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J Clin Invest. 1987;80(5):1472-1478. https://doi.org/10.1172/JCI113228.

Research Article

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Glycoprotein Hormone Genes Are Expressed in Clinically Nonfunctioning Pituitary Adenomas

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Abstract

Approximately 25% of patients with pituitary adenomas have no clinical or biochemical evidence for excess hormone secretion and are classified as having null cell or nonfunctioning adenomas. To characterize the cell type of these tumors, we analyzed pituitary hormone gene expression in clinically nonfunctioning pituitary adenomas using specific oligonucleotide probes for the messenger (m)RNAs encoding growth hormone, prolactin, ACTH, and the glycoprotein hormone subunits, α, luteinizing hormone (LH)β, follicle-stimulating hormone (FSH)β, and thyroid-stimulating hormone (TSH)β. Expression of one or more of the anterior pituitary hormone genes was found in $^{12}/_{14}$ (86%) of the patients with clinically classified nonfunctioning adenomas. Expression of one or more of the glycoprotein hormone genes (α, LHβ, FSHβ, TSHβ) was identified most commonly (79%) with expression of multiple β-subunit genes in many cases. Expression of α-subunit mRNA was found in each of the adenomas from patients expressing one of the β-subunit mRNAs and in three patients with no detectable β-subunit mRNA. Although FSHβ and LHβ mRNAs were found with similar frequencies in nonfunctioning adenomas, expression of FSHβ mRNA was generally much more abundant. TSHβ mRNA was detected in only one adenoma. The levels of glycoprotein hormone subunit mRNAs were variable in different adenomas, but the lengths of the mRNAs and transcriptional start sites for the α- and β-subunit genes were the same in the pituitary adenomas and in normal pituitary. Growth hormone and prolactin gene expression were not observed in the nonfunctioning adenomas, but ACTH mRNA was found in a single case. Immunohistochemistry of the adenomas confirmed production of one or more pituitary hormones in $^{13}/_{14}$ (93%) nonfunctioning tumors, with a distribution of hormone production similar to that of the hormone mRNAs. These data indicate that pituitary adenomas originating from cells producing glycoprotein hormones are common, but are difficult to recognize clinically because of the absence of characteristic endocrine syndromes and defective hormone biosynthesis and secretion.

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Received for publication 6 February 1987 and in revised form 18 May 1987.

J. Clin. Invest.

0021-9738/87/11/1472/07 $2.00
Volume 80, November 1987, 1472–1478

Introduction

Pituitary adenomas are classified according to characteristic clinical syndromes that result from excess secretion of hormones and by cellular phenotypes based upon immunohistochemistry or ultrastructure (1, 2). The dramatic clinical manifestations of acromegaly and Cushing's disease led to the early recognition of excess growth hormone and ACTH secretion. Owing to the development of the prolactin immunoassay, a majority of chromophobe adenomas of previously unknown phenotype were shown to secrete prolactin (3). The identification of prolactin-secreting adenomas has been helpful in the selection of certain of these patients for treatment with bromocriptine (4). Despite these advances in diagnostic techniques, clinically nonfunctioning adenomas still constitute ~ 25% of all pituitary tumors (2).

Although 15% of cells in the normal pituitary gland produce luteinizing hormone (LH)[1], follicle-stimulating hormone (FSH), or thyroid-stimulating hormone (TSH) (5), pituitary adenomas secreting these hormones are diagnosed infrequently (6, 7). With the exception of TSH-secreting adenomas, which can cause thyrotoxicosis (7), glycoprotein hormone-secreting adenomas do not result in characteristic endocrine syndromes and the detection of glycoprotein hormone-secreting adenomas using serum immunoassays is often difficult (6, 7).

The glycoprotein hormones are heterodimers consisting of two different subunits called α and β. The α-subunit is common to all of the glycoprotein hormones and the unique β-subunits confer biological and immunological specificity to the hormones (8). Many glycoprotein hormone adenomas secrete uncombined and biologically inert α and/or β-subunits (6, 7). Moreover, the excess production of uncombined α-subunit in TSH-producing adenomas has been used to distinguish adenomatous from nonadenomatous causes of inappropriate TSH secretion (7, 9). In contrast to normals, many patients with gonadotropin-producing adenomas secrete FSH and LH in response to thyrotropin-releasing hormone (TRH) (6). Interestingly, although FSH is released primarily as intact hormone, LH is secreted largely in the form of uncombined LHβ subunit (6). These observations indicate that there are both biosynthetic and secretory defects in glycoprotein hormone-producing adenomas and may explain, in part, the rare occurrence of endocrine manifestations by these tumors.

