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Research Article

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Gonadotropin-releasing Hormone Receptor mRNA Expression by Human Pituitary Tumors In Vitro

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Abstract

An important question in the pathogenesis and regulation of human gonadotroph adenomas is whether heterogeneous gonadotropin responses to gonadotropin-releasing hormone (GnRH) are due to dysregulation of GnRH receptor biosynthesis and/or cell-signaling pathways. We investigated gonadotropin responsiveness to pulsatile GnRH in 13 gonadotroph adenomas. All tumors had evidence of follicle-stimulating hormone (FSH) β and α subunit biosynthesis using reverse transcriptase/polymerase chain reaction (RTPCR) techniques. Four tumors significantly increased gonadotropin and/or free subunit secretion during pulsatile 10^{-8} M GnRH administration. The GnRH antagonist Antide (10^{-6} to 10^{-8} M) blocked secretory increases in all GnRH-responsive tumors. Gonadotropin and/or free subunit secretion increased after 60 mM KCl, confirming that GnRH nonresponsiveness was not due to intracellular gonadotropin depletion. We hypothesized that GnRH nonresponsiveness in these tumors may be due to GnRH receptor (GnRH-Rc) biosynthetic defects. RTPCR analyses detected GnRH-Rc transcripts only in responsive tumors and normal human pituitary. This is the first demonstration of a cell-surface receptor biosynthetic defect in human pituitary tumors. We conclude (*a*) one third of gonadotroph tumors respond to pulsatile GnRH in vitro, (*b*) GnRH-Rc mRNA is detected in human gonadotroph adenomas and predicts GnRH responsiveness, and (*c*) GnRH-Rc biosynthetic defects may underlie GnRH nonresponsiveness in gonadotroph tumors. (*J. Clin. Invest.* 1994. 93:2332–2339.) Key words: gonadotropin • adenoma • pathogenesis • pulsatile • perifusion

Introduction

The majority of clinically nonfunctioning pituitary adenomas synthesize gonadotropin subunit mRNAs and secrete intact gonadotropins and/or their free subunits (1, 2) and are therefore considered to be of a gonadotroph cell origin. There are several lines of evidence suggesting dysregulation of gonadotropin and free subunit secretion by gonadotropin-releasing hormone (GnRH)[1] in such tumors. GnRH consistently stimulates gonadotropin secretion in normal individuals (3). In contrast, < 50% of patients with gonadotropin-secreting tumors exhibit in vivo increases in serum levels of intact gonadotropins and free subunits after an intravenous bolus of GnRH (4, 5). Second, desensitization rarely occurs in neoplastic gonadotrophs. Although gonadotropin secretion decreases during continuous GnRH administration in normals, patients with GnRH-responsive pituitary adenomas demonstrate a persistent agonist effect with sustained increases in gonadotropin secretion (5). GnRH-induced secretory responses have been demonstrated in static cell cultures of pituitary tumors (6–8). However, the physiological regulation of neoplastic gonadotrophs by pulsatile GnRH has not been examined in vitro, and little is known about the mechanism underlying GnRH responsiveness in neoplastic gonadotrophs.

Although human pituitary adenomas are clonal in origin, it is unknown how hypothalamic or other factors may potentiate tumor development or lead to specific tumor phenotypes (9, 10). An important question in the pathogenesis and regulation of human neoplastic gonadotrophs is whether the lack of GnRH responsiveness in a subset of tumors is due to dysregulation of GnRH receptor (GnRH-Rc) biosynthesis and/or cell-signaling pathways. The recent cloning of human receptors for hypothalamic releasing peptides has offered the potential for new insights into receptor expression and function in neoplastic pituitary cells (11–13). Data indicate that GnRH-Rc biosynthesis can be regulated by physiological factors such as gonadal steroids as well as homologous regulation by GnRH itself (14). Therefore, the investigation of GnRH-Rc expression is critical for determining both GnRH regulation and potential cell-signaling defects in human tumors of gonadotroph origin. Sequence analysis of the human GnRH-Rc has revealed that it is a member of the G protein–coupled receptor family (13). The conserved structure of the cloned GnRH-Rc confirms biochemical data that its effects on phospholipases and subsequent phosphatidylinositol hydrolysis is likely mediated by interactions with the $Gq\alpha$ family of GTP-binding proteins (15, 16). Constitutive activation of this cell-signaling pathway has mitogenic and transforming effects in NIH3T3 mouse fibroblasts (17). Therefore, GnRH-Rc could potentially modulate tumor growth as well as gonadotropin biosynthesis and secretion.

Because of the potential importance of GnRH in pituitary tumor pathogenesis, we investigated (*a*) intact gonadotropin and free subunit responses to pulsatile physiological GnRH in human gonadotropin-secreting adenomas, (*b*) blockade with a GnRH antagonist, and (*c*) whether the presence of GnRH-Rc mRNA predicts GnRH responsiveness in neoplastic gonadotrophs.

Methods

Clinical data. Adenoma tissue was obtained from 13 patients with gonadotropin hormone–producing pituitary tumors who underwent transsphenoidal surgery. Patients ranged in age from 48 to 80 yr (median age 58 yr, see Table I). All patients presented with visual field

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1. *Abbreviations used in this paper:* AMV, avian myeloblastosis virus; GapDH, glyceraldehyde phosphate dehydrogenase; GH, growth hormone; GnRH, gonadotropin-releasing hormone; GnRH-Rc, gonadotropin-releasing hormone receptor; GPH, glycoprotein hormone; PRL, prolactin; RTPCR, reverse transcriptase/polymerase chain reaction; TSH, thyroid-stimulating hormone.

