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Expression of the Growth Hormone Secretagogue Receptor in Pituitary Adenomas and Other Neuroendocrine Tumors*

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ABSTRACT

Synthetic GH secretagogues (GHSs; GH-releasing peptides and their nonpeptide mimetics) stimulate GH release, activate the hypothalamo-pituitary-adrenal axis, and release PRL *in vivo*. Patients with acromegaly show an exuberant GH response to GHSs, whereas patients with pituitary-dependent ACTH-secreting tumors show an exaggerated rise in ACTH and cortisol. We, therefore, studied the presence of GHS receptor (GHS-R) messenger ribonucleic acid (RNA) in 38 human pituitary tumors of different cell types, 3 ectopic ACTH-secreting tumors, a pancreatic gastrinoma, 3 insulinomas, and a nonsecreting thymic carcinoid as well as in 7 normal pituitary glands. Certain pituitary tumors were also studied by *in vitro* cell culture with measurement of secreted GH, ACTH, PRL, FSH, LH, α-subunit, and TSH. RNA was extracted from tissue samples and, after RT, a duplex PCR reaction with primers for the GHS-R gene and for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase was performed, allowing semiquantitation of GHS-R expression.

All the somatotroph adenomas (n = 8) showed a 2–10 times higher expression of the GHS-R gene compared to normal pituitaries. Higher than normal expression was shown in 5 of 18 tumors from patients with ACTH-secreting pituitary adenomas and in 1 of 3 ectopic ACTH-secreting carcinoid tumors. Two of the pituitary ACTH-secreting adenoma samples showed completely absent expression of the GHS-R, 8 showed expression similar to that of normal pituitary tissue, and 3 of the corticotroph adenoma tissue samples and 2 ectopic ACTH-secreting tumors showed a very low level of expression. One of 4 prolactinoma samples showed a high level of expression, 1 showed expression similar to that of normal pituitary, and 2 samples showed a very low level of expression. Nonfunctioning pituitary adenoma samples showed either absent or very low level expression of the GHS-R. The pancreatic gastrinoma sample showed expression similar to that of normal pituitary tissue, whereas 3 insulinomas showed low level expression of the GHS-R gene; a nonsecreting thymic carcinoid tumor showed no detectable expression.

In summary, although GHS-R messenger RNA is abundant in human somatotroph adenomas, it is also present in other pituitary adenomas, particularly ACTH-secreting tumors. These findings may explain the *in vivo* responses to GHSs in patients harboring such tumors. It also appears from our study that GHS-R may be expressed in other neuroendocrine tumors. (*J Clin Endocrinol Metab* **83:** 3624–3630, 1998)

GH SECRETAGOGUES (GHSs), including GH-releasing peptide-6 (GHRP-6) and nonpeptide pharmacological analogs, stimulate GH release *in vivo*. These effects occur through mechanisms that involve the hypothalamus and the pituitary, although the hypothalamic effect is probably predominant (1, 2). They also stimulate the hypothalamo-pituitary-adrenal axis, causing an elevation in circulating ACTH and cortisol levels (1, 3–7). This effect is thought to occur via the hypothalamus, at least in the rat, as GHRP-6 does not cause ACTH release from the rat pituitary *in vitro* (8, 9). In the human, the magnitude of the rise in ACTH and cortisol levels in healthy volunteers is similar to that after the injection of either CRH or arginine vasopressin, the two main hypothalamic releasing factors for ACTH (10). GHSs also stimulate PRL release and have been shown to produce sleepiness in humans (6). A seven-transmembrane domain, G protein-coupled receptor for GHSs has been identified in human pituitary and hypothalamic tissue; its gene is located on chromosome 3 and codes for a receptor that shows little resemblance to other G protein-coupled hormone receptors (11). This GHS receptor (GHS-R) is present in several brain nuclei of the rat, but the principal sites of expression in humans are the hypothalamus, pituitary gland, and hippocampus, although using a sensitive ribonuclease protection assay it has also been detected in the pancreas (12, 13).

The effects of GHSs have been studied in patients with pituitary adenomas. GHS stimulate GH release in patients with acromegaly, but do not stimulate further PRL release in patients with prolactinomas (14–17). *In vitro*, human somatotroph adenoma cells also respond to stimulation with GHSs (18). The most striking results, however, came from patients with Cushing's syndrome. Ten patients with pituitary-dependent ACTH-secreting adenomas each showed a markedly exaggerated ACTH and cortisol response to hexarelin, one of the peptide GHSs (19). The rise in ACTH

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and cortisol after hexarelin was considerably higher than that after a near-maximal dose of CRH, whereas two patients with ectopic ACTH-secreting tumors showed no response.