The availability of nucleic acid sequences encoding human ACTH (10), growth hormone (11), prolactin (12), the glycoprotein hormone common α-subunit (13), and each of the

1. *Abbreviations used in this paper:* LH, luteinizing hormone; FSH, follicle-stimulating hormone; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

glycoprotein hormone β-subunits (LHβ [14], FSHβ [15], and TSHβ [16]) provided us the opportunity to analyze hormone biosynthesis in pituitary adenomas at the level of gene expression (15, 17). Using specific oligonucleotide cDNAs complementary to each of these anterior pituitary hormone mRNAs, we found that expression of one or more of the glycoprotein hormone genes is a common occurrence in clinically nonfunctioning pituitary adenomas.

Methods

Patients. Over a 2-yr period, 195 patients with pituitary tumors were referred for transsphenoidal surgery. Baseline endocrine evaluation included serum prolactin, thyroxine, α-subunit, TSH, LH, FSH, and when clinically indicated, somatomedin C and dexamethasone suppression testing. Serum hormone levels were measured in the clinical endocrinology laboratories of the Massachusetts General Hospital (18). The clinical diagnosis of a nonfunctioning pituitary adenoma was made in 54 of the 195 patients (27%). Tumor tissue was frozen at the time of transsphenoidal surgery in 21 of the 54 patients with a clinical diagnosis of nonfunctioning adenoma.

Of these 21 patients, 14 had sufficient RNA (> 20 μg) in the tissue samples to allow hybridization analyses. Patient 1 underwent emergency surgery for progressive visual loss and stupor secondary to a recurrent nonfunctioning pituitary adenoma. Although previous serum LH levels had been normal (LH, 7.4 mIU/ml), preoperative blood samples were retrieved and demonstrated an elevated LH level (99 mIU/ml) consistent with LH secretion by the tumor that was not apparent at the time of his clinical diagnosis. Patient 9 presented with galactorrhea and infertility and was found to have a pituitary macroadenoma on CT scan. Although her postoperative tumor analysis demonstrated ACTH production (see Results), at the time of transsphenoidal surgery, clinical features of hypercortisolemia were not apparent and she was thought to have a poorly functioning prolactinoma (prolactin, 18.8 ng/ml) or nonfunctioning tumor. The remainder of the patients presented with visual field loss secondary to suprasellar extension of tumor and compression of the optic chiasm.

Analyses of pituitary adenomas. Tissue obtained at the time of transsphenoidal surgery was immediately frozen in liquid nitrogen. As control specimens, normal pituitaries were obtained at autopsy from postmenopausal women within 12 h postmortem. Total RNA was isolated by extraction in guanidinium thiocyanate followed by centrifugation through cesium chloride (19). The yield of total RNA was typically 1 μg RNA/mg of tumor. Expression of specific mRNAs were assessed by the Northern blot technique. After denaturation with glyoxal (20), RNA was subjected to electrophoresis through 1.2% agarose gels, and electroeluted onto membranes (Genescreen Plus, New England Nuclear, Boston, MA). Ethidium bromide staining of the RNA before electrophoretic transfer showed no evidence of degradation. Membranes were hybridized with ^{32}P-labeled oligonucleotides and washed as described previously (15). Using the translation start codon as position 1, the locations of the oligonucleotide cDNA probes are: growth hormone (codons 154-162), prolactin (codons 65-74), ACTH (codons 146-154), α-subunit (codons 63-69), LHβ (codons 108-112), FSHβ (codons 30-39), TSHβ (codons 70-79), and CGβ (codons 108-112). The specificities of the glycoprotein hormone β-subunit cDNAs have been described elsewhere (15).

S1-nuclease mapping of the transcriptional start sites of the glycoprotein hormone genes was performed as described previously (21). ^{32}P-labeled oligonucleotides (3,000 cpm) that overlap the transcriptional start sites were hybridized to 20 μg total RNA and digested with 40 U of S1-nuclease (Sigma Chemical Co., St. Louis, MO) (21). The lengths of the oligonucleotide fragments protected from S1-nuclease digestion were analyzed by autoradiography after electrophoresis through 20% polyacrylamide-urea gels.

For immunohistochemistry, tissue was fixed in formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin for histologic evaluation. Immunoperoxidase staining was performed using the avidin-biotin-peroxidase complex technique (22) on 5-μm sections after incubation with specific antisera (supplied by Dr. S. Raiti of the National Pituitary Agency) against growth hormone (1:1,500), prolactin (1:1,500), ACTH (1:400), TSH (1:1,000), LH (1:500), and FSH (1:400). A monoclonal antibody against the free α-subunit was used at a 1:100 dilution of mouse ascites fluid. This antibody does not cross-react with intact glycoprotein hormones.