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Table I. *Patient Data and Preoperative Serum Levels*

No.	Age	Sex	α Subunit	LH	FSH	FSHβ	Prolactin
	yr		*μg/liter*	*IU/liter*	*IU/liter*	*μg/liter*	*μg/liter*
1	58	Male	0.6	n/d	n/d	0.3	<0.8
2	80	Male	1.5	1.4	2.9	<0.2	20.5
3	75	Female	0.7	6.0	10.4	0.8	16.5
4	58	Female	0.4	0.4	1.5	<0.2	22.1
5	76	Female	1.1	9.8	28.2	3.4	27.7
6	48	Male	0.6	1.0	1.6	<0.2	18.9
7	49	Male	1.3	1.9	2.0	0.2	4.4
8	65	Male	1.0	0.2	1.6	0.3	16.3
9	58	Male	0.2	3.4	6.9	0.9	23.7
10	55	Female	1.2	27.4	58.4	1.0	n/d
11	72	Female	1.6	3.0	8.0	0.6	7.5
12	68	Female	1.4	23.6	54.6	2.9	9.1
13	48	Male	1.6	2.7	3.4	<0.2	7.3
Normal serum levels							
Males			0.5–2.5	3.0–18.0	3.0–18.0	<0.2–0.5	0–10
Females Premenopausal			0.5–2.5	2.4–187.2	4.6–41.2	<0.2–0.8	0–15
Females Postmenopausal			2.5–5.0	30.0–150.0	30.0–150.0	1.3–3.7	0–15

abnormalities, headaches, and/or reproductive dysfunction, including oligomenorrhea, amenorrhea, or hypogonadism. A pituitary macroadenoma with extrasellar extension was diagnosed by magnetic resonance imaging in all patients. Serum prolactin levels were mildly elevated in seven patients and ranged from 16.3 to 27.7 μg/liter (median, 20.5 μg/liter), consistent with pituitary stalk compression. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and free glycoprotein hormone (GPH) α subunit levels were normal in all patients. One patient had an elevated serum-free FSHβ subunit level of 0.9 μg/liter.

Pituitary tissue culture and perifusion methods. Pituitary adenomas were obtained in phosphate-buffered saline after transsphenoidal surgery, enzymatically dispersed according to our previously described methods (18), and incubated overnight in suspension culture containing 10% fetal calf serum/DME (Gibco/BRL, Bethesda, MD). Cells were then pelleted, resuspended in DME/0.1% BSA (Fraction V; Collaborative Research Inc., Lexington, MA), and aliquoted at equal concentrations. Preswollen Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, CA) was used as an attachment matrix for tumor cells. Cells were coincubated with Bio-Gel matrix (500 mg/column; Bio-Rad Laboratories) for 1 h at 37°C in DME/0.1% BSA, loaded into two columns at equal densities, and perifused at a rate of 200 μl/min with DME/0.1% BSA. Each perifusion column contained an average of 5×10^5 cells. Samples collected during the first 30 min containing unattached red blood cells, cell debris, and gonadotropin stores from lysed cells were discarded. Samples obtained during the next 30 min were used to establish a secretory baseline before pulse administration. Hourly 10-min GnRH pulses (10^{-8} M) were delivered. Simultaneous antide was administered at variable concentrations, ranging from 10^{-6} to 10^{-10} M (19). In each perifusion experiment, one column received simultaneous antide/GnRH and one control column received GnRH alone. To determine tumor intracellular gonadotropin stores, perifusion columns were administered 60 mM KCl for 10 min in isotonic culture media.

RNA extraction and reverse transcriptase/polymerase chain reaction (RTPCR) analysis of GnRH receptor from perifused tumors. Total RNA was successfully extracted after perifusion from 12 of 13 tumors using guanidinium isothiocyanate/phenol/chloroform as described previously (20). 400 ng of total RNA was then reverse transcribed in 50 mM Tris-HCl (pH 8.3), 5 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 0.25 mM Spermidine, and 200 μM dNTPs using avian myeloblastosis virus (AMV) RT (12 U/reaction) and random hexamers (1 μg/reaction) as first-strand cDNA primers (Promega, Madison, WI). Reverse transcriptase reactions were carried out at 25°C for 10 min, followed by a 10-min step at 42°C, and AMV-RT heat inactivation at 99°C for 5 min. Control reactions without AMV reverse transcriptase were also performed to exclude genomic DNA contamination as a source of amplified signal. Human GnRH receptor was amplified from tumor cDNA using the following primer set: sense 5′ CAG CAA AGT CGG ACA GTC 3′; antisense 5′ AGT TGT AGT TCG TGG GGG 3′. Human glyceraldehyde phosphate dehydrogenase (GapDH) was amplified as a positive control from tumor cDNA (21) using the following primer set: sense 5′ (exon 2) GAG CCA CAT CGC TCA GAC 3′; antisense 5′ (exon 5) TTC TCC ATG GTG GTG AAG 3′.