Messenger ribonucleic acid (mRNA) quantitation has previously been used extensively to study gene expression in human pituitary adenomas (20, 21). Recently, human fetal pituitary cells has been shown to express and respond to GHRP-6 (22). We speculated that the GHS-R might be expressed in human pituitary adenomas arising from different cell types, particularly in tumors from patients with pituitary-dependent Cushing's syndrome, and Cushing's disease. Carcinoid tumors are also known to secrete a wide range of neuroendocrine hormones and contain a number of neurohormonal receptors (23). We therefore studied 41 pituitary tumors, 3 ectopic ACTH-secreting tumors, 4 pancreatic tumors, 1 gastrinoma, and 3 insulinomas, as well as a nonsecreting thymic carcinoid tumor, and compared the results with those from 7 normal pituitary glands. Our results clearly show the presence of GHS-R mRNA in a variety of different tumor types and suggest that the presence of GHS-R in corticotropinomas may well be responsible for the aberrant ACTH and cortisol responses to GHSs seen in patients with this condition.

Materials and Methods

Tissues studied

Pituitary adenomas were obtained at the time of transsphenoidal surgery (except for an ACTH-secreting pituitary carcinoma, which was obtained at autopsy). The tumor type was determined on the basis of clinical and biochemical findings before surgery, from morphological and immunocytochemical data, and with *in vitro* cell culture studies in some cases. The size of the pituitary tumors was determined by computed tomography/magnetic resonance imaging. Carcinoid tumors and pancreatic tumors were obtained at surgery. Seven normal human pituitaries were collected at autopsy (4–24 h postmortem) from patients with no evidence of endocrine abnormality.

A total of 41 pituitary adenomas was studied: 8 somatotroph adenomas, 4 lactotroph adenomas, 10 nonfunctioning pituitary adenomas (NFPA), 18 corticotroph tumors including 1 corticotroph carcinoma, and 1 FSH-secreting adenoma. Three ACTH-secreting ectopic tumors (of the bronchus, thymus, and pancreas), 3 pancreatic insulinomas, 1 gastrinoma, and 1 thymic nonsecreting carcinoid tumor were also studied. In addition, normal human kidney, liver, and peripheral lymphocytes and a parasellar meningioma were analyzed.

RT-PCR

Total RNA was obtained and reverse transcribed into complementary DNA (cDNA) by a standardized technique as described previously (24). The integrity of mRNA from each specimen was verified by RT-PCR for the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH; GenBank accession no. M33197). RT-PCR, with omission of reverse transcriptase and with water replacing template, was used as the negative control. The PCR was performed using primers spanning one or more introns of the genes studied to allow for exclusion of genomic DNA contamination (Table 1). Primers for the GHS-R gene (GenBank accession no. U60179) gave rise to a product of 484 bp; this product was analyzed by restriction enzyme analysis (Fig. 1a) and then by direct sequencing, confirming the expected product. A PCR reaction for the Pit-1 (GenBank accession no. D10216) gene, which is only expressed in somato-, lacto-, and thyrotroph cells, was also performed on ACTH- and FSH-secreting tumors and on NFPAs. We used previously published primers that gave rise to a product of 560 bp (25).

