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Multifunctional Cells in Human Pituitary Adenomas: Implications for Paradoxical Secretion and Tumorigenesis

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Pituitary adenomas are very common in humans. They are of monoclonal origin, very heterogeneous, and produce frequently paradoxical secretion. The normal anterior pituitary (AP) contains some unorthodox multifunctional cells able to store more than one AP hormone (polyhormonal) and/or to express multiple hypothalamic-releasing hormone receptors (multiresponsive). Multifunctional AP cells seem to be involved in plasticity processes such as transdifferentiation or paradoxical secretion. Here, we have characterized the single-cell phenotypes of 15 human pituitary tumors, including prolactinomas, nonfunctioning adenomas, and adenomas from multiple endocrine neoplasia type I (MEN-I) and pituitary Cushing's disease patients. Individual tumor cells were typed according to expression of AP hormones and hypothalamic-releasing hormone receptors by combination of calcium imaging and multiple sequential immunocytochemistry in the same cells. We found a large heterogeneity among the different tumors. In eight of the 15 tumors studied, more than 80% of the cells presented a multifunctional phenotype. This may explain the occurrence of paradoxical secretion. In addition, our results suggest that human pituitary adenomas might derive from multifunctional cells. This is consistent with the existence of a link between pituitary plasticity and tumorigenesis. (*J Clin Endocrinol Metab* 89: 4545–4552, 2004)

PITUITARY ADENOMAS ARE benign neoplasms of adenohypophysial cells. They represent the most common neoplasm of the sellar region, amounting approximately 15% of all primary intracranial tumors. In studies of unselected adult autopsy material, their frequency as an incidental finding varies between 5 and 20% in different studies. Pituitary adenomas cause considerable morbidity due to local invasion, hypopituitarism, or hormone hypersecretion (1, 2). Evidence indicates that pituitary adenomas are of monoclonal origin (3). This pathogenesis should give rise to five well-defined tumor types, derived from each one of the normal anterior pituitary (AP) cell types. Contrary to this expectation, pituitary adenomas are extremely heterogeneous and have been difficult to type (4). They are classified according to morphometric and secretory characteristics (1, 4) as prolactinomas [excess prolactin (PRL) secretion], GH-omas or somatotrophinomas (excess GH secretion leading to gigantism or acromegalia), ACTHomas (resulting in pituitary Cushing's disease), nonfunctioning adenomas (those not leading to excess hormone secretion or endocrine changes), and the least frequent adenomas secreting either TSH or gonadotropins. Finally, although less common, some pituitary tumors produce more than one pituitary hormone. A comprehensive characterization of pituitary tumors according to their cell phenotypes is lacking.

Pituitary tumors are also frequently characterized by the so-called paradoxical secretion (5, 6), which is secretion of a given AP hormone induced by a noncorresponding hypothalamic-releasing hormone (HRH). For instance, paradoxical secretion of GH induced by TRH and/or LHRH has been variably reported in pituitary tumors (5, 7). Multihormonal responses to CRH have been reported in Cushing's disease (8). GHRH has been reported to induce PRL secretion both in acromegalic (9) and Cushing's syndrome patients (10). The underlying mechanisms are not known. Paradoxical secretion has also been sporadically reported in the normal pituitaries, both *in vivo* and *in vitro*, including in healthy humans (10–12).

It has been shown recently that normal mouse and rat AP contain cells expressing multiple HRH receptors (13–16), which could be responsible for paradoxical secretion of PRL (15). A growing body of evidence suggests that, contrary to the orthodox view, 20–40% of the rat and the mouse AP cells are multifunctional and exhibit mixed phenotypes, storing multiple AP hormones (polyhormonal) and/or expressing multiple HRH receptors (multiresponsive) and/or multiple AP hormone mRNAs (15–17). It has been suggested that multifunctional AP cells may be involved in cell plasticity processes directed to increase hormone production during demanding physiologic or pathophysiologic situations such as lactation, ovulation, hypothyroidism, *etc.* (18–20). Whether human pituitary adenomas do contain cells with

Abbreviations: AP, Anterior pituitary; $[Ca^{2+}]_i$, cytosolic calcium concentration; HRH, hypothalamic-releasing hormone; MEN-I, multiple endocrine neoplasia type I; MSPI, multiple sequential primary immunocytochemistry; PRL, prolactin.

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mixed phenotypes is not known, but if they do, that could help to explain paradoxical secretion and/or tumorigenesis.

