Mono- and plurihormonal thyrotropic pituitary adenomas: pathological, hormonal and clinical studies in 12 patients

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Abstract
In a series of 12 patients (eight women and four men, aged between 20 and 62 years), operated on for a pituitary adenoma shown to be thyrotropic by immunocytochemistry, we performed a retrospective and comparative analysis of clinical and biological data, tumor studies including immunocytochemistry with double labeling, and proliferation marker (proliferative cell nuclear antigen (PCNA) and Ki-67) detection, electron microscopy and culture. Our study leads us to confirm that thyrotropic tumors are rare (12 of 1174 pituitary adenomas: 1%). The main points arising were that: (1) high or normal plasma TSH associated with an increase in plasma α-subunit and high thyroid hormone levels is the best criterion for diagnosis; (2) the failure of TSH to respond to TRH or Werner’s test is not a reliable criterion for diagnosis; (3) thyrotropic adenomas may be ‘silent’, without clinical signs of hyperthyroidism and with only slight increase in TSH, tri-iodothyronine and thyroxine concentrations; (4) mitoses and nuclear atypies are frequently detected in large tumors, which are invasive in more than 50% of cases – the first analysis of two proliferation markers (PCNA and Ki-67) bears out the relative aggressiveness of thyrotropic adenomas; (5) thyrotropic adenomas are frequently plurihormonal. Immunocytochemical double labeling, complemented by in vitro study, showed that thyrotropic tumor cells sometimes can or sometimes cannot cosecrete TSH, GH or prolactin. The pathological identification of monohormonal and plurihormonal adenomas seems to be supported by clinical and biological differences.

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Introduction
Thyrotropic pituitary adenomas are true neoplasias, to be distinguished from simple thyrotropic cell hyperplasia occurring in reaction to a neglected, usually congenital, hypothyroidism. They are rare tumors, with a prevalence rate of only 1:1 000 000 in the population as a whole, and represent between just 0.2 and 2.8% of all pituitary adenomas (1–4). Two reviews of the literature documented 69 cases in 1987 (5) and 280 cases 10 years later (6). Only three series of fewer than 20 cases have previously been described (3, 4, 7). Diagnosis has often been late – sometimes, indeed, made only on anatomopathological examination.

These are often large, radiologically invasive tumors. Only 28 cases of microadenomas have been reported (6, 8). It is difficult to say whether this is due to diagnostic delay or to the peculiar aggressiveness of these tumors; there has not yet been any analysis of tumor proliferation markers. Finally, some 30% of cases in the clinical series involve associated secretion of other hormones, notably growth hormone (GH) and prolactin (PRL) (9). This is in agreement with findings from anatomopathological series, in which thyrotropic adenomas have frequently proved plurihormonal (2).

We report here a retrospective study involving 12 thyrotropic adenomas from an anatomopathological series of 1174 pituitary tumors (Laboratoire d’Histologie et Embryologie Moléculaires, Lyon). Analysis of two proliferating markers, proliferative cell nuclear antigen (PCNA) and Ki-67, bears out the relative aggressiveness of thyrotropic adenomas. The immunocytochemical identification of monohormonal and plurihormonal thyrotropic adenomas seems to be supported by clinical and biological differences. Double-labeling techniques complemented by in vitro study show that the thyrotropic tumor cell sometimes can and sometimes cannot cosecrete thyrotropin (TSH), GH or PRL.

Patients and methods

Patients
Between 1971 (10) and 1996, 12 cases of pituitary adenoma were immunocytochemically shown to be
Hormone measurements

Hormone measurements were performed in the laboratories of each institution using standard radioimmunoassay (RIA) or immunoradiometric assay (IRMA) methods. In view of the heterogeneity of the techniques, TSH concentrations are expressed as percentages of the upper limit of normality. For the other hormones, normal ranges were as follows: free triiodothyronine (fT₃) 3.4–7.2 pmol/l; total triiodothyronine (tT₃) 0.85–2.3 nmol/l; free thyroxine (fT₄) 11–23 pmol/l; total thyroxine (tT₄) 53–153 nmol/l; PRL < 22 mIU/l; anti-hPRL m (164–22–12) and anti-hFSHm (11, 12).

Tumor studies

All the adenomas were studied by light and electron microscopy and immunocytochemistry.

For light microscopy, pieces of tumor tissue were fixed in Bouin–Hollande–Sublimate for 4 days and embedded in paraffin. Sections 5 µm thick were stained with Herlant’s tetrachrome and periodic acid–Schiff–orange G methods.

