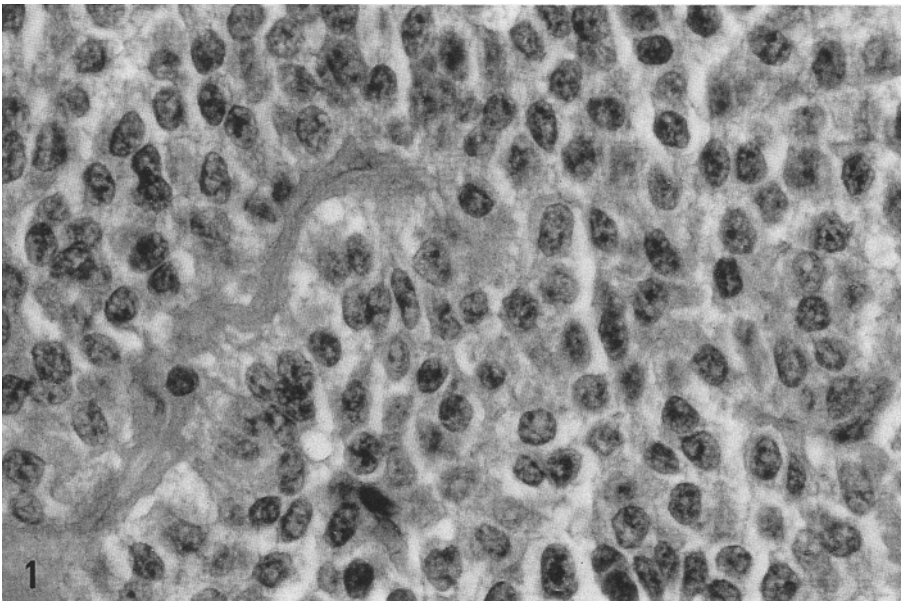

Fig. 1. Light micrograph of pure alpha-subunit-producing adenoma consisting of isomorphous cells with clear cytoplasm. There are only few strands of interposed connective tissue. Case 5, H & E, × 720

Table 1. Immunocytochemical findings in 11 endocrine-inactive pituitary adenomas

Adenoma type	Reaction						
Case	GH	PRL	ACTH	beta-FSH	beta-LH	beta-TSH	alpha-subunit
Oncocytoma							
1	–	–	–	–	–	–	+
2	–	–	–	–	–	–	+
3	–	–	–	–	–	–	+
4	–	–	–	–	–	–	+
Pure alpha-subunit producing adenoma							
5	–	–	–	–	–	–	+
6	–	–	–	–	–	–	+
7	–	–	–	–	–	–	+
Adenoma with hormone production shown by immunostaining only							
8	–	–	–	–	(+)	–	–
9	–	–	–	+	–	–	+
10	–	+	–	–	–	–	+
Adenoma without hormone production							
11	–	–	–	–	–	–	–

– negative reaction; (+) rare positive cell; + positive reaction

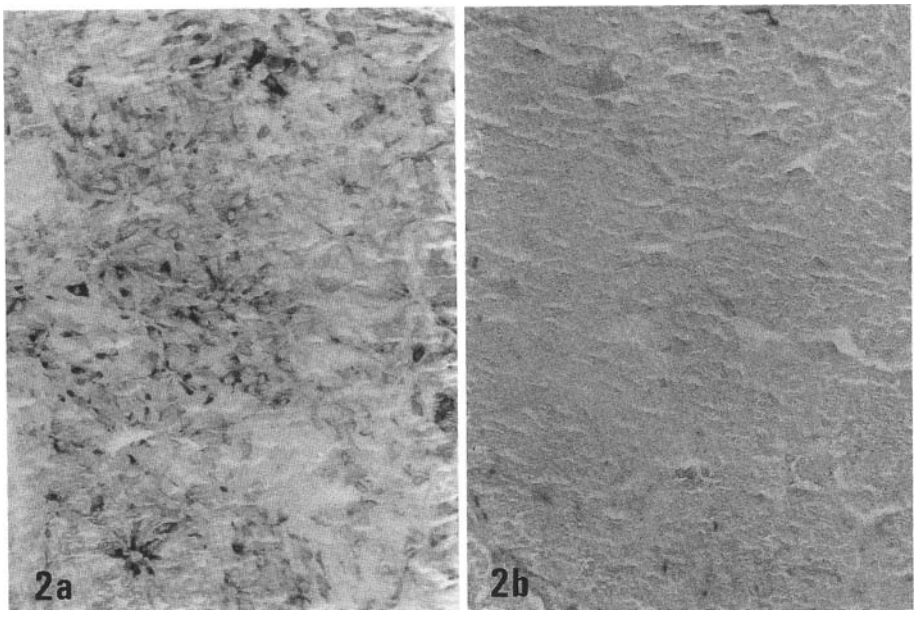

Fig. 2a, b. Alpha-subunit immunoreactivity. **a** Large group of adenoma cells with positive alpha-subunit immunoreactivity. **b** Absence of reaction product in adjacent section after incubation with anti-alpha-subunit serum adsorbed with alpha-hCG (10^{-6} M) before incubation. Case 5, formaldehyde fixation, paraffin embedding, unlabelled antibody method, antibody dilution 1:800, ×125

cytoplasm of the seven remaining tumours (Tables 1 and 2, Cases 5–11), showed only a few cells with a faint acidophilic staining.