Here we have characterized the cell phenotypes present in human pituitary adenomas according to the AP hormones stored and the HRH receptors expressed. This was achieved by a novel approach that combines calcium imaging of freshly dispersed cells and multiple sequential primary immunocytochemistry (MSPI) of the six AP hormones in the same cells (16). Using this methodology, we have typed individual cells from 15 pituitary tumors including five prolactinomas, three pituitary adenomas from multiple endocrine neoplasia type I (MEN-I) patients, four nonfunctioning adenomas, and three adenomas from pituitary Cushing's disease patients. The phenotypic characteristics were very heterogeneous not only among different types of tumors but also among different tumors of the same class. Many tumor cells expressed multiple receptors for different HRHs providing the basis for paradoxical secretion in human pituitary adenomas.

Materials and Methods

Materials

Antisera against human AP hormones FSHβ (AFP891891), GH (AFPC11981A), LHβ (AFP55951889), PRL (AFP55781789), TSHβ (AFP55741789), and ACTH (AFP39032082Rb) were generous gifts from the National Hormone and Pituitary Program (Torrance, CA) and Dr. A. F. Parlow. The human HRHs (GHRH, TRH, LHRH, and CRH) were purchased from Sigma (Madrid, Spain). Fluorescent antibodies were prepared by labeling with Oregon Green 488, Cascade Yellow, or Alexa 350 and purified over a protein A-Sepharose column (16). Fura-2/AM, Oregon Green 488-isothiocyanate, Cascade Yellow succinimidyl ester, and Alexa 350 succinimidyl ester were purchased from Molecular Probes (Eugene, OR).

Pituitary tumor cell culture

All the procedures used here were approved by the university hospitals and School of Medicine ethical committees. Selected patients were asked to read carefully and, if pertinent, to sign the informed consent form approved by the university hospital ethical committee. Fresh pituitary tumoral tissue was obtained from patients from the two Valladolid University hospitals (Valladolid, Spain) at the time of surgery. Extreme care was taken to ensure that samples for analysis were devoid of any contaminating normal tissue. The adenoma nature as well as the lack of contamination of the samples was confirmed later by pathological analysis of the samples. Although it is possible that a few normal cells contaminated our adenoma cultures, we believe that their contribution to the results shown here is not significant. The tissue was transferred to MEM (Invitrogen, Carlsbad, CA) at 4 C and quickly dispersed with trypsin (1 mg/ml) for 15–30 min at 37 C. Dispersed cells were plated on coverslips previously coated with 0.01 mg/ml poly-L-lysine and cultured in DMEM (Invitrogen) supplemented with 10% fetal bovine serum and antibiotics until use.

Calcium imaging

Calcium imaging was carried out as previously reported (15, 16). Briefly, cells were incubated with fura-2/AM (4 μM) for about 1 h at room temperature in standard medium: 145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, (pH 7.4), and 10 mM glucose. Then, cells were washed in the same medium, placed in a thermostatically controlled (37 C) stage of an inverted microscope (Diaphot; Nikon, Tokyo, Japan), and perifused with standard medium, prewarmed at 37 C. Cells were epi-illuminated alternately at 340 and 380 nm, and light emitted above 520 nm was recorded by using a Magical Image Processor (Applied Imaging, Newcastle, UK). Pixel-by-pixel ratios of consecutive frames were produced, and cytosolic calcium concentration ($[Ca^{2+}]_i$) was estimated from these ratios by comparison with fura-2 standards. Figure 1Aa shows an image during stimulation with TRH. Test solutions containing HRHs at 10 nM were perifused for 30 sec at the times indicated. A depolarizing solution containing high K^+ (75 mM) was perifused for 15 sec at the end of the experiment. Cells not responding to the high K^+ stimulus (usually <5% of the total) were excluded from analysis.