The overall expression of the gene was determined by duplex PCR using the GHS-R primers relative to the expression of GAPDH, according to a previously validated semiquantitative technique (26, 27). All PCRs were performed before the plateau phase of the synthesis curve. A cycling curve with the GHS-R and GAPDH primers shows parallel amplification (Fig. 1b). We used 100 ng cDNA, 200 μmol/L deoxynucleotides (Promega Corp., Southampton, UK), 0.5 μmol GHS-R primers, 0.1 μmol GAPDH primers, 1.5 mmol/L $MgCl_2$, 0.125 U *Taq* (Promega Corp.), and TaqStart antibody (Clontech, Heidelberg, Germany), according to the manufacturers' guidelines, in a 25 μL PCR reaction. For GHS-R and GAPDH duplex PCR reaction, 28 cycles were performed at 94 C for 1 min, 54 C for 1 min, and 72 C for 1 min after a denaturing cycle of 95 C for 5 min. For the Pit-1 gene reaction, we used a 0.2 μmol primer concentration, and 30 cycles were performed at 94 C for 30 s, 55 C for 30 s, and 72 C for 45 s after a denaturing cycle of 95 C for 3 min. A final extension cycle of 10 min at 72 C was used. The PCR products were run on 2% ethidium bromide-stained agarose gels. The absorbance values were measured for each band by densitometry (model DS670 image densitometer, Bio-Rad Hemel-Hempstead, Hertfordshire, UK), using the Molecular Analyst PC software for Bio-Rad's Image Analysis systems, and expressed as optical density units. A ratio between GHS-R and GAPDH was obtained for each sample. Each cDNA sample was assayed with duplex PCR on at least 2 separate occasions. Our normal pituitary tissues defined a normal range of expression of the GHS-R gene relative to the expression of GAPDH. If no expression of the GHS-R gene was shown at 28 cycles in the duplex PCR, a single PCR with the GHS-R primers only was performed at 34 cycles to determine whether any expression was present at a lower level. In the description of our data we use the terms similar, very low, high, and undetectable levels of expression to describe tumors with a level of expression (GHS-R/GAPDH ratio) within the range of the normal samples, below this range but still detectable at 34 cycles, above this range (with a ratio >2 SD above the mean of the normal samples), or no detectable level of expression (no band observed at 34 cycles), respectively.

Contamination of ACTH- and FSH-secreting tumors and NFPAs by somato-, lacto-, or thyrotroph cells of nontumorous tissue was excluded by confirming undetectable expression of the Pit-1 gene. In normal pituitary tissue cDNA we were still able to detect Pit-1 and GHS-R expression at a 1:50 dilution, suggesting that contamination of tumor samples with normal pituitary would be detected at this level. Three NFPA were considered noninformative because Pit-1 mRNA was expressed, indicating possible contamination with nontumorous tissue in the adenoma specimen; these were excluded from further analysis. The final analysis, therefore, included 38 pituitary adenomas. Clinical details are shown in Tables 2a and 2b.

Pituitary tumor cell culture

Pituitary adenoma tissue was transported to the laboratory in DMEM containing 10% heat-inactivated FCS, 0.06 g/L penicillin, 0.1 g/L streptomycin, and 2.5 g/L fungizone and buffered with HEPES (0.02 mmol/L), hereafter referred to as culture medium. Tumor tissue was washed three times with phosphate-buffered saline, cut into small pieces with a sterile scalpel, and dispersed by incubation for 40 min at 37 C in phosphate-buffered saline containing ethylenediamine tetraacetate (0.5 mmol/L) and 0.125% trypsin with periodic titration. Dispersed cells were harvested by centrifugation, washed once, and subsequently resuspended in culture medium. Cell viability was assessed using trypan blue exclusion and was more than 90% of the cells in all tumors studied

TABLE 1. Primer sequences

Gene	Sense primer	Antisense primer	Product size
GHS-R	5′-GAACTTCGGCGACCTCCT-3′	5′-AAACACCACTACAGCCAGCA-3′	484
PIT-1[a]	5′-AGTGCTGCCGAGTGTCTACCA-3′	5′-TTTCTTTTCCTTTCATTTGCT-3′	560
GAPDH	5′-CCATGGAGAAGGCTGGGG-3′	5′-CAAAGTTGTCATGGATGACC-3′	196

[a] Primers were published by Haddad *et al.* (25).