Immunohistological analysis showed the following results (Table 1): The four oncocytomas reacted exclusively with anti-alpha-subunit antibodies. Three adenomas reacted exclusively with anti-alpha-subunit antibodies. Large groups of cells containing alpha-chain-immunoreactivity were scattered throughout the tumours (Fig. 2a, b). The immunoreactivity was found in large areas of the tumour cell cytoplasm (Fig. 3). Of the three adenomas with clinically silent hormone production, one reacted with anti-PRL, one with anti-beta-FSH, and one, faintly, with anti-beta-LH. The first two adenomas displayed a positive reaction for the anti-alpha-subunit, whereas in the latter this reaction was lacking. The plasma levels of the hormones visualized immunocytochemically (FSH, LH, PRL) were normal according to preoperative testing in all three patients (Cases 8–10). One adenoma was without detectable hormone production.

Electron microscopy differentiated two types of adenomas: the first, oncocytomas (Cases 1–4) consisting of typical oncocytes with a few dispersed light cells, as described shortly (Kovacs and Horvath 1973; Landolt and Oswald 1973). The rare secretory granules were well preserved after glutaraldehyde-osmium fixation (average diameter, 125–180 nm) but were destroyed by direct osmium fixation (Table 2). The second type were ad-

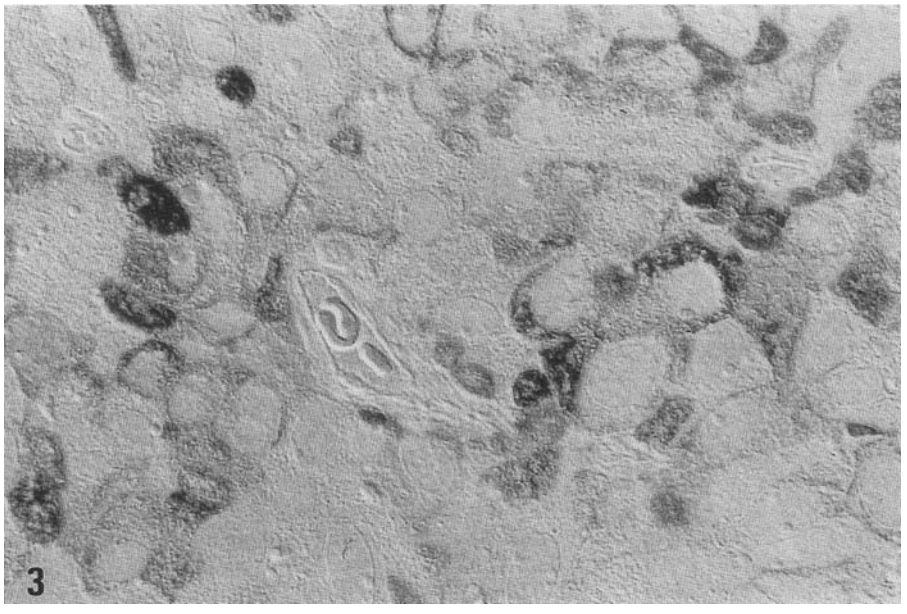

Fig. 3. Alpha-subunit immunoreactivity is confined to the cytoplasm of the adenoma cells. Case 5, glutaraldehyde-osmium fixation, Epon embedding, semithin (2 μm) section, differential intereference contrast optics, × 800

Table 2. Ultrastructural features of 11 endocrine inactive pituitary adenomas

Adenoma type Case	Cell type	Osmium-induced granule destruction	Average diameter of secretory granules (nm)
Oncocytoma			
1	Half of cells oncocytic	+	127
2	Most cells oncocytic	+	129
3	Most cells oncocytic	+	180
4	Most cells oncocytic	+	123
Pure alpha-subunit-producing adenoma			
5	Normal	+	124
6	Normal	+	142
7	Normal	+	160
Adenoma with hormone production shown immunocytochemistry only			
8	Majority normal, few oncocytic cells	+	180
9	Normal	+	142
10	Normal	+	138
Adenoma without hormone production			
11	Normal	+	132