MSPI

At the end of the calcium imaging experiment, cells kept in the microscope stage were fixed with 4% paraformaldehyde in PBS, permeabilized with 0.3% Triton X-100, and washed with PBS. Then, 10% goat serum in PBS was added. After 5 min, cells were incubated with antibodies against three human AP hormones (TSH, FSH, and LH) labeled with Oregon Green 488, Cascade Yellow, and Alexa 350, respectively. After washing, specific fluorescence images corresponding to each fluorophore were captured to reveal stained cells with the following fluorescence settings: Oregon Green (FSH): excitation, 490 nm; emission, greater than 510 nm; Cascade Yellow (TSH): excitation, 380 nm, emission, greater than 510 nm; and Alexa 350 (LH): excitation, 340 nm; emission, greater than 450 nm. This step enables typing cells storing TSH, LH, or FSH as well as cells costoring combinations of these AP hormones. Once the first series of images were captured and stored, cells were washed and incubated again with antibodies against GH, PRL, and ACTH labeled with Oregon Green 488 (PRL), Cascade Yellow (GH), and Alexa 350 (ACTH), respectively, and the incubation was continued for 30 min. Then, cells were washed, and three new fluorescence images were taken with the same fluorescence settings described above. This new series of images revealed cells stained by the first antibody plus those newly stained by the second one. Cells stained by the second series of antibodies were revealed by subtracting the first series from the second one. Figure 1Ab shows the merger of the staining with the different antibodies in a representative experiment. Finally, nuclei were stained with Hoechst 33258 (0.5 μg/ml, 10 min; Fig. 1Ac), and another fluorescence image was acquired (excitation, 340 nm; emission, >420 nm). The nuclear images permitted distinguishing individual cells that were physically close. In all cases, the analysis was performed within 4 h after surgery and on the next day to achieve one to five independent experiments for each tumor cell dispersion. In most cases, an additional multiple sequential immunocytochemistry was carried out on cells neither loaded with fura-2 nor subjected to sequential stimulation with HRHs. Finally, a classic immunocytochemical analysis in tissue sections was carried out by the pathologists. All three procedures yielded similar results.

This procedure has been tested and established previously in normal pituitary cells from mice (16). Specific controls included the following: 1) perfusion of the cells with hypothalamic releasing factors did not alter responsiveness and 2) multiple sequential immunocytochemistry analysis carried out in cells from the same cultures before and after sequential stimulation with HRHs did not affect either the distribution or the relative abundance of hormone-positive cells (for a more detailed description of this procedure, see Ref. 16).

Results

Most relevant clinical and pathological details of the 15 human pituitary adenomas studied here are summarized in Table 1. Figure 1 summarizes the strategy followed for phenotypic characterization of the cells, which has been established and validated previously with normal mouse pituitary cells (16). First, cells were loaded with fura-2 and subjected to a protocol of sequential stimulation with the four HRHs and Ca^{2+} imaging (Fig 1Aa). The increase of the $[Ca^{2+}]_i$ in target cells (21–24) reveals expression of functional HRH receptors. The image shown here was taken during stimulation with TRH and shows that six of the eight cells present in the field responded to this releasing hormone. At the end of the imaging experiment, the cells were subjected to MSPI (Fig. 1Ab) to reveal storage of the