All amplifications were carried out in 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 3.5 mM $MgCl_2$, 0.1% Triton X-100, 40 μM dNTPs, and 0.5 U of Taq1 polymerase (Perkin-Elmer, Norwalk, CT). 50 pM of PCR primers were used for each reaction, and amplification of specific cDNAs were carried out for 30 cycles (1 min 94°C, 1 min 50°C, 1.25 min 72°C) in a thermocycler (MJ Research, Watertown, MA). PCR products were visualized by incorporation of 5 μCi [α^{32}P]dCTP for direct visualization on 6% polyacrylamide gels. Autoradiographic exposures were for 30 min.

RTPCR analysis of gonadotropin subunit mRNAs from perifused tumors. Analysis of gonadotropin subunit mRNAs was performed as described above for the GnRH receptor, except that PCR products were visualized by 1.5% agarose gel electrophoresis and Southern blot hybridization with human GPH α subunit, FSHβ, or GapDH cDNA probes. PCR primers used for gonadotropin subunit mRNAs were as follows: α-SU-1, CCC TCT TCG GAT CCA CAG; α-SU-2, GCA CTG AAG TAT TGG GGC; FSHβ-1, GCT GCA ATA GCT GTG AGC; FSHβ-2, CTG AAT CAT GAT GGA GTC.

RIAs of intact gonadotropins and free subunits. Serum α subunit levels were determined by RIA (22). The α subunit assay has a detection limit of 0.2 μg/liter and cross-reactivities of LH, FSH, and thyroid-stimulating hormone (TSH) are 2.7%, 15%, and 10%, respectively. Media α subunit was measured by a monoclonal RIA (Biomerica, Newport Beach, CA) (23). The detection limit for α subunit is 0.05 μg/liter. Cross-reactivities with each of the intact glycoprotein hormones and free β subunits are < 0.2% and < 0.1%, respectively. Media FSHβ subunit was measured by RIA (24, 25). The detection limit of the FSHβ assay is 0.2 μg/liter, and the cross-reactivity of intact FSH is

14%. Cross-reactivities of the assay with each of the other intact glycoprotein hormones and free β subunits are < 0.2%. Intact LH and FSH were measured by RIA using polyclonal antibodies specific for the β subunit, and quantitated using World Health Organization IU Standards IRP 78/549 (FSH) and IRP 68/40 (LH). The detection limit of both RIAs is 0.8 IU/liter. Cross reactivities of the LH assay are 3.9% for both FSH and TSH, and 1.2% and 1.3% for FSHβ and TSHβ, respectively. The cross-reactivities of the FSH assay are < 0.03% for all glycoprotein hormones and free subunits.

RIAs of other pituitary hormones. TSH and prolactin (PRL) hormone concentrations in perifusion media were determined using commercially available double-antibody chemiluminescent assays (Nichols Institute, San Juan Capistrano, CA). Cross-reactivities of each assay with all other pituitary hormones are < 0.1%. Growth hormone (GH) concentrations in perifusion media were determined using a commercially available double-antibody immunoradiometric assay (Nichols Institute). Cross-reactivities of each assay with all other pituitary hormones are < 0.1%.

Statistical analysis. Secretory baselines were determined as the mean hormone level released per column fraction during the 20 min preceding each GnRH pulse. Secretory peaks were calculated as maximal hormone released after the GnRH pulse minus the preceding secretory baseline. Secretory responses that were ≥ 2 SDs above preceding baseline were considered significant.

Table II. Basal Secretion of GH, PRL, and TSH by Perifused Pituitary Tumors

Tumor No.	GH	PRL	TSH
	µg/liter	*µg/liter*	*mIU/liter*
1	<1.0	0.2	0.07
2	<1.0	0.3	0.06
3	<1.0	0.4	0.13
4	<1.0	1.5	0.09
5	<1.0	0.2	0.07
6	<1.0	0.3	0.07
7	<1.0	0.2	0.07
8	<1.0	0.1	0.06
9	<1.0	0.3	0.08
10	<1.0	0.5	0.09
11	<1.0	0.4	0.06
12	<1.0	0.2	0.07
13	<1.0	0.2	0.07
Detection limit	<1.0	<0.1	<0.01

Results

Basal hormone secretion and immunocytochemistry. All 13 tumors secreted one or more intact gonadotropins and/or free subunits during perifusion experiments. Detectable levels of FSH were found in 10 tumors with mean secretory rates ranging from 1.1 to 10.0 IU/liter · min^{-1} (median 2.6 IU/liter · min^{-1}). Detectable levels of LH were found in 11 tumors with mean secretory rates ranging from 1.7 to 7.9 IU/liter · min^{-1} (median 2.6 IU/liter · min^{-1}). Free α and FSHβ subunit levels were detected in eight and two tumors, respectively. The mean α subunit secretory rate ranged from 0.2 to 0.6 µg/liter · min^{-1} (median 0.4 µg/liter · min^{-1}). The mean FSHβ subunit secretory rates were 3.3 and 1.7 µg/liter · min^{-1} in tumor 9 and 10, respectively. Immunocytochemical staining was performed on pituitary tumor tissue from each patient using specific antibodies for LHβ, TSHβ, FSHβ, α subunit, GH, PRL, and ACTH (26). Nine of 13 tumor specimens immunostained positively for one or more gonadotropin subunits. Immunocytochemical staining for GH, PRL, and ACTH was negative in all tumors, and 2 of 13 tumors had rare TSHβ immunostaining.