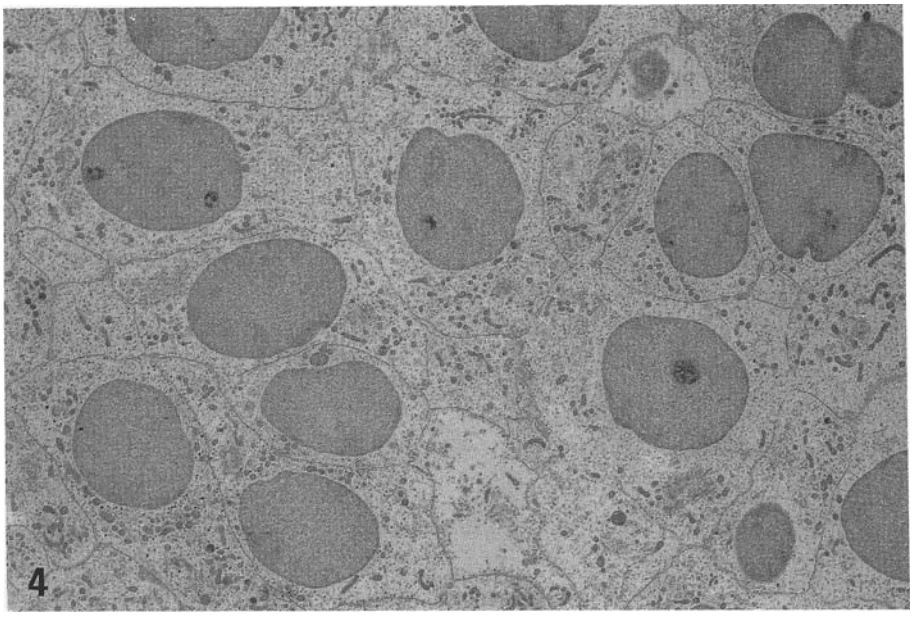

Fig. 4. Low-power electron micrograph of pure alpha-subunit-producing adenoma. The polygonal cells contain round or oval nuclei with few indentations, clearly visible nucleoli, and a light cytoplasm with few organellae. Case 5, direct osmium fixation, × 3,200

enomas (Cases 5–11) consisting of polygonal cells with clear cytoplasm (Fig. 4). The centrally located nuclei usually were round or oval. The cytoplasm appeared more electron-dense after glutaraldehyde-osmium fixation than after direct osmium fixation (Fig. 5a, b). A few normal mitochondria, small Golgi cisterns, and sparse lipid bodies were dispersed in the cytoplasm. The rough-surfaced endoplasmic reticulum was inconspicuous and consisted mainly of a few vesicular cisterns. There was a moderate number of free ribosomes. The secretory granules were electron-dense and round; their average diameter was between 125 and 180 nm in glutaraldehyde-osmium-fixed material (Table 2; Fig. 5b, 6b). Upon direct osmium fixation, only granule "shadows" with ruptured and distorted membranes were left. Their content was finely granular and spilled into the cytoplasm (Figs. 5a, 6a). No sites of granule release could be observed. However, coated pits were observed frequently (Fig. 6a, b); coated pits may indicate granule release immediately preceding fixation (Douglas et al. 1971). Only one biopsy specimen (Case 8) contained few dispersed oncocytes.

Among the 21 GH-secreting adenomas, none showed osmium-induced granule destruction. Eleven of these adenomas reacted with the anti-alpha-subunit (Fig. 7), while only two reacted simultaneously with anti-FSH and anti-TSH (Table 3). Only one of these 11 specimens also reacted with anti-LH. Immunoreactive GH and PRL were found in equal numbers in alpha-subunit-positive and in alpha-subunit-negative specimens.