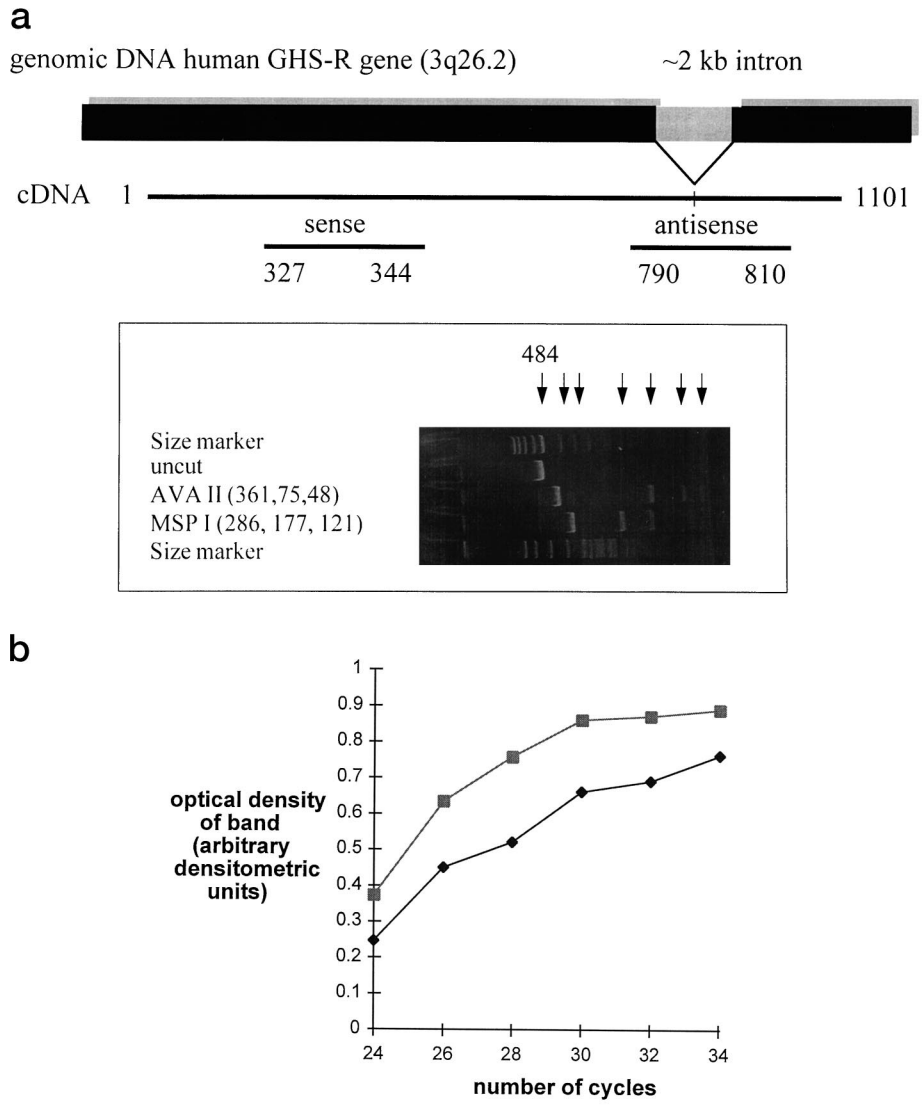

FIG. 1. a, Schematic representation of the GHS-R gene; the sense and antisense primers are marked by the base numbers framing the oligonucleotides. Restriction enzyme analysis of the GHS-R PCR product is shown with the expected fragment sizes in *brackets* and marked by *arrows*. b, Cycle curve for GHS-R and GAPDH primers in normal pituitary tissue. ■, GAPDH; ◆, GHS-R.

after cell dispersion. Cell yield from each tumor varied from 1–65 × 10^6 cells.

The cells were plated in 24-well plates at approximately 10^5 cells/well in 2 mL medium. Cultures were incubated at 37 C in a humidified atmosphere of 95% air and 5% CO_2 for 48 h to allow cell attachment to occur, after which time the medium was collected. All pituitary hormones, including the glycoprotein α-subunit, were measured in medium, as described previously (28).

Statistical analysis

Absorbance ratios of the GHS-R and the GAPDH bands were calculated in the duplex PCR reaction. ANOVA followed by *post-hoc* tests were used to calculate differences between groups of tissues. Significance was taken at $P < 0.05$.

Results

All tissues from patients with acromegaly showed an increased ratio of expression of the GHS-R gene compared to the normal pituitary samples ($P < 0.001$; Figs. 2-4). The GHS-R/GAPDH absorbance ratio for normal pituitary tissue ranged between 0.09–0.4; in acromegalic tissue, the GHS-R/GAPDH ratio ranged between 0.85–1.5, resulting in 2- to 10-fold expression of the GHS-R gene in acromegalic tissue compared to normal pituitary. A representative gel of a duplex PCR is shown in Fig. 3, comparing normal pituitary tissue with sample from patients with acromegaly or nonfunctioning tumors. Figure 4 shows the *in vivo* GH response to iv administration of 2 μg/kg hexarelin in 12 healthy volunteers and 1 of the acromegalic patients whose tumor tissue was also studied [subject 4; details of the protocol of hexarelin administration have been published (6)]. The *lower part* of Fig. 4 shows a cycling curve of a normal cadaver pituitary and of acromegalic pituitary tissue. An increased GHS-R/GAPDH ratio was still present when 2-fold diluted acromegalic samples were used to control for the fact that normal pituitary tissue comprises approximately 50% somatotrophs (29).