To exclude the possibility that normal pituitary tissue contamination was responsible for the observed tumor secretory phenotype in perifusion, medias were assayed for the nongonadotroph hormones, GH, PRL, and TSH (Table II). Secretion of these nongonadotroph pituitary hormones from perifused gonadotroph tumors was negligible for both GnRH-responsive and -unresponsive tumors. GH was undetectable in all tumors (< 1.0 µg/liter). TSH levels were near the detection limit in all tumors, and ranged from 0.06 to 0.13 mIU/liter (median, 0.07 mIU/liter), whereas PRL levels were also low, ranging from 0.1 to 1.5 µg/liter (median, 0.2 µg/liter).

Gonadotropin subunit biosynthesis in perifused tumors. To confirm the gonadotroph origin of these tumors, RTPCR analysis was performed for a gonadotropin-specific marker, FSHβ, as well as GPH α subunit and GapDH. As shown in Fig. 1, all 12 perifused tumors showed positive RTPCR amplification for FSHβ mRNA, as well as GPH α subunit and GapDH. RTPCR reactions with and without reverse transcriptase using RNA from normal human pituitary were included as positive and negative controls, respectively.

GnRH responsive tumors. Intact gonadotropins and/or free subunits increased significantly in response to pulsatile GnRH administration in 4 of 13 tumors (tumors 3, 5, 9, and 10). LH was secreted in the perifusion media from all four of the GnRH-responsive tumors. There was a mean increase in LH of 1.5- to 20-fold after GnRH administration. Three of the four responding tumors also secreted FSH (tumors 3, 9, 10), with a mean increase ranging from 1.8- to 5.4-fold after GnRH administration. Pulsatile GnRH also stimulated α- and FSHβ-free subunit secretion. The mean increase in α subunit and FSHβ secretion ranged from 1.2- to 1.8-fold (tumors 3, 9, 10) and from 1.2- to 2-fold (tumors 9 and 10), respectively. GnRH stimulation of FSHβ and α subunit was accompanied by stimulation of intact gonadotropin in all cases.

Coordinate secretion of intact FSH and free FSHβ subunit in response to pulsatile GnRH. Two of the four GnRH-responsive tumors secreted both intact FSH and FSHβ free subunit, whereas FSHβ was undetectable in all nonresponders. In two responding tumors (tumors 9 and 10), FSH secretory increases were accompanied by increased release of FSHβ-free subunit. Coordinate secretion of intact FSH and free FSHβ subunit in one tumor (tumor 9) in response to pulsatile GnRH is shown in Fig. 2. FSH and FSHβ free subunit mean secretory responses were 1.8- and 2-fold, respectively.

GnRH nonresponsive tumors. 9 of 13 tumors were unresponsive to pulsatile GnRH in vitro. When responders were compared with nonresponders, there were no significant differences in the mean baseline secretion of intact gonadotropins or α subunit. However, FSHβ was undetectable in all GnRH nonresponders. Mean secretory rates of LH, FSH, and α subunit ranged from < 0.8 to 4.2 IU/liter · min^{-1}, 1.1 to 5.1 IU/liter · min^{-1}, and 0.2 to 0.6 µg/liter · min^{-1}, respectively. A 5-h FSH secretory profile for one nonresponding tumor (tumor 2) is shown in Fig. 3. To confirm that GnRH nonresponsiveness was not due to the absence of intracellular gonadotropins, a membrane-depolarizing pulse of 60 mM KCl was ad-

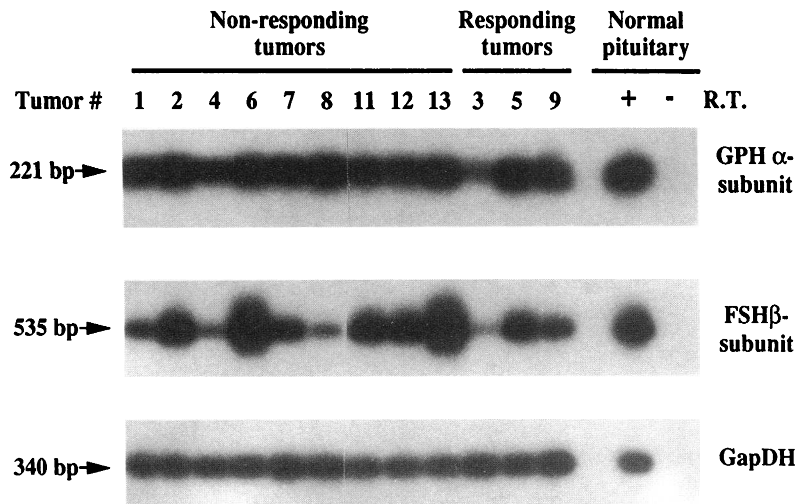

Figure 1. RTPCR analysis of gonadotropin subunit mRNAs from perifused tumors. Analysis of gonadotropin subunit mRNAs was examined by RTPCR amplification of tumor total RNA, followed by Southern blot hybridization with human GPH α subunit, FSHβ, or GapDH cDNA probes. Normal pituitary total RNA was used as a control. RT was omitted from one normal pituitary PCR reaction to assure that the observed amplified products were RNA specific. All RTPCR products were of the expected size.

ministered to four of the nine GnRH-nonresponsive tumors. In each case, a significant transient secretory burst of gonadotropins and/or free subunits was observed. The response in tumor 2 is also shown in Fig. 3. Secretion of LH as well as α- and FSHβ-free subunit were undetectable in this pituitary tumor throughout the perifusion experiment.