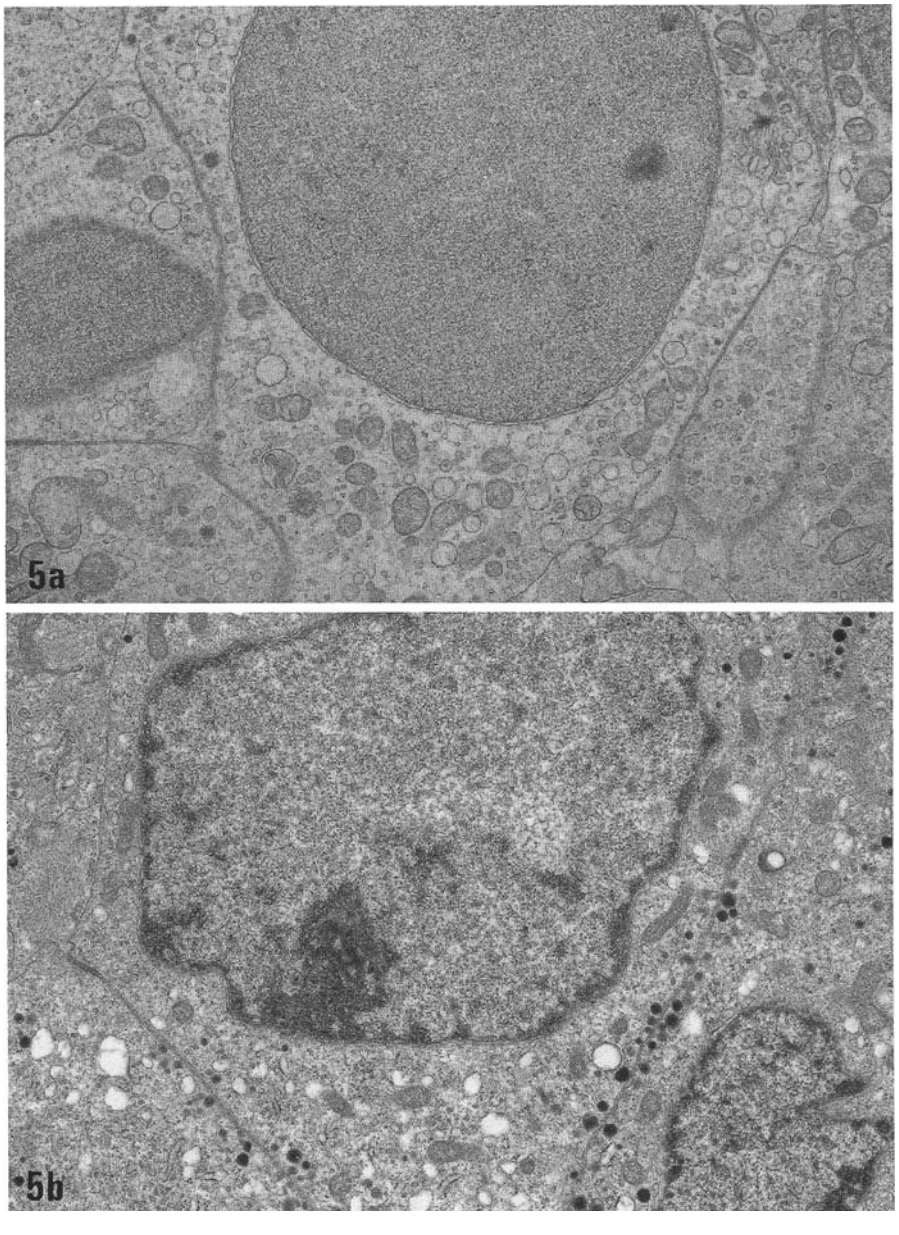

Fig. 5a, b. Electron micrographs of pure alpha-subunit-producing adenoma cells after direct osmium fixation (**a**) and after glutaraldehyde-osmium fixation (**b**). The glutaraldehyde prefixation causes irregular nuclear outlines, coarser chromatin structure, higher electron density of the cytoplasm. It demonstrates a large number of electron-dense secretory granules that are invisible after direct osmium fixation. Case 6, × 11,900

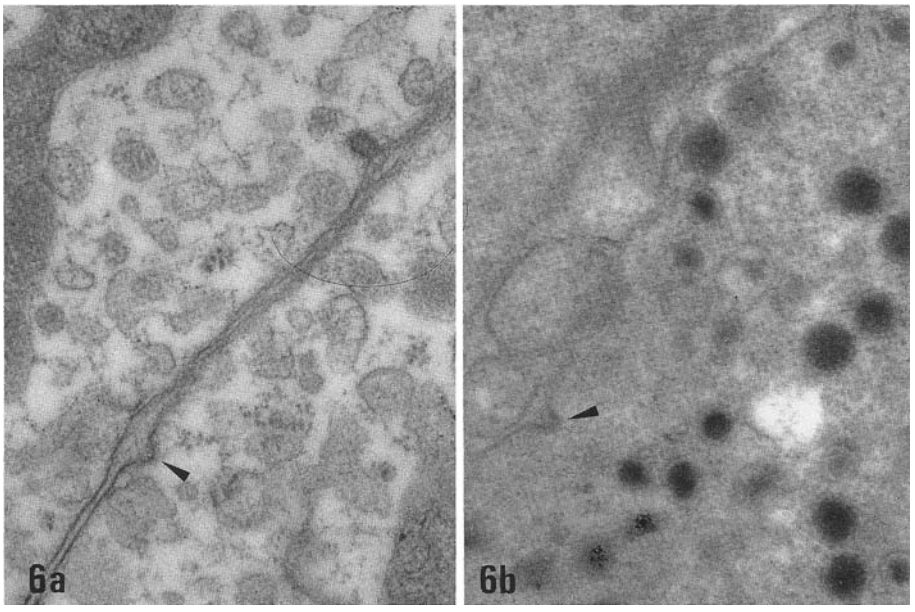

Fig. 6a, b. Detail electron micrograph of a pure alpha-subunit-producing pituitary adenoma cell after direct osmium fixation (**a**) and after glutaraldehyde-osmium fixation (**b**). The glutaraldehyde prefixation preserves the electron dense secretory granules that are destroyed by direct osmium fixation and spill their granular contents into the cytoplasm. Note coated pits (*arrowheads*). Case 7, magnification ×43,600