Results

Clinical features. The clinical features of the patients included in this study are summarized in Table I. There were seven men (35–85 yr old; median 48 yr) and seven women (32–82 yr old; median 58 yr), five of whom were postmenopausal. Visual field loss was the presenting complaint in 93% of the patients. Additional symptoms included galactorrhea in one premenopausal woman and diminished libido in three patients. Computerized axial tomographic scanning demonstrated pituitary macroadenomas with extrasellar extension in all of the patients.

Endocrine evaluation was notable for the presence of mild hyperprolactinemia (range, 18.8–54.4 ng/ml; normal < 15 ng/ml) in 77% of the patients. Low serum testosterone levels were found in four of the seven men (range, 43–249 ng/dl; normal, 300–1,000 ng/dl), all of whom had normal serum LH levels. All of the postmenopausal women had inappropriately low serum levels of gonadotropins. Serum thyroid hormone values were normal in all patients. Serum LH levels were normal in all patients except one male (patient 1) who had elevated LH (99 mIU/ml; normal, 3–18 mIU/ml) and testosterone (1,661 ng/dl) levels. The serum FSH level was minimally elevated (21.7 mIU/ml; normal, 3–18 mIU/ml) in patient 13. Serum free alpha subunit levels were elevated in two patients (14%).

Analyses of pituitary hormone gene expression. Northern blot analyses of RNA were used to characterize expression of pituitary hormone genes in nonfunctioning adenomas and to assess the relative amounts of the different hormone mRNAs in pituitary adenomas compared with normal pituitary RNA. RNA prepared from normal pituitary, placenta, and the nonfunctioning pituitary adenomas was hybridized with cDNA probes specific for the mRNAs encoding α-subunit, prolactin, growth hormone, or ACTH (Fig. 1). Hybridization of α-subunit mRNA, which is common to each of the pituitary glycoprotein hormones (TSH, LH, and FSH), was detected in normal pituitaries and in most of the pituitary adenomas. However, the levels of α-subunit mRNA were variable in adenomas from different patients (Fig. 1 *A*). The same blot was rehybridized with prolactin-specific cDNA. Although abundant prolactin mRNA was found in normal pituitary tissue, none was detected in the nonfunctioning pituitary adenomas (Fig. 1 *B*). Inasmuch as prolactin mRNA was abundant in the normal pituitary (Fig. 1 *B*, lane *pit*), these findings indicate that fragments of normal pituitary tissue do not account for the α-subunit mRNA observed in the pituitary adenomas. Similarly, growth hormone mRNA was absent from the nonfunctioning adenomas (Fig. 1 *C*), but was abundant in both normal pituitary tissue (Fig. 1 *C*, lane *pit*) and an adenoma from a patient with acromegaly (lane *GH*). ACTH mRNA was present in one of the pituitary adenomas (Fig. 1 *D*), a finding consistent with

Table I. Clinical Features of Patients with Pituitary Tumors

Patient No.	Age/Sex	Clinical presentation	Prolactin	TSH	FSH	LH	Testosterone	Estradiol	α-Subunit
			ng/ml	*μU/ml*	*mIU/ml*	*mIU/ml*	*ng/dl*	*pg/ml*	*ng/ml*
1	48/M	Visual field loss	8.1	1.2	5.4	99.0	1,661	ND	7.8
2	45/M	Visual field loss	9.6	0.7	12.0	12.0	153	ND	0.9
3	76/F	Visual field loss	25.0	<0.5	13.2	6.7	ND	<20	0.4
4	42/M	Visual field loss	19.5	6.8	2.0	5.5	43	ND	1.0
5	47/F	Visual field loss	53.0	<0.5	10.5	43.0	ND	ND	1.7
6	67/M	Visual field loss	26.5	<0.5	7.3	7.2	302	ND	1.4
7	35/M	Visual field loss	23.0	3.3	6.8	5.5	310	ND	ND
8	65/F	Visual field loss	ND	ND	ND	ND	ND	ND	ND
9	32/F	Galactorrhea	18.8	1.2	9.2	30.0	ND	113	1.9
10	82/F	Visual field loss	29.7	2.2	2.6	1.0	ND	<20	0.6
11	32/F	Visual field loss	54.4	1.1	2.0	17.7	ND	<20	0.6
12	58/F	Visual field loss	41.2	2.7	18.1	15.8	ND	<20	0.9
13	85/M	Visual field loss	15.4	1.3	21.7	17.7	249	ND	1.5
14	79/M	Visual field loss	4.2	1.2	9.1	14.1	225	ND	8.3
Normal range									
Male			2–10	0.5–5.0	3–18	3–18	300–1,000	ND	0.5–2.5
Female (postmenopausal)			2–15	0.5–5.0	>30	>30	ND	<20	0.5–5.0

a silent corticotroph adenoma that was not appreciated clinically (23). The detection of prolactin (Fig. 1 *B, plac*) and ACTH (Fig. 1 *D, plac*) related mRNAs in placenta has been described previously (24, 25).