Higher than normal expression (GHS-R/GAPDH ratio >2 SD above the mean value in normal tissue) was also shown in five patients with ACTH-secreting pituitary adenomas (GHS-R/GAPDH ratio range, 0.53–1.1) and in the ectopic ACTH-secreting bronchial carcinoid (GHS-R/GAPDH ratio,

TABLE 2a. Clinical data of patients with pituitary tumors

Diagnosis	Patient no.	Age (yr)	Sex	*In vitro* hormone secretion	Immunocytochemistry	Tumor size
Acromegaly	1	26	M	GH, TSH, αSU	LH, FSH, TSH, GH, PRL	Macro
	2	36	M	GH, LH		Macro
	3	44	F	GH, PRL, LH, FSH, αSU	GH, PRL, TSH	Macro
	4	61	F	GH, PRL, TSH, αSU	GH, PRL, αSU	Micro
	5	29	F	GH, PRL, LH	GH	Macro
	6	64	F	GH, PRL, αSU	GH, PRL, FSH, TSH	Macro
	7	22	M	GH	GH, αSU	Macro + SSE
	8	24	F	GH (PRL, αSU)	GH	Macro
Prolactinoma	9	36	M	PRL, TSH	PRL, TSH (GH)	Macro
	10	53	M		PRL	Macro
	11	23	F	PRL, αSU	PRL	Micro
	12	37	M		PRL	Macro
NFPA	13	59	M	LH, FSH, αSU	All negative	Macro + SSE
	14	52	M	All negative	(αSU)	Macro + SSE
MEN 1	15	38	M	(αSU)	All negative	Macro + SSE
	16	64	M	LH, FSH, αSU	αSU, FSH, LH	Macro
	17	80	F	All negative	(LH, αSU)	Macro + SSE
	18	68	M	All negative	All negative	Macro + SSE
	19	41	M	LH, FSH, αSU	All negative	Macro
Cushing's disease	20	34	M		ACTH	Macro, metastatic
	21	59	M		ACTH	Macro
	22	24	F		ACTH	Micro
	23	25	F		ACTH	Micro
	24	44	F		ACTH	Micro
	25	21	F		ACTH	Micro
Nelson's syndrome	26	28	F		ACTH	Macro
	27	42	F		ACTH	Micro
	28	69	F		ACTH	Macro
	29	24	F		ACTH	Micro
	30	21	M		ACTH	Macro, invasive
	31	26	F		ACTH	Macro, invasive
Nelson's syndrome	32	47	F		ACTH	Micro
	33	60	F		ACTH	Macro
	34	64	F		ACTH, αSU	Macro
Nelson's syndrome	35	49	M		ACTH	Macro, invasive
	36	31	M		ACTH	Macro
	37	74	F		ACTH	Macro
FSHoma	38	74	M	FSH (αSU)	FSH, GH, PRL	Macro

NFPA, Nonfunctioning pituitary adenoma; Macro, macroadenoma (>10 mm); Micro, microadenoma (<10 mm); SSE, suprasellar extension. Hormone name in *parentheses* indicates minimal presence (that is, for *in vitro* experiments: hormone secretion for αSU, 0.2–4.2 ng/24 h · 10^6 cells; for PRL, 50–240 μU/24 h·10^6; and for immunocytochemistry, only rare/scattered cells showing positivity).

TABLE 2b. Clinical data of patients with nonpituitary tumors

Diagnosis	Patient no.	Age (yr)	Sex	Immunocytochemistry
Ectopic ACTH-secreting tumor				
Bronchial	39	49	F	ACTH, chromogranin, bombesin, serotonin
Thymus	40	55	M	ACTH, chromogranin
Pancreas, metastatic	41	66	F	ACTH, chromogranin
Gastrinoma, metastatic, MEN 1	42	42	F	Gastrin, chromogranin, glucagon, PPP, (insulin)
Insulinoma MEN 1	43	26	F	Insulin, VIP
Insulinoma, metastatic	44	57	M	Insulin
Insulinoma	45	69	M	Insulin, chromogranin, (somatostatin)
Nonsecreting thymic carcinoid MEN 1	46	56	M	Chromogranin

PPP, Pancreatic polypeptide; VIP, vasoactive intestinal polypeptide.