Gonadotropin responses to GnRH stimulation during Antide blockade. In all four GnRH-responsive tumors, gonadotropin secretory pulses were blocked by 10^{-6}–10^{-8} M Antide. The LH responses to GnRH in tumor 3 during coadministration of increasing doses of Antide are shown in Fig. 4. LH responses after each GnRH pulse in column A were significantly increased over basal levels. LH stimulation after GnRH was blocked by simultaneous administration of 10^{-8} M GnRH with either 10^{-7} or 10^{-8} M Antide. However, lower concentrations of Antide (10^{-9} and 10^{-10} M) failed to block GnRH-stimulated LH secretion. Mean basal FSH secretion rates in columns A and B were 1.2 and 1.1 IU/liter · min^{-1}, respectively. After GnRH administration, mean FSH secretion rates in columns A and B were significantly increased to 2.5 and 2.0 IU/liter · min^{-1}, respectively. However, FSH secretory baselines in both columns, as well as responses to GnRH, were attenuated after the first two GnRH pulses, probably because of depletion of intracellular FSH. After 60 mM KCl, there was a significant increase in both LH and FSH secretion. LH increased over baseline 4.2- and 5.0-fold in columns A and B, respectively. Release of intracellular FSH after KCl administration was less pronounced, but secretion increased over baseline 0.7- and 2.4-fold in columns A and B, respectively.

Recovery of gonadotropin responses after Antide blockade. GnRH responses of tumor 9 after administration of Antide are shown in Fig. 5. LH secretory responses to GnRH (column A) ranged from 7.7 to 28.1 IU/liter · min^{-1}, with a mean response of 260% over the preceding secretory baseline. LH secretory responses to GnRH and 10^{-6} M Antide (column B) ranged from 5.2 to 9.1 IU/liter · min^{-1}, with a mean response of 45%. FSH secretory responses to GnRH ranged from 5.4 to 7.3 IU/liter · min^{-1}, with a mean response of 85% over the preceding

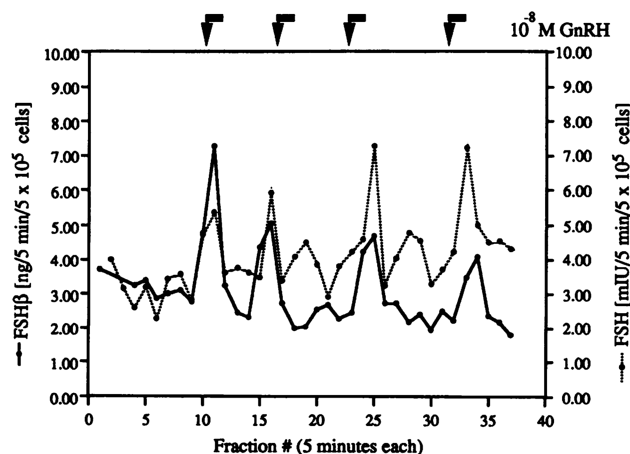

Figure 2. Coordinate secretion of intact FSH and free FSHβ subunit by tumor 9 in response to pulsatile GnRH. 10-min GnRH pulses were administered hourly. Arrows indicate the timing and duration of each GnRH pulse. Fractions were collected every 5 min.

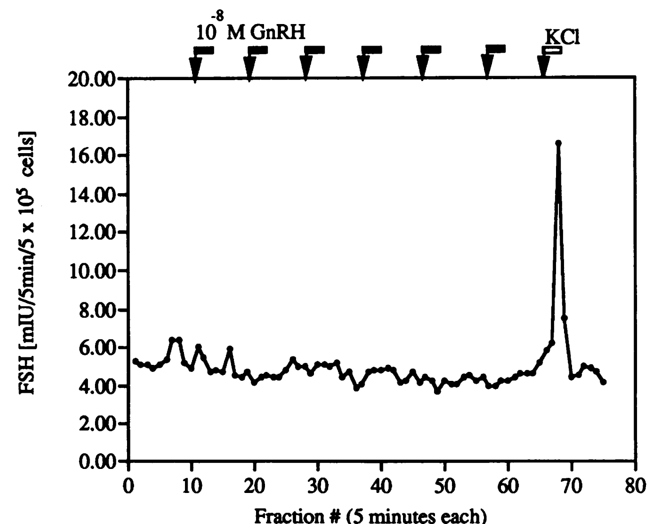

Figure 3. Secretion of FSH in perifusion by GnRH-nonresponsive pituitary tumor 2. 5-min fractions of perifusion media were assayed for intact FSH secretion by tumor 2 during the 5-h experiment. 10-min GnRH pulses were given every 45 min, followed by a single 10-min pulse of 60 mM KCl at the conclusion of the experiment. Arrows indicate the timing and duration of each GnRH pulse.