Because the glycoprotein hormone β-subunit genes are structurally related to one another (8, 26), we prepared specific cDNA hybridization probes to distinguish expression of the different β-subunit mRNAs. Short oligonucleotides comple-

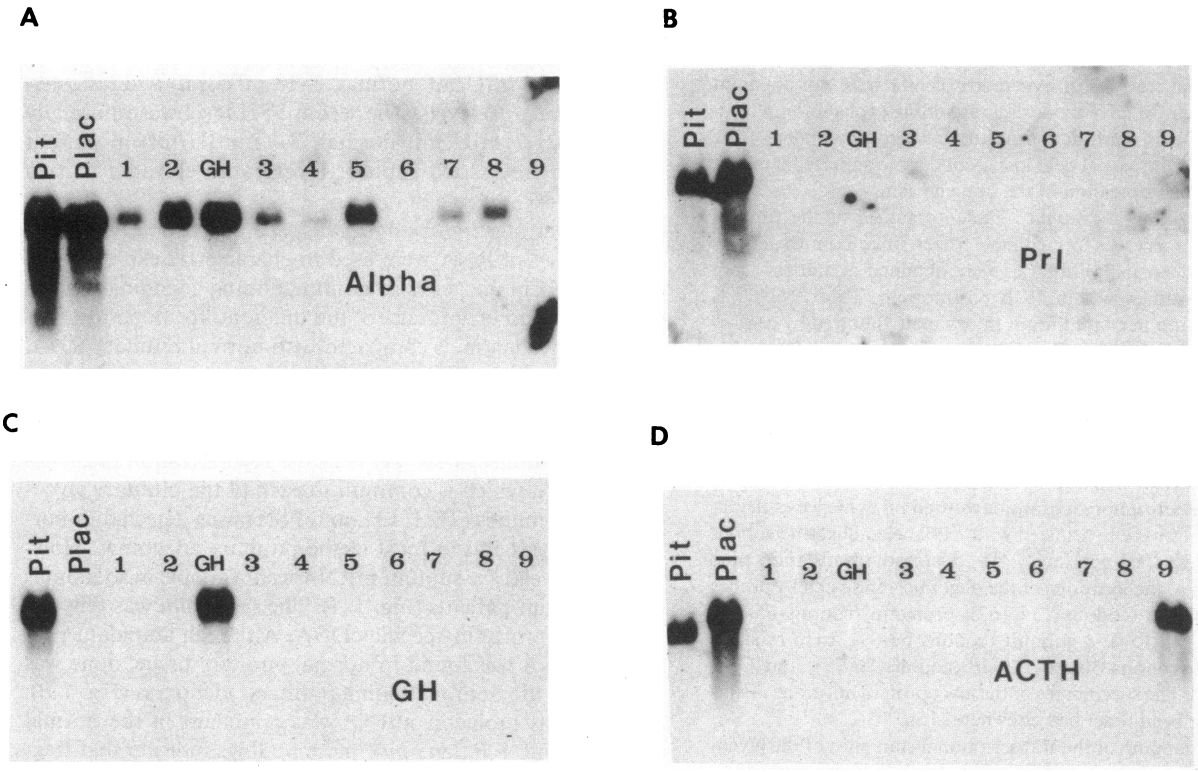

Figure 1. Northern blot analysis of pituitary hormone mRNAs in pituitary adenomas. The same RNA blot was sequentially hybridized with labeled cDNAs specific for (*A*) α-subunit mRNA, exposure, 3 d; (*B*) prolactin (*Prl*) mRNA, exposure, 16 h; (*C*) growth hormone (GH) mRNA, exposure, 3 h; (*D*) adrenocorticotrophin (ACTH) mRNA, exposure, 5 h. Normal pituitary (*Pit*) RNA (20 μg) and first-trimester placental (*Plac*) RNA (1 μg) controls are shown in the left lanes of the figures. The lanes containing pituitary adenoma RNA (20 μg) from patients 1–9 are indicated. The lane *GH* contains RNA from a patient with a growth hormone, α-subunit, and TSH-secreting pituitary adenoma, included on the blot as a control for RNA hybridization but not included in the analyses of the group of clinically nonfunctioning adenomas.