2.5). Figure 6 shows a representative ACTH-secreting pituitary sample with GHS-R expression and no detectable Pit-1 expression. Two ACTH-secreting pituitary adenoma samples showed absent expression of the GHS-R. Eight showed similar expression to normal pituitary tissue. Three corticotroph tumors, including the corticotroph carcinoma and pancreatic and thymic ACTH-secreting tumors, showed low levels of expression (present at 34 PCR cycles, but not at 28

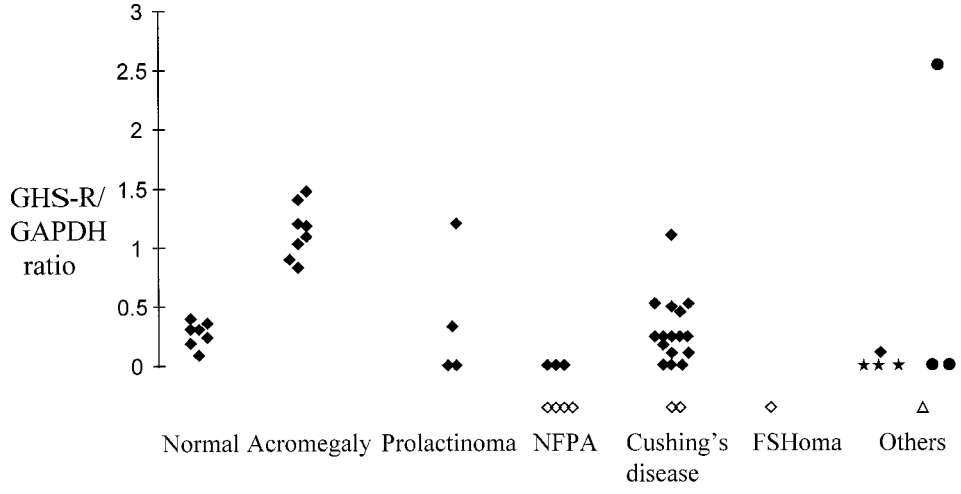

FIG. 2. Relative expression of the GHS-R gene at 28 cycles in tissue from 7 normal pituitaries, 8 somatotroph tumors, 4 prolactinomas, 7 nonfunctioning pituitary adenomas, 18 corticotroph adenomas, an FSHoma, and nonpituitary tumors, including 3 ectopic ACTH-secreting tumors (●), 3 insulinomas (★), 1 gastrinoma (♦), and 1 nonsecreting thymic carcinoid tumor (▲). *Open symbols* represent samples with no detectable expression under any conditions; *filled symbols* at the zero GHS-R/GAPDH ratio represent samples with very low level expression.

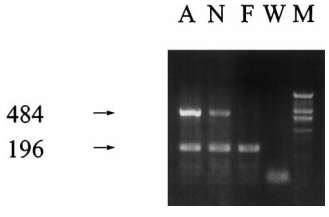

FIG. 3. A representative 2% agarose gel of a duplex PCR 28 cycles of the GHS-R gene (*upper band*) and of GAPDH (*lower band*). Product size is shown on the *left*. A, An acromegalic sample (subject 3); N′, normal pituitary; F, nonfunctioning pituitary adenoma (subject 16, with no detectable expression of the GHS-R gene); W, water control; M, size marker Phi X174 DNA/*Hinf*-1 digest.

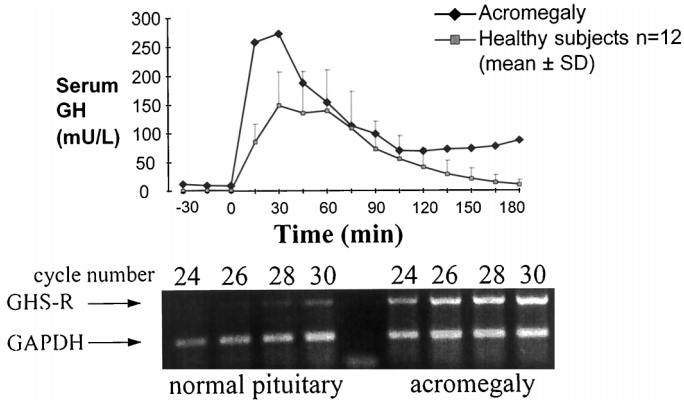

FIG. 4. The *upper part* of the figure shows the effect of 2 μg/kg hexarelin in 12 healthy volunteers (■) and in a patient with acromegaly (♦, subject 4). To convert the value for GH to micrograms per L, multiply by 0.37. The *lower part* of the figure shows two cycling curves for the GHS-R-GAPDH duplex PCR to represent the difference between normal and acromegalic samples. The number of cycles is shown *above the lanes*.

cycles). One of 4 prolactinoma samples showed expression similar to normal, 2 showed very low level of expression, and the fourth showed high expression (GHS-R/GAPDH ratio, 1.2). Although it is difficult to exclude that this sample contained normal somatotrophs, the expression of the gene was higher than that of normal tissue, suggesting that the increased expression was unlikely to be due to contaminating normal tissue. Four NFPA samples showed no expression of the GHS-R, whereas the remaining 3 showed a very low level of expression. Figure 5 shows representative cycling curves of the pituitary tumor samples.