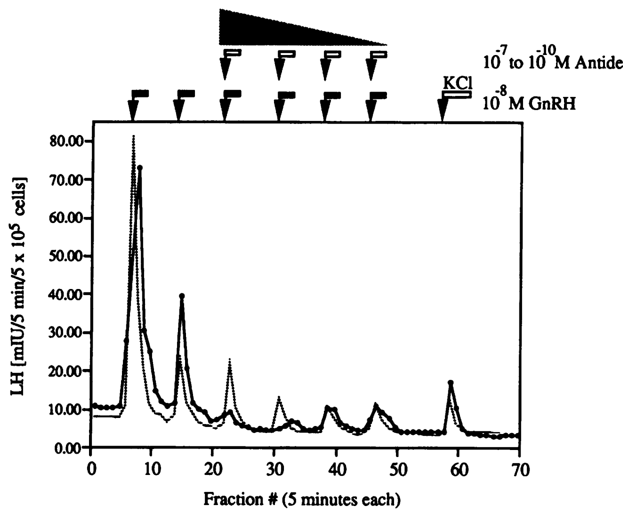

Figure 4. Dose-dependent Antide inhibition of secretory responses by tumor 3. 10-min GnRH pulses were given every 45 min. Antide [10^{-7}-10^{-10} M] was coadministered. A single 10-min pulse of 60 mM KCl was given at the conclusion of the experiment. Arrows indicate the timing and duration of each GnRH, Antide, or KCl pulse. (-----) Control perifusion column A profile of GnRH alone. (——) Perifusion column B profile of GnRH + simultaneous Antide.

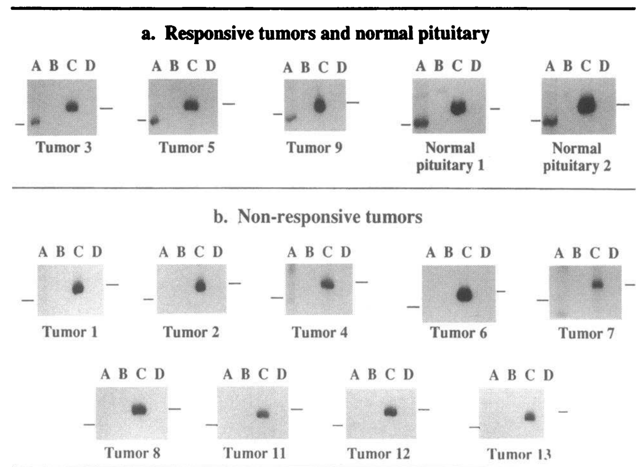

Figure 6. RTPCR analysis of human GnRH receptor mRNA from perifused pituitary tumors and normal pituitary. Lane *A*, GnRH receptor RTPCR reactions. The demarcated band represents the expected 293-bp PCR product. Lane *B*, control GnRH receptor RTPCR without RT. Lane *C*, GapDH RTPCR reactions. The demarcated band represents the expected 340-bp PCR product. Lane *D*, control GapDH RTPCR reactions without RT.

secretory baseline. FSH secretory responses to GnRH and 10^{-6} M Antide ranged from 3.5 to 4.1 IU/liter · min^{-1}, with a mean response of 8%. Pretreatment with 10^{-6} M Antide significantly decreased the secretory responses of both LH and FSH to GnRH.

RTPCR of human GnRH receptor from perifused gonadotroph tumors and normal human pituitary. The results of RTPCR analyses of GnRH-Rc mRNA from perifused tumors and normal pituitary controls are shown in Fig. 6. GnRH-Rc was identified in all GnRH-responsive tumors, and GnRH-Rc amplification was similar to that seen in two normal human pituitary samples. GnRH-unresponsive tumors failed to show amplification of the GnRH-Rc mRNA comparable to levels detected for either responsive tumors or normal pituitary. All tumor mRNA preparations had detectable levels of control GapDH PCR product, and GapDH mRNA from unresponsive tumors amplified with comparable signal intensity as that seen for GnRH-responsive tumors and normal pituitary.

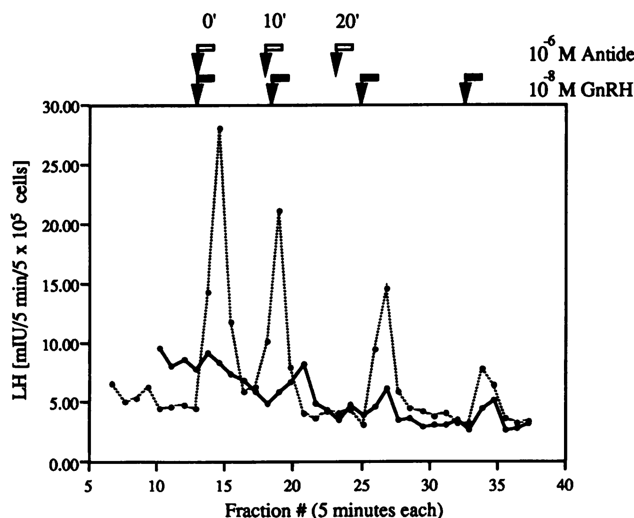

Figure 5. Recovery of secretory responses by tumor 9 after antide blockade. 10-min GnRH pulses were coadministered with Antide or at 10 or 20 min after Antide treatments. Arrows indicate the timing and duration of each GnRH and Antide pulse. (-----) Perifusion column profile of GnRH alone. (——) Perifusion column profile of GnRH preceded by Antide.