mentary to regions of the β-subunit sequences with low homology were prepared for the TSHβ, FSHβ, LHβ, and CGβ mRNAs (15). Glycoprotein hormone β-subunit mRNAs were detected in many of the clinically nonfunctioning adenomas (Fig. 2). As occurs in normal pituitary (15), the levels of β-subunit mRNAs in most of the adenomas were lower than the levels of the α-subunit mRNA when hybridizing bands were compared using cDNAs of similar specific activity. Considerable variability existed in the levels of β-subunit mRNAs expressed in different adenomas and more than one glycoprotein hormone β-subunit gene was expressed in some of the adenomas (Fig. 2, Table II). For example, an adenoma (patient 2) in which high levels of α-subunit mRNA were observed (Fig. 1 *A*) also contained both FSHβ (Fig. 2 *A*) and LHβ (Fig. 2 *B*) mRNAs.

Northern blot analyses showed no major differences in the lengths of the glycoprotein hormone α- or β-subunit mRNAs expressed in the pituitary tumors and in normal pituitary tissue (Figs. 1 and 2). S1-nuclease mapping was used to characterize the 5′-ends of the α and LHβ mRNAs (21) and provided an independent method for analyses of gene expression in the nonfunctioning adenomas (Fig. 3). Using an α-subunit–specific cDNA that overlaps the 5′-end of the α-subunit mRNA (21), 29 of 36 bases of the labeled oligonucleotide were protected from S1-nuclease digestion by hybridization to adenoma RNA (Fig. 3 *A*). These findings confirmed that the hybridized mRNA (Fig. 1 *A*) was α-subunit mRNA and demonstrated that the transcriptional start site of the α-gene was identical in normal pituitary and in each of the pituitary adenomas that were studied. Similarly, the transcriptional start site of the LHβ gene in the adenomas and normal pituitaries was identical (Fig. 3 *B*), distinguishing expression of LHβ mRNA from the structurally related CGβ mRNA and the excluding transcription from an upstream promoter site as occurs in the CGβ gene (17, 21). Primer-extension analyses (15, 21) of FSHβ mRNA were used in the absence of available gene sequence for S1-nuclease mapping. The primer-extended FSHβ cDNAs (15) using pituitary adenoma RNA (patients 2 and 13) as a template were identical in length to those obtained using normal pituitary RNA (not shown), providing additional evidence for the specificity of the hybridized FSHβ mRNA in the pituitary adenomas.

The results of the pituitary adenoma mRNA hybridization for all 14 patients are summarized in Table II. Expression of pituitary hormone genes was found in $^{12}/_{14}$ patients with clinically nonfunctioning adenomas. Thus, in only two of the patients, (patients 6 and 12) could no pituitary hormone gene expression be identified. Expression of one or more of the glycoprotein hormone genes was found in $^{11}/_{14}$ (79%) patients. Expression of α-subunit mRNA was found in all of the patients expressing one of the β-subunit mRNAs and in three patients in whom no β-subunit mRNA was found. In no case was glycoprotein hormone β-subunit mRNA expressed in the absence of α-subunit mRNA. Patient 14 expressed α and CGβ mRNAs, consistent with ectopic expression of the CGβ gene in a pituitary adenoma (17). Although FSHβ and LHβ mRNAs were found with similar frequencies, expression of FSHβ mRNA was generally much more abundant. TSHβ mRNA was detected in only one patient.

Immunohistochemistry. Analysis of hormone production by immunohistochemistry indicated that the majority (86%) of the clinically nonfunctioning adenomas contain one or more glycoprotein hormones (Table II). Moreover, production of prolactin, growth hormone, or ACTH was rare. For the hormones that were detected, there was ~ 70% concordance between the analysis by gene expression and immunohistochemistry.