A pituitary sample (not included in the analysis above) that was removed during transsphenoidal surgery and showed a normal *in vitro* secretion pattern, normal histology, and normal immunocytochemistry, had a GHS-R/GAPDH ratio similar to that of normal pituitaries removed at autopsy, suggesting that excessive RNA degradation probably did not confound the data of the autopsy samples. The results from the *in vitro* secretion data, which were all concordant with the clinical diagnosis and immunocytochemistry data, are shown in Table 2a.

Full clinical details of the nonpituitary tumors are given in Table 2b. Three patients presented with classical recurrent hypoglycemic attacks and after the removal of the pancreatic lesion(s), consistent with insulin-secreting islet cell tumors, showed clinical and biochemical cure; all three insulinoma samples showed low level expression of the GHS-R gene. The gastrinoma sample, from a patient presenting with recurrent peptic ulceration, showed expression similar to that of normal pituitary. None of the patients with nonpituitary tumors showed signs of GH excess. A nonsecreting thymic carcinoid tumor, from a patient with the multiple endocrine neoplasia syndrome type 1, showed no detectable expression of the GHS-R gene. No GHS-R expression was found in human kidney, lymphocytes, or liver or in a tissue sample from a parasellar meningioma (data not shown).

Discussion

Our data confirm that GHS-R mRNA is present in normal human pituitary cells. We also found increased expression of the GHS-R gene in all of the somatotroph tumors, even when controlling for the number of somatotroph cells in the sample. Recently, it has been shown that the amount of GHRH expressed in somatotroph adenomas correlates with tumor aggressivity and prognosis (30). Although GHSs do not seem to be as directly involved in cell proliferation and growth as GHRH, the expression of the GHS-R might also influence local tumor growth in pituitary adenomas, especially if the putative ligand is also expressed.

It has recently been reported that GHS-R is present only on

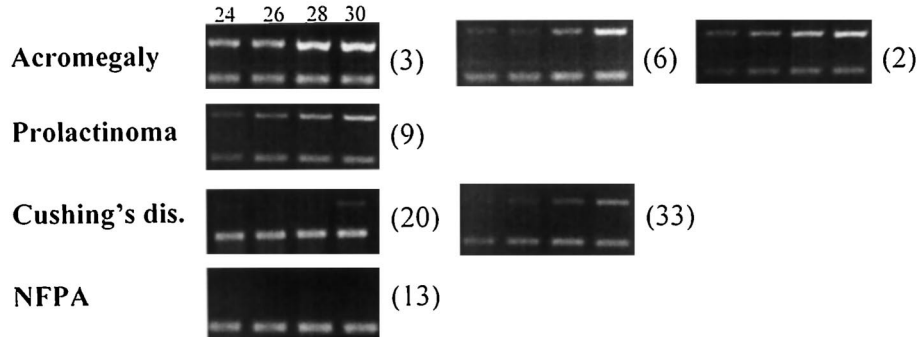

FIG. 5. Representative cycling curves for the GHS-R-GAPDH duplex PCR between 24 and 30 cycles for 3 acromegalic, 1 prolactinoma, 2 Cushing's disease, and 1 NFPA sample. Numbers in *brackets* refer to subject numbers in Table 2a.

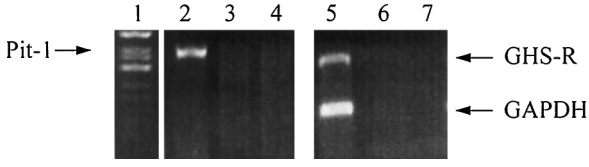

FIG. 6. A representative 2% agarose gel shows size marker *Phi*X 174 DNA/*Hinf*-1 digest in lane 1. An ACTH-secreting pituitary adenoma (subject 28) shows no Pit-1 gene expression (lane 3), suggesting that no somatotroph cells are present in the sample, but the presence of GHS-R is detectable in a duplex PCR with GAPDH (lane 5). A positive control for the Pit-1 gene from a prolactinoma sample (lane 2), a water control for the Pit-1 PCR reaction (lane 4), RT-PCR reaction without the RT enzyme on sample 28 (lane 6), and a water control for the GHS-R-GAPDH duplex PCR reaction (lane 7) are shown.