Tumor type was identified by immunocytochemistry. Serial sections were treated by the indirect immuno-peroxidase method with streptavidin–biotin–complex (Dako A/S, Copenhagen, Denmark), as described previously (13). The following monoclonal (m) and polyclonal (p) antibodies were used: anti-β-follicle-stimulating hormone (anti-β-hFSHm; 300–10-E-14–3), anti-hPRLm (164–22–12) and anti-α-subunit (anti-α-hSUm; 326–2–1; lot f 1079) (Immunotech, Marseille, France), anti-hGHp and anti-β-luteinizing hormone (anti-β-LH₃p) (donated by Dr A F Perlow, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases (NIDDK)), and anti-β-hTSHm (Dako). The dilutions used were 1/200 to 1/500 for the monoclonal antibodies and 1/1000 to 1/10 000 for the polyclonal antibodies and anti-α-hSUm.

To study the colocalization of TSH, GH and PRL, double labelings were performed in the five plurithyrotrophic adenomas. Two antibodies directed against human hormones, raised in different species, were tested by indirect immunofluorescence. The sections were first treated with mouse first primary antibody, then by biotinylated anti-mouse IgG (Dako, E433, lot No. 093) and by tetramethyl rhodamine isothiocyanate (TRITC)-conjugated streptavidin (Jackson, West Grove, PA, USA, O16–2–084, lot No. 32 008). A second immunostaining was carried out with rabbit second primary antibody followed by goat anti-rabbit IgG conjugated to fluorescein isothiocyanate (FITC; Jackson, 111–095–006, lot No. 30 816). The first primary antibody was polyclonal anti-hGH in TSH–GH adenomas and monoclonal anti-β-hTSH in TSH–PRL adenomas. Control experiments showed that the various immunoreagents did not crossreact; in particular, the specificity of anti-α-hSUm and anti-β-hTSHm antibodies was assessed by liquid-phase absorption tests with α-hFSH antigen (NIDDK) and β-TSH antigen (NIDDK). Extinction of the reactions was obtained with α-hFSH (0.012 µg/ml) for anti-α-hSUm diluted 1/10 000 and β-hTSH (0.05 µg/ml) for anti-β-hTSH diluted 1/200. No modification of the reaction was observed when anti-β-hTSH antibodies were preabsorbed with α-hFSH (0.025 µg/ml).

The immunocytochemical detection of proliferation markers PCNA and Ki-67 was performed on paraffin sections with anti-Ki-67 (MIB 1, Immunotech) and anti-PCNA (lot No. 515, Tebu Novocastra, Newcastle upon Tyne, UK) at a dilution of 1/400. For detection of Ki-67, the sections were heated in a microwave oven in 10 mmol/l citric acid (pH 6.0) at 750 W for three 5-min cycles and were blocked in 0.5% H₂O₂/methanol for 10 min. To determine the Ki-67 and PCNA labeling index, cell counts were performed at high power (×400). Five high-power fields were enumerated for each tumor. An average of approximately 1000 nuclei were evaluated in each specimen. The Ki-67 and PCNA labeling indexes were expressed as percentages of labeled nuclei.

For electron microscopy, tissues were fixed in 2% glutaraldehyde in 0.1 mol/l cacodylate buffer, postfixed in 2% osmium tetroxide, and embedded in araldite. Ultrathin sections were contrasted with uranyl acetate and lead citrate, and examined with a Jeol-type 1200 EX electron microscope.

In vitro studies were performed on three tumors (Nos 8, 9 and 11). They were cultured as explants in medium 199 with 20% fetal calf serum. Culture medium was renewed twice a week. From these tumors, dispersed thyrotrophic. This series comprises eight women and four men aged between 20 and 62 years. Except in one case, transsphenoidal pituitary surgery was indicated by radiological evidence of a pituitary tumor. Four of the cases (Nos 1, 2, 3 and 11) have already been published (11, 12).
cells were also cultured in medium containing $T_3$ (46.2 nmol/l) and $T_4$ (38.7 nmol/l) and in medium without $T_3$ and $T_4$. Hormone release was measured over a 72-h period between days 5–8 and days 12–15 and under TRH administration ($10^{-7}$ mol/l). Pituitary hormones were assayed in culture medium by RIA. TSH was measured with a commercial kit (International CIS, CEAS, gif sur Yvette, France). $\alpha$-Subunit was measured using a commercial antibody raised against the common $\alpha$-subunit of glycoprotein hormones (Valbiotech, Paris, France) or anti-$\alpha$hFSH (donated by NIDDK).

**Results**

Thyrotropic adenoma frequency in our anatomopathological series was 1%; 12/1174.