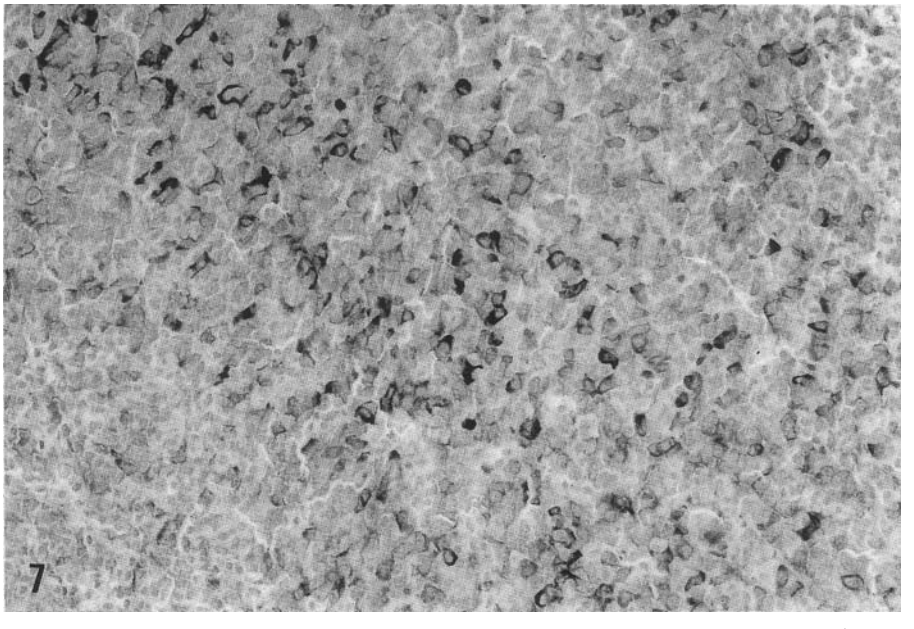

Fig. 7. Alpha-subunit immunoreactivity in a growth hormone-secreting pituitary adenoma. Formaldehyde fixation, paraffin embedding, unlabelled antibody method, antibody dilution 1:800, ×125

Table 3. Immunocytochemical findings in 21 pituitary adenomas causing acromegaly

Positive immunoreactions	Alpha-subunit positive ($N=11$)	Alpha-subunit negative ($N=10$)
GH	9	6
PRL	9	5
ACTH	3	3
FSH	2	0
LH	1	0
TSH	2	0

Table 4. Immunocytochemistry findings in 38 prolactinomas

Positive immunoreactions	Alpha-subunit positive	Alpha-subunit negative
Atypical prolactinomas	$N=2$	$N=1$
GH	0	1
PRL	2	0
ACTH	0	0
FSH	0	0
LH	0	0
TSH	0	0
Typical prolactinomas	$N=5$	$N=30$
GH	1	4
PRL	5	30
ACTH	3	0
FSH	0	0
LH	0	0
TSH	0	0

Only three of the 38 specimens of PRL-secreting adenomas showed osmium-induced granule destruction (Table 4); two of these were obtained from female patients whose preoperative serum PRL levels were 51.5 and 71 µg/l, respectively (normal, <25 µg/l). These two specimens reacted with the anti-alpha-subunit and with the anti-PRL antibody. The third specimen contained neither immunoreactive hormones nor immunoreactive alpha-subunit (preoperative serum PRL value, 107 µg/l).

The other 35 biopsy specimens showed an ultrastructure typical of prolactinomas (Horvath and Kovacs 1980; Landolt 1978, 1980, 1984). The reaction with the anti-alpha-subunit antibody was positive in five and negative in 30 specimens. The five alpha-subunit-positive specimens contained either small groups of cells or single cells reacting with the alpha-subunit antibody. All 35 specimens reacted with anti-PRL and did not react with anti-FSH, with anti-LH, or with anti-TSH. However, three of the five biopsy specimens showed positive reactions with anti-ACTH, and one of these reacted also with anti-GH.