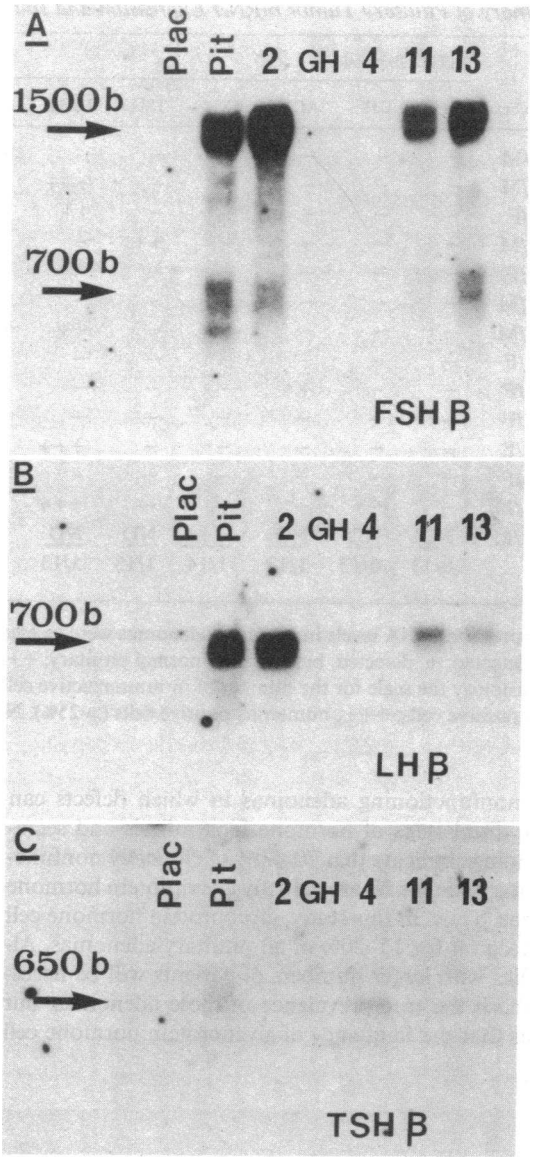

Figure 2. Northern blot analysis of glycoprotein hormone β-subunit mRNAs in pituitary adenomas. Replicate RNA blots were hybridized with labeled cDNAs specific for (*A*) FSHβ mRNA exposure, 5 d; (*B*) LHβ mRNA exposure, 1 wk; (*C*) TSHβ mRNA exposure, 1 wk. First-trimester placental (*Plac*) RNA (1 μg) and normal pituitary (*Pit*) RNA (10 μg) controls are shown in the left lanes of the figures. The lanes containing pituitary adenoma RNA (10 μg) from patients 2, 4, 11, and 13 are indicated. The lane labeled "GH" contains RNA from a patient with a growth hormone, α-subunit, and TSH-secreting pituitary adenoma. The lengths of the RNAs are indicated at the left of the figures (*arrows*). FSHβ mRNAs are heterogeneous (15).

Discussion

Analysis of hormone gene expression in pituitary adenomas provides a definitive determination of the cell phenotype, par-

Table II. Summary of Pituitary Tumor mRNA Expression and Immunohistochemistry

		mRNA Expression								Immunohistochemistry						
Patient No.	Age/Sex	Prl	GH	ACTH	CGβ	TSHβ	FSHβ	LHβ	α	PRL	GH	ACTH	TSH	FSH	LH	α
1	48/M	−	−	−	−	−	−	+++	+	−	−	−	−	−	+++	++
2	45/M	−	−	−	−	−	+++	+++	++	−	−	−	+	++	+++	−
3	76/F	−	−	−	−	−	++	−	+	−	−	−	−	++	++	++
4	42/M	−	−	−	−	++	−	−	+	−	−	−	++	−	−	−
5	47/F	−	−	−	−	−	−	−	++	−	−	−	−	+	+	−
6	67/M	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7	35/M	−	−	−	−	−	++	+	+	−	−	−	−	+	+++	+
8	65/F	−	−	−	−	−	−	−	+	−	−	−	−	+++	+	+
9	32/F	−	−	++	−	−	−	−	−	−	−	+++	−	−	−	−
10	82/F	−	−	−	−	−	−	−	+	−	−	−	+	++	+	−
11	32/F	−	−	−	−	−	+++	+	++	−	−	−	+	+++	+++	−
12	58/F	−	−	−	−	−	−	−	−	+	+	−	−	+	+	+
13	85/M	−	−	−	−	−	+++	+	+++	−	−	−	−	++	++	+
14	79/M	ND	ND	ND	+++	ND	ND	−	+++	−	−	−	−	+	−	++
TOTAL		0/13	0/13	1/13	1/14	1/13	5/13	5/14	11/14	1/14	1/14	1/14	4/14	10/14	10/14	7/14

For mRNA expression, RNA levels in pituitary adenomas were assessed relative to that in normal pituitary. The scale for mRNA expression was: −, none detected; +, detected, but less than normal pituitary; ++, comparable to normal pituitary; +++, greater than normal pituitary. For immunohistology the scale for the number of immunoreactive cells was: −, no immunostaining; +, rare (~ 5%) positive cells; ++, scattered (5–25%) positive cells; +++, numerous positive cells (> 25%). ND, not done.

ticularly in nonfunctioning adenomas in which defects can occur in the distal steps of hormone biosynthesis and secretion. Our findings indicate that 70–80% of clinically nonfunctioning pituitary adenomas are actually glycoprotein hormone cell adenomas. Thus, in this study, glycoprotein hormone cell adenomas account for 15–20% of all pituitary adenomas. Although studies with larger numbers of patients will be necessary to establish the true prevalance of these adenomas, our data suggests that the frequency of glycoprotein hormone cell adenomas approaches that of Cushing's disease and acromegaly, but is less than that of prolactinomas (2).