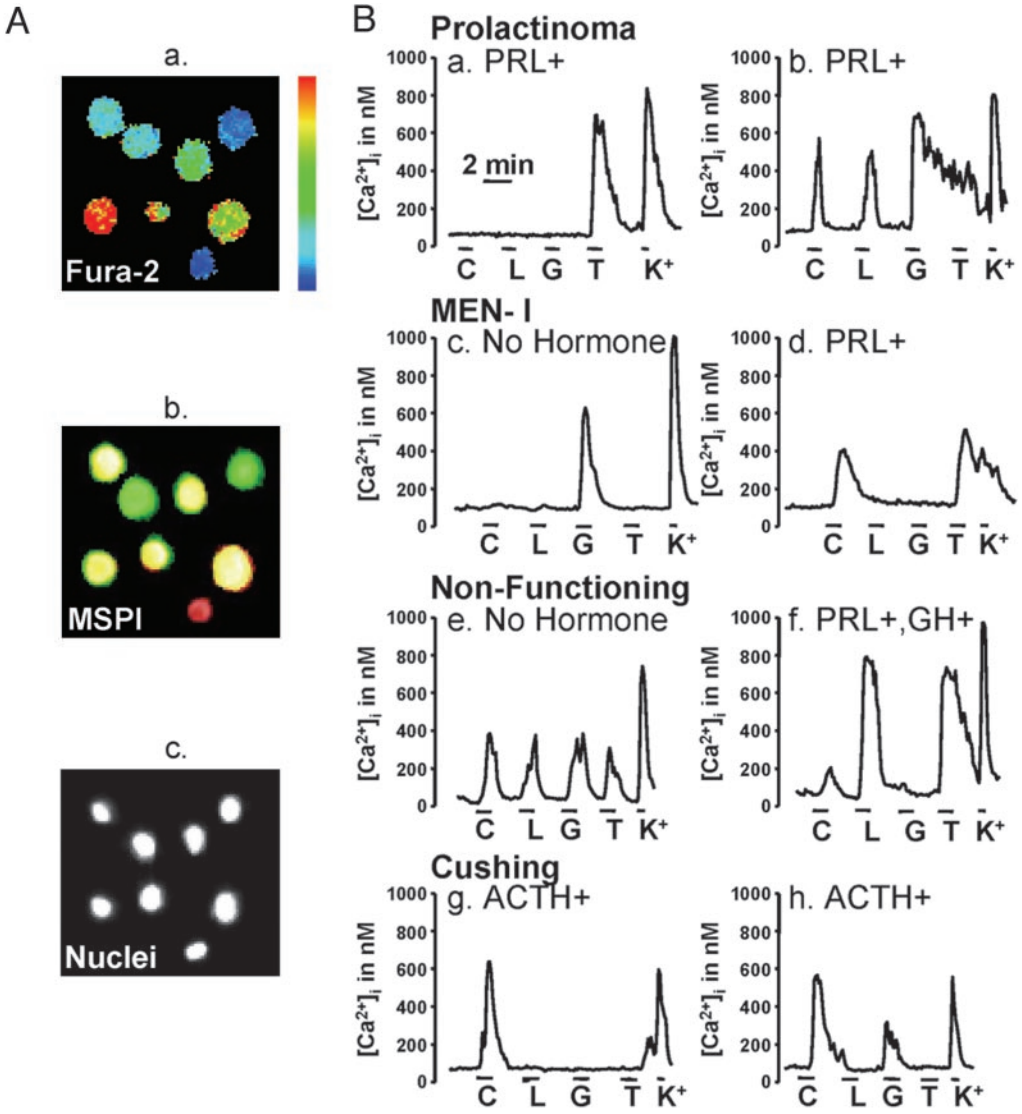

FIG. 1. Strategy for typing of human pituitary tumor cells. Freshly dispersed cells plated on poly-L-lysine-coated coverslips were loaded with fura-2 for 45 min, and expression of HRH receptors was evidenced in single-cell $[Ca^{2+}]_i$ measurements performed by digital imaging fluorescence microscopy. Cells were then fixed and subjected to MSPI against the six AP hormones as detailed in *Materials and Methods*. A, Fura-2 image (a) shows calcium levels, coded in pseudocolor (0–1000 nM, scale at *right*) during stimulation with 10 nm TRH in a 70- × 70-μm field. The *middle* image (b) shows a multiple immunocytochemistry image of the same cell field. Cells storing PRL (PRL+) are shown in *green*. Cells storing GH (GH+) are shown in *red*. Cells storing both PRL and GH are shown in *yellow* (PRL+, GH+). The *bottom* image (c) shows nuclear staining of the same cells by HOECHST 33258. B, Typical $[Ca^{2+}]_i$ recordings from eight individual cells during sequential perfusion with the four HRHs at 10 nM (C, CRH; L, LHRH; G, GHRH; and T, TRH) and high-K^+ medium from two prolactinomas, two MEN-1-related adenomas, two nonfunctioning adenomas, and two Cushing's disease adenomas, as shown. The AP hormone content of each cell is shown in the *top left corner* of each trace. Cells with $[Ca^{2+}]_i$ responses larger than 50 nM were considered responsive. Cells lacking responses to high K^+ were excluded from analysis. Data are representative of 1862 cells studied in 40 independent experiments.

different AP hormones. In this case, two cells stored PRL (in *green*), one GH (in *red*), and five stored both PRL and GH (in *yellow*). Finally, the total number of cells was established by staining the nuclei (Fig. 1Ac), here confirming that there were eight different cells in the field. Figure 1B shows illustrative examples of the cell phenotypes found in different tumors. In prolactinomas, the cells generally stained only for PRL and responded either only to TRH (Fig. 1Ba) or to several HRHs (Fig. 1Bb). Cells from MEN-1 patients showed no staining or stored PRL and responded only to TRH (Fig. 1Bc) or to both CRH and TRH (Fig. 1Bd). Nonfunctioning adenomas usually contained cells that responded to several HRHs and either showed no staining for any AP hormone (Fig. 1Be) or stained with multiple antibodies (Fig. 1Bf). Finally, cells from Cushing's disease patients may store either ACTH alone or several hormones and respond either solely to CRH (Fig. 1Bg) or to several HRHs (Fig. 1Bh). These examples document the heterogeneity found among different tumors and also among tumors of the same type. In addition, our data show clearly that cells from human pituitary tumors are multifunctional and may respond to multiple HRHs.