The granules of the pituitary adenomas causing Cushing's disease and Nelson's syndrome are typically destroyed by direct osmium fixation (Landolt 1975, 1978); because their average diameter is usually larger than 200 nm (Landolt et al. 1984) they can be differentiated easily from the granules of the endocrine-inactive adenomas. All six biopsy specimens in this group showed a positive reaction for ACTH. In one tumour, there was also a positive reaction for the anti-alpha-subunit. This adenoma, obtained from a patient who had Nelson's syndrome, reacted also with anti-PRL. Anti-PRL reactions were negative in the other 5 specimens.

All control reactions were negative, with the exception of the immunostaining by anti-alpha-hCG after absorption with the beta-subunits of hCG, the beta-subunits of the other glycoprotein hormones, and with GH. In addition, beta-hCG could not be detected in the immunocytochemical reaction system using alpha-hCG-antibody. In the placenta, hydatidiform moles, and choriocarcinomas of the testis, the anti-alpha-hCG serum stained the same cells (cytotrophoblast and syncytiotrophoblast) as did the anti-beta-hCG serum. In addition, the anti-alpha-hCG serum yielded a positive reaction in a large number of cells located in the central area of the normal anterior pituitary. The same cells were immunostained by the antisera specific for the beta-subunits of glycoprotein hormones. We were not able to detect cells immunoreactive to anti-alpha-hCG or anti-beta-hCG in normal pancreas, lung, and liver.

Discussion

The results of this study confirm the basic assumptions of our proposed subdivision of apparently endocrine-inactive pituitary adenomas. Some considerations are necessary, however. All tumour tissue shows certain variations that may be an intrinsic characteristic of neoplasia or the result of previous treatment. The implications of such minor variations should not be overestimated (Landolt 1984). It must be borne in mind that overclassification may be as great a deterrent to the understanding of tumours as is a lack of classification.

The pure alpha-subunit-secreting adenoma has been accepted as a new clinical entity since elevated serum levels of this hormone subunit were found in patients' serum by radioimmunoassay (Klibansky et al. 1983; Macfarlane et al. 1980; Ridgway et al. 1981). To our knowledge, no extensive immunocytochemical and ultrastructural data regarding this entity have been published to date. The results of our immunohistological evaluations in one subgroup of endocrine-inactive adenomas showed that the adenomas apparently contained only the alpha-subunit. Therefore, we can classify Cases 5–7 of our series as pure alpha-subunit-producing adenomas unless additional, presently unrecognized hormones, hormone fragments, or secretory products are found. The ultrastructure of the three tumours corresponds to that of "undifferentiated adenoma" (Horvath and Kovacs 1980) or of "endocrine-inactive adenomas with signs of secretion" (Landolt 1975, 1978). They apparently cannot be considered truly "undifferentiated", as

they are still sufficiently differentiated to produce an immunologically reactive alpha-subunit. They might represent tumours unable to produce complete glycoprotein hormones or a stage of degeneration occurring in a pituitary adenoma that originally secreted complete, active glycoprotein hormones. A mouse pituitary adenoma cell line secreting only the alpha-subunit which was derived from a cell line secreting the entire TSH molecule has been described recently (Ross et al. 1985). The group of pure alpha-subunit-producing adenomas only partially corresponds to the "argyrophil" pituitary tumours, which comprise not only the pure alpha-subunit-secreting adenomas but also adenomas producing the beta-subunits of glycoprotein hormones (Capella et al. 1983).

The cytoplasm of cells in pure alpha-subunit-producing adenomas contains only a few organells. Their small secretory granules disrupt after direct osmium fixation, but are well preserved after glutaraldehyde prefixation. This phenomenon is not restricted to this type of adenoma, however. It can also be observed in endocrine-inactive adenomas producing additional hormones although not secreting them in amounts elevating the serum levels, and in adenomas in which no alpha-subunit can be detected. It was found in three prolactinomas in this series. Osmium-induced granule disruption, therefore, is merely an indicator – but by no means proof – of a pure alpha-subunit-secreting adenoma. It should be noted that the positive immunohistochemical reaction for PRL in two of the three atypical prolactinomas suggests that the elevated PRL levels in blood resulted from PRL secretion by these two adenomas.

The four oncocytomas in this series (Cases 1–4) produced the alpha-subunit exclusively. Although these tumours might be included among the pure alpha-subunit-producing adenomas they deserve a separate classification on the basis of their peculiar morphology.

Oncocytomas have been considered to be nonsecretory adenomas (Horvath and Kovacs 1973; Landolt 1975, 1978; Landolt and Oswald 1973). The tumours observed in this study clearly synthesized the alpha-subunit. Further endocrinological studies are needed to determine if this substance is ever released in quantities large enough to cause a rise of alpha-subunit levels in the blood. Additionally, isolated oncocytes are found dispersed in many endocrine-active pituitary adenomas, and their presence in large tumours is interpreted as a sign of degeneration (Horvath and Kovacs 1980; Landolt 1980). This suggests the possibility that the alpha-subunit producing oncocytomas might develop from pure, non oncocytic alpha-subunit-producing adenomas. The higher mean age of patients with oncocytomas (64 years) compared with the mean age of patients suffering from non oncocytic, alpha-subunit-producing adenomas (44 years) would correspond with this hypothesis. To substantiate this notion, it is necessary to demonstrate the presence of an increased number of oncocytes in tumours of patients with recurrent pure alpha-subunit-producing adenomas.