The cDNAs used for hybridization to pituitary mRNAs were highly specific, allowing distinction of related sequences such as prolactin (12) and growth hormone (11), or the members of the glycoprotein hormone family (15). Thus, in a somatotroph adenoma producing large amounts of growth hormone mRNA (Fig. 1 *C*, "GH"), no cross-hybridization was observed with the prolactin cDNA probe. Although some of

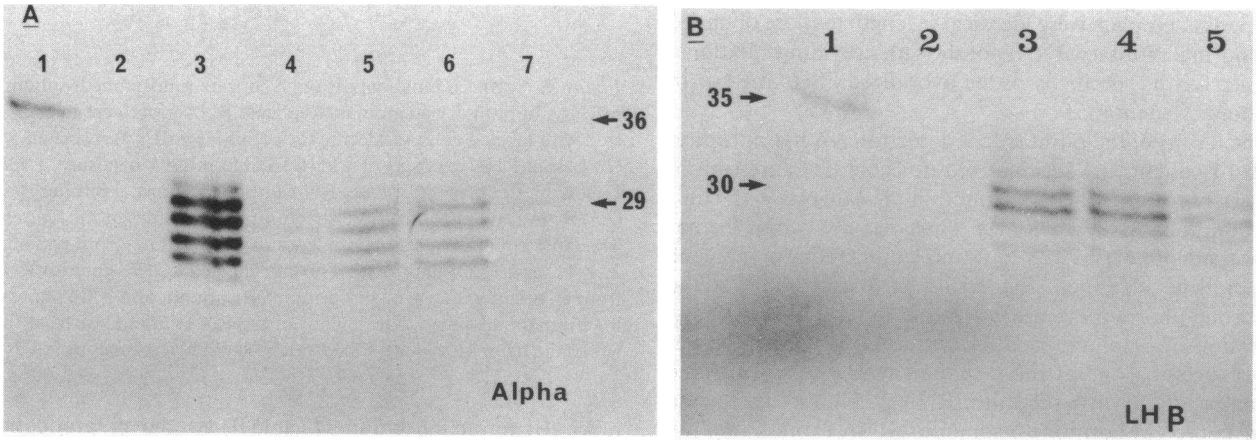

Figure 3. S1-nuclease mapping of the transcriptional start site for the α-subunit and LHβ-gene in pituitary adenomas. (*A*) S1-nuclease mapping of α-subunit mRNA. A 36-base oligonucleotide that overlaps the transcriptional start site of the α-subunit gene was hybridized to RNA, digested with S1-nuclease, and analyzed by electrophoresis through a 20% polyacrylamide-urea gel. Lane *1*, oligonucleotide prior to S1-nuclease digestion, diluted 100-fold; lane *2*, no RNA; lane *3*, normal pituitary RNA; lane *4*, RNA from patient 4; lane *5*, RNA from patient 2; lane *6*, RNA from patient 13; lane *7*, RNA from patient 11. (*B*) S1 nuclease mapping of LHβ-mRNA using a 35-base oligonucleotide that overlaps the transcriptional start site of the LHβ-gene (15). Lane *1*, oligonucleotide before S1-nuclease digestion, diluted 200-fold; lane *2*, no RNA; lane *3*, normal pituitary RNA; lane *4*, RNA from patient 2, lane *5*, RNA from patient 13. Oligonucleotides of known lengths were used as size markers to determine the lengths of the labeled fragments protected from S1-nuclease digestion by hybridized mRNA (*arrows*).

the glycoprotein hormone cell adenomas were well-differentiated into predominantly LH (patient 1), FSH (patient 3), or TSH (patient 4) adenomas, others were plurihormonal (patients 2, 11, and 13) and exemplify the efficacy of these analyses to specifically define the cellular phenotype of pituitary adenomas. The fact that none of the adenomas expressing glycoprotein hormone genes were found to contain growth hormone, prolactin, or ACTH mRNAs reflects the specificities of the hybridization probes and strongly supports the view that the expressed mRNAs are derived from adenoma tissue and not from fragments of normal pituitary. Furthermore, the lack of prolactin mRNA expression in adenomas from several patients with mild hyperprolactinemia (Table II), indicates that these adenomas are not prolactinomas, allowing distinction of excess prolactin secretion due to tumor mass effects as opposed to prolactin secretion by tumor cells.