the somatotrophs of rat pituitary and is absent from other cell types (2). Our data clearly show that in corticotroph pituitary adenomas, GHS-R may be variably expressed, although some ACTH-secreting tumors show no detectable expression. Five ACTH-secreting pituitary adenomas showed higher than normal expression of the GHS-R gene. A significant proportion of the remaining tumors showed a level of expression similar to that of normal pituitary tissue despite the fact that no detectable Pit-1 gene expression was found, suggesting that the samples were not contaminated with nontumorous pituitary tissue (Fig. 6). These data suggest that the receptor must be present in some of the corticotroph adenoma cell population and are in accordance with clinical data showing exaggerated ACTH responses to GHSs in this group of patients (19). Adams *et al.* recently reported their findings of the presence of GHS-R mRNA and *in vitro* responsiveness to GHRP-2 in human somatotropinomas, prolactinomas, and nonfunctioning pituitary tumors; however, they did not report any data from corticotroph adenomas (31). Their findings are concordant with our demonstration of GHS-R expression, although double labeling immunochemistry would be necessary to show the precise cell types expressing the GHS-R.

Little is known concerning the regulation of expression of the GHS-R gene, although it has been reported that estrogens may have a positive effect on GHS-R expression (32). It has also been shown that GH-deficient rats show overexpression of the receptor (33). Interestingly, one of our patients with a macroprolactinoma (no. 9) had been GH deficient over the preceding 2 yr before surgery, with subnormal GH reserve on dynamic tests and low IGF-I levels compared to an age-matched reference range; this patient showed a higher than normal expression of the GHS-R gene. However, some of our patients with NFPA were also GH deficient at the time of the transsphenoidal surgery, with very low or undetectable GHS-R expression. An alternative explanation for the overexpression of the GHS-R gene in patient 9 is possible: during the *in vitro* hormone secretion test, this tissue released TSH as well as PRL, and TSH was also positive in the tumor tissue on immunocytochemistry. It is possible that this tumor arose from a progenitor of the somato-, lacto-, and thyrotroph cell population that might express the GHS-R gene.

Ectopic ACTH-secreting tumors may arise from many sites, including the lung, pancreas, and thymus. These tumors characteristically produce and secrete a whole range of neuroendocrine peptides. We can now add another member to this list of these peptides and receptors: the GHS-R. Low level expression was found in a thymic and a pancreatic ACTH-secreting carcinoid, and very high expression was found in a bronchial carcinoid sample, whereas no expression was found in a nonsecreting thymic carcinoid tumor. A GHRH- and ACTH-secreting carcinoid tumor has recently been reported to respond to GHRP-6 stimulation with calcium influx (34), confirming our data demonstrating the presence of the GHS-R in such tumors. Ghigo *et al*. (19) have suggested that GHSs may be useful in the differential diagnosis of pituitary *vs.* ectopic ACTH-secreting tumors, because their two patients with ectopic ACTH-secreting tumors did not respond to GHS with any stimulation of the pituitary-adrenal axis, whereas patients with pituitary-dependent Cushing's disease showed an exaggerated response. Our finding of the presence of the GHS-R in ectopic tissue suggests that patients with tumors expressing this receptor may also respond to GHSs. Indeed, suggestions that other neuropeptide secretagogues may completely discriminate between ectopic and eutopic ACTH secretion have generally been disproved; thus, desmopressin responsiveness was recently reported in a bronchial carcinoid that expressed vasopressin receptors (35), and such absolute distinction may never be possible. However, detection of GHS-R transcripts in human pituitary adenomas and other tumorous tissue does not necessarily imply translation and functional protein expression, and further studies are needed to detect the GHS-R protein in these samples. The presence of GHS-R in ectopic endocrine tumors is not surprising, but the role of these receptors is unclear; the possible targeting (blocking) of the receptor suggests that this could be the basis for new therapeutic approaches.

In summary, we have shown enhanced expression of the GHS-R in a proportion of pituitary tumors, especially soma-

totroph and corticotroph tumors. We speculate that this expression is responsible for the marked GH and pituitary-adrenal responses, respectively, to GHSs seen in many patients with these tumors. GHS-R may also be expressed in nonpituitary neuroendocrine tumors.

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