The three adenomas characterized by a clinically insignificant hormone production demonstrated only by immunocytochemistry were immunoreactive for beta-LH, beta-FSH, and PRL. Similar adenomas exhibiting positive

reactions with anti-ACTH, anti-beta-MSH (melanocyte stimulating hormone), anti-GH, anti-FSH, anti-LH, anti-PRL, and anti-TSH have been described by others (Girod et al. 1980; Kirsch and Nakane 1973; Kujas et al. 1984; Zimmerman et al. 1974). In no case did these adenomas show clinical or endocrinological evidence of hypersecretion of these hormones. This group of adenomas represents a mixture of tumour types which can be subdivided only by immunohistochemical analyses.

Certain findings in this study emphasize the point that every classification system must admit exceptional cases. The positive reaction with anti-alpha-subunit in Case 9, which reacted also with anti-beta-FSH, is not surprising. It is apparent that the adenoma produced both subunits of this glycoprotein hormone. The negative reaction for the alpha-subunit in the presence of beta-LH in Case 8 might be explained by the presence of very small amounts of the alpha-subunit not detectable by the technique used in these investigations. The presence of both PRL and the alpha-subunit in case 10 was unexpected. The adenoma corresponds to prolactinomas producing PRL and the alpha-subunit; however, in this case, PRL is released in amounts too small to elevate the serum PRL levels.

In one adenoma (Case 11) no known pituitary hormone or subunit could be detected, although the electron microscopic observations showed formation and release of secretory products. The findings might be explained as follows: firstly, the adenoma may secrete a hormone fragment devoid of epitopes reacting with our antibodies and/or secondly the adenoma produces and stores a known hormone or hormone fragment in quantities to small to be detected either in the patient's blood or in the tumour tissue.

Coproduction of the alpha-subunit and the nonglycoprotein pituitary hormones ACTH and PRL is apparently rare. It was observed in only one-fifth to one-sixth of the tumours of each group. Its presence in one of the atypical prolactinomas demonstrates the limitations of a purely ultrastructural classification of pituitary adenomas. To obtain a valid classification, with ultrastructural distinction, electron microscopy and immunocytochemical criteria must be applied simultaneously.

Coproduction of the alpha-subunit and GH, in contrast, was found in about half of the adenomas causing acromegaly. There were no detectable ultrastructural differences between adenomas with or without alpha-subunit production. This finding corresponds with the observation of Macfarlane (1980), who noted an elevated serum concentration of the alpha-subunit 13 of 46 acromegalic patients. Multihormonal adenomas causing acromegaly apparently have a tendency to coproduce the alpha-subunit also; although this tendency cannot be explained as yet, the possibility of a cross-reaction has been excluded by our control experiments.

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References

Capella C, Buffa R, Usellini L, Frigerio B, Jehenson P, Sessa F, Solcia E (1983) Alpha and beta subunits of glycoprotein hormones in argyrophil pituitary tumors with small granule cells. Ultrastruct Pathol 4:35–50

Douglas WW, Nagasawa J, Schulz R (1971) Electron microscopic studies on the mechanism of secretion of posterior pituitary hormones and significance of microvesicles (synaptic vesicles). In: Heller H, Lederis K (eds) Subcellular organization and function of endocrine tissues. Cambridge University Press, Cambridge, pp 353–377

Erlandsen PL, Parsons JA, Rodning CB (1979) Technical parameters of immunostaining of osmicated tissue in epoxy sections. J Histochem Cytochem 27:1286–1289

Girod C, Mazzuca M, Trouillas J, Tramu G, Lhéritier M, Beauvillain J-L, Claustrat B, Dubois M-P (1980) Light microscopy, fine structure and immunohistochemistry studies of 278 pituitary adenomas. In: Derome PJ, Jedynak CP, Peillon F (eds) Pituitary adenomas: biology, physiopathology and treatment. Asclepios Publishers, France, pp 3–18

Guesdon JL, Ternynck T, Avrameas S (1979) The use of avidin-biotin interaction in immunoenzymatic techniques. J Histochem Cytochem 27:1131–1139

Heitz PhU, Kaspar M, Klöppel G, Polak JM, Vaitukaitis JL (1983) Glycoprotein-hormone alpha-chain production by pancreatic endocrine tumors: A specific marker of malignancy. Immunocytochemical analysis of tumors of 155 patients. Cancer 51:277–282