The immunocytochemistry studies complement the analyses of gene expression, demonstrating glycoprotein hormone antigens in most (86%) of the nonfunctioning adenomas. In a few cases, glycoprotein hormones were found by immunostaining without detection of glycoprotein hormone β-subunit mRNAs, suggesting that the mRNA levels may be below the limits of detection by cDNA hybridization. It is important to emphasize that among the pituitary hormone mRNAs, the levels for the glycoprotein hormone β-subunit mRNAs are the lowest and are therefore the most likely to be below detection using Northern blot hybridization analyses. By comparison, the levels of α-subunit mRNAs are typically two- to fivefold greater than any given β-subunit mRNA and the levels of GH, ACTH, and Prl mRNAs in normal pituitary are approximately an order of magnitude greater than α-subunit mRNA. Solution hybridization assays are currently being developed to improve the sensitivity of the β-subunit mRNA assays.

Although several adenomas contained α-subunit mRNA without immunostaining for free α-subunit, in each case there was immunostaining for the intact glycoprotein hormones in which the α-subunit is combined with β-subunit. The prevalence of glycoprotein hormone immunostaining reported previously for nonfunctioning adenomas varies widely (27–31), likely reflecting differences in fixation techniques or the sensitivities of different antisera. However, characterization of adenomas by analyses of gene expression and by immunohistochemistry in this study provides convincing evidence for the common occurrence of the glycoprotein hormone cell phenotype using independent techniques.

Although nonfunctioning adenomas express glycoprotein hormone genes and contain glycoprotein hormone antigens, it is unclear why excess hormone secretion is not observed more commonly in vivo. Effective glycoprotein hormone biosynthesis and secretion requires balanced expression of the α- and β-subunit genes, translation of the mRNAs into proteins, complex posttranslational processing, and regulated secretion of the hormones (8). Several lines of evidence indicate that glycoprotein hormone cell adenomas contain defects in one or more of these steps in hormone biosynthesis. The relative amounts of α- and β-subunit mRNAs expressed in different pituitary adenomas varied widely. Furthermore, in comparison with normal pituitary and placenta, in which the levels of α-subunit mRNA were more abundant than β-subunit mRNAs (Figs. 1 and 2) (15), several adenomas (patients 1 and 11) expressed relatively high levels of β-subunit mRNA (Table II). One adenoma (patient 14), described previously in detail (17), ectopically expressed α and CGβ mRNA, but did not produce hCG, suggesting a defect in the posttranslational processing or stability of the hormone. In addition to these abnormalities in glycoprotein hormone gene expression, previous studies have demonstrated secretion of uncombined α- and β-subunits in both thyrotrope and gonadotrope-cell adenomas (6, 7, 32, 33). Although electron microscopy was not performed on these adenomas, previous studies have demonstrated that nonfunctioning or null cell adenomas contain sparse secretory granules, consistent with ongoing hormone gene expression and biosynthesis. However, in addition to altered granule size and number, many of these adenomas contain other morphologic changes such as oncocytic changes and poorly developed cellular organelles (2, 28, 31). Thus, the biosynthetic abnormalities in combination with the morphological changes in cellular structure seen by electron microscopy likely account for the diminished secretion of glycoprotein hormones in vivo by this group of "nonfunctioning" adenomas.

Because of variability in the biosynthesis and secretory potential of glycoprotein hormone cell-type adenomas, patients with these types of pituitary tumors can exhibit a wide spectrum of serum glycoprotein hormone levels. Although some patients have elevated levels of intact hormones or uncombined α or β subunits (6, 7), many patients with glycoprotein hormone cell adenomas documented by analyses of gene expression or immunocytochemistry (Table II) actually have low or normal serum levels of LH and FSH. For example, inappropriately low serum levels of LH and FSH were found in several of the postmenopausal women and low serum testosterone levels were commonly found in men (Table I). Thus, in addition to the biosynthetic and secretory abnormalities in the adenomas, secretion by normal gonadotropes is also impaired, presumably due to mass effects of the tumor on the normal pituitary and/or the hypophyseal-portal circulation. Decreased or normal serum LH and FSH levels are therefore not useful criteria for excluding of the diagnosis of a gondadotrope-cell adenoma. We conclude that studies of gene expression and/or immunocytochemistry in clinically nonfunctioning adenomas can be used to define the cellular phenotype of glycoprotein production in pituitary adenomas. These analyses may be useful for characterizing the natural history of different subtypes of pituitary adenomas and for selecting patients with glycoprotein hormone adenomas for adjunctive medical therapies that are currently under evaluation (34–37).

Acknowledgments

This work was supported in part by National Institutes of Health grants AM 25532 and HD 21204.

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