Discussion

We have shown that approximately one third of gonadotroph tumors are responsive to pulsatile GnRH and that GnRH-induced secretory increases can be blocked in a dose-responsive and time-dependent manner by administration of a GnRH antagonist. We report the first identification of human GnRH-Rc mRNA in neoplastic pituitary tissue using sensitive RTPCR techniques. All GnRH-responsive tumors studied had detectable levels of GnRH-Rc mRNA comparable to those found in normal human pituitary tissue. In contrast, the majority of gonadotroph tumors were unresponsive to pulsatile GnRH in perifusion and did not synthesize detectable levels of GnRH-Rc mRNA. Therefore, the presence of GnRH-Rc mRNA predicts tumor responsiveness to GnRH stimulation in vitro. The lack of detectable GnRH-Rc mRNA in GnRH-unresponsive tumors suggests that tumor receptor biosynthesis is a critical determinant of gonadotroph responses to GnRH stimulation. These data are consistent with the hypothesis that cell-surface receptor biosynthetic defects are present in human pituitary tumors.

What are the differential effects of GnRH on normal versus neoplastic gonadotrophs? This question has been addressed in vivo by studies investigating gonadotroph desensitization in patients with gonadotropin-secreting pituitary tumors. In normal patients, administration of a GnRH agonist leads to pituitary desensitization and subsequent decreases in gonadotropin secretion. In contrast, patients with gonadotropin-secreting pi-

tuitary tumors receiving chronic GnRH agonists typically show a persistent agonist effect (5). These data suggest that gonadotroph tumors may be associated with abnormalities in receptor structure and/or function. It is unknown whether continuous GnRH administration in vitro will lead to desensitization of gonadotropin secretion in human pituitary tumors. Experiments using normal perifused rat pituitary cells have demonstrated that gonadotropin secretion decreases during continuous GnRH administration for 30–120 min (27). Recovery of secretory responses is not immediate, and complete recovery can require up to 30 min. Studies investigating gonadotropin responses to continuous GnRH in human gonadotroph tumors in vitro will be necessary to investigate this question.

Administration of a GnRH antagonist to normal subjects (28) and normal rat pituitary cells in vitro (19) causes a consistent decrease in intact gonadotropin and α subunit secretion without a stimulatory agonist phase. In one study, a 3–12-mo course of the Nal-Glu GnRH antagonist to five men with FSH-secreting pituitary tumors significantly decreased serum FSH, LH, and testosterone levels, without affecting adenoma size (29). In vivo studies are limited by several factors. First, it cannot be determined whether changes in serum hormone levels in treated patients represent tumor versus normal gonadotropin responses. Second, how pulsatile GnRH stimulation controls neoplastic gonadotrophs has not been investigated. Therefore, the physiological regulation of human pituitary tumors by GnRH is unknown. Our present data demonstrate responsiveness to pulsatile GnRH in approximately one third of gonadotroph tumors. We found that all tumors included in this series had evidence of FSHβ biosynthesis using RTPCR techniques. Gonadotropin secretion was found in GnRH nonresponsive, as well as GnRH-responsive tumors. The presence of FSHβ biosynthesis in tumors nonresponsive to GnRH and lacking GnRH-Rc mRNA suggests that FSHβ biosynthesis in these tumors may be regulated by GnRH-independent mechanisms. In all GnRH-responsive tumors, administration of the GnRH antagonist Antide blocked gonadotroph secretory responses, consistent with effects seen in normal pituitary tissue. Recovery times of up to 20 min were tested, however, GnRH responsiveness after Antide blockade could not be demonstrated in the tumors studied. These experiments suggest that longer recovery periods are required to reestablish GnRH responsiveness of neoplastic gonadotrophs after Antide blockade.

Altered GnRH pulse characteristics are one mechanism of generating differential gonadotropin subunit gene expression and secretion in normal gonadotrophs. In vitro pituitary perifusion systems have facilitated the precise study of hormone and subunit regulation in response to physiological GnRH in experimental animals (27, 30–32). Recent studies using perifused normal rat pituitary cells have shown that variations in GnRH pulse amplitude have preferential effects on FSHβ subunit mRNA steady state levels (33). Therefore, gonadotropin subunit secretory phenotype in normal pituitary tissue may reflect underlying GnRH pulse patterns. Imbalanced gonadotropin subunit biosynthesis and secretion in gonadotroph tumors have been confirmed in vivo and in vitro. Elevated serum FSHβ-free subunit levels have been shown in patients with gonadotroph adenomas. We have recently demonstrated excess FSHβ subunit mRNA expression and FSHβ-free subunit secretion relative to α subunit (34). This finding is in contrast to normal pituitary tissue where α subunit is synthesized in excess of β subunit. Two of four of the GnRH-responsive tumors secreted FSHβ, whereas none of the GnRH-nonresponsive tumors had detectable levels of FSHβ-free subunit secretion during perifusion. It is unknown whether different frequencies of GnRH administration will alter gonadotropin-free subunit biosynthetic and/or secretion patterns in human gonadotroph tumors. The responses of normal and neoplastic gonadotrophs to variable intervals of GnRH administration may differ. Therefore, alterations in pulsatile GnRH may favor FSHβ secretion and play a role in the FSHβ phenotype observed in a large subset of tumors. However, we have not investigated whether GnRH-responsive tumors have higher levels of FSHβ mRNA levels than nonresponsive tumors. RTPCR data on FSHβ mRNA in perifused tumors in Fig. 1 are designed to be a qualitative marker of the gonadotroph origin of these tumors and do not reflect FSHβ mRNA steady state levels. Future perifusion studies using quantitative RTPCR techniques will be necessary to determine if GnRH pulsatility affects FSHβ mRNA levels in neoplastic gonadotrophs, as has been shown for normal individuals.