Horvath E, Kovacs K (1980) Pathology of the pituitary gland. In: Ezrin C, Horvath E, Kaufmann K, Kovacs K, Weiss MH (eds) Pituitary diseases. CRC Press, Boca Raton, Florida, pp 1–83

Kirsch WM, Nakane PK (1973) A hitochemical examination of pituitary adenomas with enzyme-labelled antibodies. In: Carrea R, Ishii S, Le Vay D (eds) 5th Int Congr Neurol Surg – Int Congr Series, vol 293. Excerpta Medica, Amsterdam, p 35

Klibansky A, Ridgway EC, Zervas NT (1983) Pure alpha subunit-secreting pituitary tumors. J Neurosurg 59:585–589

Kovacs K, Horvath E (1973) Pituitary "chromophobe" adenoma composed of oncocytes. A light and electron microscopic study. Arch Pathol (Chicago) 95:235–239

Kujas M, Drouet Y, Pleau-Varet J, Racadot J (1984) Immunocytology of "silent" pituitary adenomas, pathology and incidence. In: Lamberts SWJ, Tilders FJH, van der Veen EA, Assies J (eds): Trends in diagnosis and treatment of pituitary adenomas. Free University Press, Amsterdam, pp 257–266

Landolt AM (1975) Ultrastructure of human sella tumors. Acta Neurochir (Wien) suppl 22:1–167

Landolt AM (1978) Progress in pituitary adenoma biology: Results of research and clinical application. In: Krayenbühl H (ed) Advances and technical standards in neurosurgery, vol 5. Springer, Wien, pp 3–49

Landolt AM (1980) Biology of pituitary adenomas. In Faglia G, Giovanelli MA, MacLeod RM (eds) Pituitary microadenomas. Academic Press, London pp 107–122

Landolt AM (1984) Secreting pituitary adenomas: Morpohological and biological aspects. In: Camanni F, Müller EE (eds) Pituitary hyperfunction. Raven Press, New York, pp 135–155

Landolt AM, Oswald UW (1973) Histology and ultrastructure of an oncocytic adenoma of the human pituitary. Cancer 31:1099–1105

Landolt AM, Rothenbühler V, Kistler GS (1978) Morphology of chromophobe adenoma. In: Fahlbusch R, von Werder K (eds) Treatment of pituitary adenomas. G Thieme, Stuttgart, pp 154–171

Landolt AM, Schäfer B, Gammert Ch (1984) Die hypophysäre Adenomektomie beim Morbus Cushing und beim Nelson-Syndrom. In: Hauri D, Schmucki O (Herausg) Erkrankungen von Nebenschilddrüsen und Nebennieren. G Fischer Verlag, Stuttgart, pp 179–194

Lane BP, Europa DL (1965) Differential staining of ultrathin sections of Epon-embedded tissues for light microscopy. J Histochem Cytochem 13:579–582

Macfarlane IA, Beardwell CG, Shalet SM, Darbyshire PJ, Hayward E, Sutton ML (1980) Glycoprotein hormone alpha subunit secretion by pituitary adenomas: Influence of external irradiation. Clin Endocrinol 13:215–222

Pierce JG (1971) The subunits of pituitary thyrotropin – their relationship to other glycoprotein hormones. Endocrinology 89:1331–1344

Ridgway EC, Klibanski A, Ladenson PW, Clemmons D, Beitins IZ, McArthur JW, Martorana MA, Zervas NT (1981) Pure alpha-secreting pituitary adenomas. N Engl J Med 304:1254–1259

Ross DS, Kieffer JD, Shupnik MA, Ridgway EC (1985) Pure alpha-subunit producing tumor derived from a thyrotropic tumor: impaired regulation of alpha-subunit and its mRNA by thyroid hormone. Mol Cell Endocrinol 39:161

Sasaki R, Misumi S, Fujii T, Takeda F, Yamamoto K (1975) Quantitative measurement of the ability of human chromophobe adenomas to synthesize and release human growth hormone. Neurol Med Chir (Tokyo) 15:7–11

Sternberger LA (1979) Immunocytochemistry, 2nd ed. John Wiley, New York, pp 1–354

Vaitukaitis JL, Robbins JB, Nieschlag E, Ross GT (1971) A method for producing specific antisera with small doses of immunogen. J Clin Endocrinol Metab 33:988–991

Vaitukaitis JL, Ross GT (1974) Subunits of human glycoprotein hormones. Isr J Med Sci 10:1280–1287

Zimmerman EA, Defendini R, Frantz AG (1974) Prolactin and growth hormone in patients with pituitary adenomas: A correlative study of hormone in tumor and plasma by immunoperoxidase technique and radioimmunoassay. J Clin Endocrinol Metab 38:577–585

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