**Clinical data (Table 1)**

All patients presented radiologically proven pituitary tumors. Only two (Nos 3 and 10) were microadenomas. The 10 macroadenomas measured less than 20 mm in diameter. In seven cases, the tumor was radiologically invasive with sellar floor erosion, lateral or suprasellar expansion, or both. Eight showed hyperthyroidism, without exophthalmos, with four cases of associated goiter (Nos 1, 8, 9 and 11). Four patients had been treated for 3 months to 11 years for hyperthyroidism, by subtotal thyroidectomy (Nos 1 and 11) or antithyroid drugs (Nos 3 and 10) which were stopped at the time of the evaluation. The other patients had intact thyroids.

Five patients showed signs of pituitary tumors: headaches (Nos 1 and 2) or altered visual field (Nos 4, 5 and 6). Secondary amenorrhea (Nos 1, 5 and 8), galactorrhea (Nos 4 and 8) and one case of acromegaly (No. 12) were also noted. To relate the severe osteoporosis of a 62-year-old woman (patient No. 7) with possible hyperthyroidism, a plasma TSH assay was performed. High TSH levels with a FT$_4$ value of 20.3 pmol/l suggested a pituitary tumoral secretion.

After pituitary adenomectomy of the eight patients with adequate follow-up, four of them (Nos 1, 5, 7 and 8) were cured recording with respect to normalization of basal TSH, T$_3$, T$_4$ and $\alpha$-subunit concentrations. Somatostatin analog treatment was prescribed for one patient (No. 9) who had failed to respond to other treatments.

**Biological data (Table 2)**

Only one of our patients (No. 5) had normal thyroid function. The others could be classified in one of three groups: high concentrations of TSH without low values of T$_3$ and T$_4$ (Nos 2 and 7); high concentrations of T$_3$, T$_4$, or both, associated with a high TSH concentration (Nos 1, 3, 4, 9 and 11); high concentrations of T$_3$, T$_4$, or both, associated with a TSH concentration which, while

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† Determined on histological criteria, bone erosion, extrasellar extension, or combination thereof.
within usual limits for a normal subject, was inappropriate in the present patients (Nos 6, 8, 10 and 12). In the two patients in whom it was studied (Nos 7 and 9), the nycthemeral TSH rhythm had been lost, but the pulsatile secretion preserved. Treatment with octreotide, by subcutaneous infusion, restored the TSH cycle and normalized the thyroid hormone concentrations (Fig. 1).

After 30 or 45 min, TRH injection increased plasma TSH concentrations in four patients: by 53% in patients Nos 1, 2 and 10, and by 67% in patient No. 6. Three had pure thyrotropic adenomas and one a thyroprolactin secretory adenoma. The TRH test was ineffective in seven patients (Nos 3, 4, 7, 8, 9, 11 and 12); three had pure thyrotropic adenomas and four had plurihormonal thyrotropic adenomas. A Werner’s test performed in seven patients gave various results. A complete inhibition of basal TSH was recorded for only one patient (No. 4). In two patients (Nos 1 and 8) the percentage of inhibition was 30%; in the others, there was no inhibition.

Circulating α-subunit concentrations were high in all six patients examined. The α-subunit/TSH ratio was high in four of the six cases as judged by the criteria of normality described by Beck Peccoz et al. (6).

Five patients showed hyperprolactinemia in the range 23.2–142 mg/l (Nos 2, 4, 5, 8 and 9). All except one (No. 2) had increased PRL concentrations in response to TRH injection. The plasma GH concentration in response to the oral glucose tolerance test was high (4.7 mg/l), as was that of insulin-like growth factor I (5.68 mg/l), in the one acromegalic patient (No. 12). The plasma GH concentration of patient No. 11 was normal (2.6 mg/l) and decreased in response to the oral glucose tolerance test.

**Pathological studies (Table 3)**

Calcifications in the form of pituitary stones and microcalcifications were observed in one tumor (No. 9) and fibrosis was present in eight tumors (Nos 1, 3, 4, 6, 7, 8, 10, 11, and 12). In the two patients in whom it was studied (Nos 7 and 9), the nycthemeral TSH rhythm had been lost, but the pulsatile secretion preserved. Treatment with octreotide, by subcutaneous infusion, restored the TSH cycle and normalized the thyroid hormone concentrations (Fig. 1).

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An invasion of the dura matter or of the juxtatumoral pituitary was histologically proved in two patients (Nos 4 and 9). The tumor cells were organized in cords or sometimes in a diffuse arrangement. They appeared large and polymorphous. Under Herlant’s tetrachrome, they were agranular with a basophilic reaction underlining the cell membrane (Fig. 2a). The nuclei were large and the nucleoli prominent. Nuclear atypies were observed in eight of 12 tumors. Mitoses were present in four tumors. Mitoses were present in four tumors.