Studies of clinically nonfunctioning human pituitary tumor responses to hypothalamic signaling peptides, such as GnRH and TRH, may provide significant insights into the pathogenesis of neoplastic gonadotrophs. Studies using molecular genetic techniques have demonstrated the monoclonal nature of clinically nonfunctioning pituitary tumors and indicate that a somatic mutation is a required event for tumor initiation (9). The single-cell origin of such adenomas makes it unlikely that circulating factors (either hypothalamic peptides or other hormones) initiate tumor pathogenesis. However, studies of tumor responses to such peptides may elucidate underlying mutations that are responsible for biosynthetic and secretory defects. In addition, genomic alterations may also give pituitary cells a distinct growth advantage, as has been shown in somatotroph tumors (35).

The lack of detectable GnRH-Rc mRNA in GnRH unresponsive tumors suggests that receptor biosynthesis is deficient in a large subset of human gonadotroph adenomas. Alterations in GnRH-Rc biosynthesis may be the result of cell-signaling defects in neoplastic gonadotrophs. In one model of constitutive activation of GnRH pathways, continuous in vitro administration of GnRH to normal rat pituitary cells downregulated pituitary GnRH-Rc mRNA levels (14). This finding suggests that one mechanism of long-term desensitization of GnRH responses by GnRH agonists is to downregulate constitutive GnRH-Rc gene expression and mRNA steady state levels. Therefore, mutations that activate gonadotroph-specific GnRH second messenger pathways could chronically downregulate GnRH-Rc biosynthesis and desensitize neoplastic gonadotrophs to GnRH stimulation. The conserved structure of the cloned GnRH-Rc confirms biochemical data that its effects on phospholipases and subsequent phosphoinositol turnover is likely mediated by interactions with the Gqα family of GTP-binding proteins (15, 16). Constitutive activation of Gqα has mitogenic and transforming effects in NIH3T3 mouse fibroblasts (17). It is unknown whether activating mutations in second messenger systems could potentially have cell proliferative effects as well as desensitizing GnRH-signaling pathways that modulate gonadotropin biosynthesis and secretion.

The gonadotroph origin and phenotype of clinically nonfunctioning pituitary tumors have been described by several groups. We have confirmed the gonadotroph phenotype of these tumors both at the biosynthetic and secretory levels. All 13 tumors secreted LH, FSH, and/or FSHβ in perifusion. We have previously shown underlying biosynthetic and secretory abnormalities in clinically nonfunctioning pituitary tumors. In contrast to the secretion of both LH and FSH by normal pituitary tissue, preferential secretion of FSH and FSHβ-free subunit is seen in a subset of tumors (34). We have confirmed these findings in this group of tumors by performing RTPCR for FSHβ mRNA. All 13 tumors synthesized FSHβ mRNA in addition to GPH α subunit and GapDH, consistent with their gonadotroph phenotype in perifusion. Several lines of evidence suggest that normal tissue contamination is not a complicating factor in this study. First, the 13 perifused gonadotroph tumors in this study do not exhibit significant in vitro secretion of GH, PRL, and TSH, an expected result if perifused gonadotroph tumors were composed of large numbers of normal pituitary cells. Second, we might also expect a histological description of normal tissue in resected tumor samples, however, all tissue received was histologically diagnosed as adenoma tissue. Third, human pituitary tumors have been demonstrated to be genetically monoclonal in origin (9, 36–40). Because contamination of normal pituitary tissue would give a polyclonal result, the consistent finding of monoclonality in both macro- and microadenomas argue strongly against normal tissue contamination in our samples. Fourth, our finding that one third of human gonadotroph adenomas are responsive to GnRH in vitro is in agreement with previous studies documenting both secretory (6, 7) and intracellular (8) responses to GnRH in static culture. These complementary approaches strongly suggest that normal pituitary tissue contamination of resected human gonadotroph tumors is not a factor in our analysis.

In contrast to the uniform LH and FSH responses to physiological GnRH in normal pituitary cells, only one third of pituitary tumors are GnRH responsive in vitro. We have demonstrated the presence of GnRH-Rc in human pituitary gonadotropin-secreting adenomas. GnRH-Rc transcripts were detected only in responsive tumors, and the levels were comparable to those seen in normal human pituitaries. Therefore, GnRH-Rc mRNA steady state levels in such tumors predict GnRH responsiveness in vitro. These studies suggest that GnRH nonresponsiveness in gonadotroph tumors may be due to GnRH-Rc biosynthetic defects. In addition, the documentation of FSHβ biosynthesis in tumors lacking GnRH-Rc mRNA and responsiveness to exogenous GnRH suggests the importance of GnRH-independent mechanisms in the regulation of gonadotropin biosynthesis in a subset of tumors. Future investigations will need to characterize such defects and determine their role in tumor pathogenesis.

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