Proliferating cell indexes as determined by PCNA and Ki-67 antibodies (Fig. 2b) ranged respectively from 0 to 37% (mean 13.6%) and from 0 to 3.8% (mean 0.8%). Mitoses were correlated with Ki-67 indexes greater than 0.7%. The oldest tumors of our series were unsuitable for measurement of proliferating marker indexes.

More than 50% of cells were immunoreactive with anti-βhTSH antibodies (Fig. 2c). The cells were positive with anti-αhSU and anti-βhTSH antibodies except for tumor No.5, which was reactive only to an anti-βTSH antibody; the percentage of immunoreactive cells with anti-α-hSU antibodies was always greater than that of those with anti-βTSH antibodies. Five tumors were plurihormonal: three TSH–PRL (Nos 8, 9 and 10) and two TSH–GH (Nos 11 and 12) adenomas. The percentage of PRL- or GH-immunoreactive cells was always equal or lower than that of TSH-immunoreactive cells. Immunocytochemistry on contiguous sections and with a double-labeling technique revealed a colocalization of PRL and TSH in the three TSH–PRL tumors (Fig. 2d). Of the two TSH–GH tumors, a colocalization of GH and TSH was demonstrated in one.

Two types of cell were found in the other tumor (No. 11). In plurihormonal TSH adenomas, a colocalization of TSH and PRL or TSH and GH was not observed in all cells; indeed, many secreted GH, PRL or TSH alone.

Under electron microscopy (Fig. 3), in all the pure adenomas, the secretory granules were round, without variation in size, shape or electron density. They were small (60–100 nm in diameter; mean diameter 80 nm) and were scattered over the cytoplasm of densely granulated cells or lining the membrane in sparsely granulated ones. The rough endoplasmic reticulum was poorly developed. In contrast, the Golgi complexes were large. In one TSH–GH adenoma (No. 11), two types of cell were observed: thyro trope cells and round somatotrope cells, orangeophile under Herlant’s tetrachrome and with numerous secretory granules, measuring 150–300 nm in diameter under electron microscopy. In the other plurihormonal TSH adenomas, the light and ultrastructural appearances were monomorphous.

In the three tumors (Nos 8, 9 and 11) studied in vitro, basal TSH secretion was increased, ranging from 3 to 45 μU/day per 2 mg tissue. PRL release rate was high (25.4–68.8 ng/day) for tumors Nos 8 and 9, but undetectable in tumor No. 11. GH release rate was low in tumors Nos 8 and 9, and reached 100 ng/day in tumor No. 11. No other pituitary secretion was detected, confirming the absence of contamination by normal cells.

There was some difference between the in vivo and in vitro response of TSH secretion to dynamic tests: TRH did not stimulate any TSH release, but T3 inhibited TSH release for cells of culture No. 9, in contrast to the Werner’s test in vivo.

**Discussion**

The present series of thyrotropic adenomas operated on from 1971 and 1996, short as it is, is nevertheless one of the largest published to date, which emphasizes how rare this pathology is. It also confirms the difficulty of diagnosis. Misdiagnoses are mainly among the older patients; the advent of systematic TSH assaying and modern imaging techniques should limit errors.

With one exception, our patients showed either high serum TSH concentrations without hypothyroidism, or high thyroid hormone concentrations without reduced...
serum TSH – that is, an inappropriate thyrotropic secretion. This inappropriate secretion, associated with an increase in circulating α-subunits and an easily detectable pituitary adenoma, secures a diagnosis of thyrotropic adenoma. Unfortunately, because the analysis was retrospective, these data were available in only six cases.

Conversely, in agreement with previous findings, failure to respond to a TRH or Werner’s test is not a sufficiently reliable criterion for diagnosis (3, 14). Persistent secretory pulsatility and altered nycthemeral TSH rhythm, seldom analysed (6), are confirmed by these tests. Administration of somatostatin analog restores a normal TSH rhythm.

In this pathological series, there was no correlation between plasma TSH concentration and thyroid hormone concentrations. In one case, normal values of TSH, T₃ and T₄ hormone may be related to the detection of only βTSH subunits in the tumor cells. In the other cases with an increase in TSH concentrations without an increase in T₃ and T₄, we suggest that TSH is not biologically active. Indeed, TSH is known for its polymorphism (15, 16), with many isoforms due to the heterogeneity of the oligosaccharide chains fraction. Thus the bioactivity/immunoactivity ratio of the released TSH may be normal, reduced (17, 18) or even increased (19), while the intratumor TSH resembles that in a normal pituitary (16). Taken together, these data suggest some inequality in rates of release of the various isoforms. Moreover, we can also suggest that, as is the case for gonadotropin adenomas (20), the TSH secretion of tumoral cells is too low to induce biological or clinical
signs of hyperthyroidism. These tumors could be considered as 'silent' thyrotropic adenomas.

Inside these large tumors, 50% of which exceeded 15 mm in diameter and which were radiologically invasive in more than 50% of cases, histological examination confirmed that nuclear atypies and mitoses were frequent, as has been noted previously (2, 11). Mitoses alone proved insufficient to evaluate the potential proliferation of the pituitary adenomas. Immunocytochemical study of Ki-67 and PCNA enables proliferating cells to be identified. In the present study, these markers were more frequently detectable than in all pituitary adenomas (21), and the PCNA and Ki-67 values were in agreement with each other and with the existence of mitosis. These two markers are still being assessed. According to some authors (22), tumor size and PCNA immunoreactivity correlate. For others, Ki-67 is a more reliable marker, correlating well with proliferation potential and invasiveness (23–25). Recent studies have shown a relation between invasive, bromocriptine-resistant macroprolactinomas in males and positive Ki-67 results (26). The predictive value of PCNA for recurrence and prognosis, although accepted by some (22), needs further confirmation, especially as no difference has yet been found between pituitary adenomas and carcinomas.

The peculiar invasive and evolutive potential of thyrotropic tumors has, as yet, no pathogenic explanation. Only one carcinoma has been reported. The search for a specific oncogenic agent for TRH receptor mutation or for dopamine receptor subtype 2 deactivation has so far been fruitless (27). Likewise, no mutation of the TR group of thyroid hormone receptors has been found (6) and, among the reported cases of TRβ1 mutation, it is notable that no particular incidence of pituitary tumors has been shown. Of the present 12 thyrotropic tumors, five were plurihormonal. Only in one case did clinical examination enable such a diagnosis to be made preoperatively: in the other four cases, the diagnosis was arrived at biologically or immunocytochemically. Scheithauer et al. (28) have already referred to this absence of clinical testimony for associated secretions. Culture and immunocytochemistry revealed that the secretions most frequently associated with TSH are PRL and GH (3, 6). The detection of GH and PRL in culture media proves that these hormones are being secreted; however, only one GH–TSH plurihormonal adenoma exhibited increased plasma concentrations of GH, and this may have been related to a gonadotropin adenoma without an increase in plasma FSH or LH (20). In four cases out of five,
colocalization of two hormones in one cell was discovered by the double-labeling method. Other less frequently associated secretions (FSH and LH) were not detected in the present series (29). In one case, on both light and electron microscopy, two separate cell populations appeared to underly the double secretion. In the other tumors, secretion colocalization was accompanied by a monomorphic cellular appearance under the electron microscope, as reported elsewhere in the literature (2, 28). Colocalized TSH–GH or TSH–PRL secretion and the monomorphic cellular appearance found in most tumors suggests that some multipotent cell underlies the tumor process (18). One tumor was examined for expression of Pit-1 by in situ hybridization (30). Pit-1 mRNA associated with TSH and PRL RNA appeared in the tumor cells and was expressed only in tumors secreting TSH, GH or PRL, and not in the corticotropic or gonadotropin adenomas studied. No Pit-1 mutation was found in these tumors (31). One possible explanation of the pathogenesis of plurihormonal thyrotropic adenomas could be overexpression of Pit-1 or the alternative gene splicing product, associated with defective inhibition of GH and PRL expression (32). Some authors (18) have considered these plurihormonal thyrotropic adenomas to be less differentiated tumors. However, they show reduced proliferation potential without any significant difference in time of diagnosis or tumor volume.

Histological findings set against clinical and biological findings lead us to suggest two varieties of thyrotropic adenoma:

(1) Monohormonal thyrotropic adenomas, usually revealed by radiologically invasive pituitary tumor symptoms. High thyroid hormone concentrations tend to be lacking. The thyrotropic response to TRH is often preserved. The cellular atypies and mitoses are numerous and proliferation markers are generally positive.

(2) Plurihormonal thyrotropic adenomas are always revealed by clinical and biological hyperthyroidism, usually associated with loss of thyrotropic response to TRH. Histological proliferation criteria tend to be lacking.

We are aware that this retrospective series is too small to allow us to assess the reality of two anatomoclinical forms and fails to include a later follow-up that could determine the evolutive potential. Even so, thyrotropic adenomas show such invisiveness and high proliferative capacities that we advocate surgical action as early as possible.

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