TABLE 1. Clinical and pathological characteristics of pituitary adenomas

No.	Sex, age (yr)	Clinical features	Size (mm)[a]	Immunocytochemistry
1	F, 27	Hyperprolactinemia (190 ng/ml PRL), amenorrhea, galactorrhea	7	PRL+++
2	F, 21	Hyperprolactinemia (574 ng/ml PRL), amenorrhea, galactorrhea	10	PRL+++
3	F, 28	Hyperprolactinemia (745 ng/ml PRL), amenorrhea	10	PRL+++
4	M,37	Hyperprolactinemia (750 ng/ml PRL), impotence	25	PRL+++, GH+
5	F, 61	Amenorrhea during last 27 yr (normal PRL), hyperlipidemia, headache, diplopia	20	PRL++, GH++
6[b]	F, 26	MEN-I, hyperparathyroidism	10	PRL+++
7[b]	M,38	MEN-I, hyperprolactinemia (3450 ng/ml PRL), hyperparathyroidism, enlarged adrenal, pancreatic tumor, panhypopituitarism, visual loss	30	PRL+++, TSH+
8[b]	M,45	MEN-I, parathyroid hyperplasia, visual loss	40	PRL+
9[c]	M,46	Nonfunctioning, visual loss	35	Negative
10[c]	M,57	Nonfunctioning, visual field defects, low cortisol and testosterone	>10	Negative
11[c]	F, 73	Nonfunctioning, visual loss, headache	30	PRL, +GH+
12[c]	M,74	Nonfunctioning, visual defects in the last 10 yr	40	Negative
13[d]	F, 25	Cushing's disease	10	All AP hormones ++
14[d]	M,46	Cushing's disease	7	ACTH+++
15[d]	F, 51	Cushing's disease	2	ACTH+ and some other +

F, Female; M, male.

[a] Maximum diameter of the tumor.

[b] All the three MEN-I patients had undergone previous surgery to remove primary tumors in other endocrine glands (parathyroid gland and/or pancreas).

[c] All the patients with nonfunctioning pituitary adenoma were diagnosed on the basis of visual defects and magnetic resonance imaging analysis revealing macroadenomas. Their plasma PRL levels were normal (between 14 and 20 ng/ml), and the remaining analytical data were normal as well.

[d] Cushing's disease patients exhibited hypercortisolism with plasma cortisol levels (0800 h) from 24.7–33.1 μg/100 ml (normal reference range 5.5–23.1 μg/100 ml) and 24-h urine cortisol from 324–1915 μg (normal reference range 24–135 μg/24 h).

Phenotypic analysis of prolactinomas

We have used the above described strategy to type cells derived from five prolactinomas (tumors 1–5; Table 1). MSPI revealed that all the cells from tumors 1 and 2 stained strongly for PRL. All the cells from tumor 3 stored PRL, but 7% of the cells also contained TSH. In tumor 4, most (>70%) cells stored PRL, and 14% also stored GH. Finally, in tumor 5, all the cells stained for both PRL and GH. Figure 2 shows the percentage of cells responding to each HRH. The *rightmost black bar* shows the percentage of multiresponsive cells (cells responding to >1 HRH). Results for two representative prolactinomas (2 and 5), and the averages of all the five studied are shown. Most cells from tumor 2 responded to TRH, and 25% were multiresponsive. Tumors 3 and 4 were very similar (data not shown). Thus, these three prolactinomas were composed essentially of cells storing only PRL and responding mostly to TRH, a phenotype similar to the normal mammotrope. Tumor 1 was similar in storing only PRL, but cells responded both to TRH (70%) and to CRH (90%). The percentage of multiresponsive cells was 72% (not shown). Finally, prolactinoma 5 was composed entirely by bihormonal cells containing both GH and PRL (mammosomatotropes); they responded not only to TRH and CRH but also to LHRH and specifically to GHRH (Fig. 2). Up to 85% of the cells of this tumor were multiresponsive. Thus, the cell phenotypes differed greatly among the different prolactinomas. Multiresponsiveness was variable but present in all the five tumors studied here.

Phenotypic analysis of pituitary adenomas from MEN-1 patients

We have also studied three pituitary tumors from patients diagnosed of type I MEN-I (6–8; Table 1). Virtually all the cells from the three tumors stained only for PRL and were negative for the rest of the AP hormones except for about 1% of the cells in tumors 7 and 8 that showed also staining for GH. Figure 3 shows characterization of tumors 7 and 8 as well as the average responses of all the three MEN-1 adenomas. In all the cases, the most prominent responses were to TRH, but responses to the other HRHs were also observed. Specifically, responses to CRH ranged between 25 and 80%, whereas responses to LHRH varied between 9 and 50%. Finally, responses to GHRH were less frequent (15–27%) in tumors 6 and 7 but increased to 80% in tumor 8. Multiresponsive cells varied between 30 and 91%. In conclusion, MEN-I-related AP adenomas were composed of cells storing only PRL but bearing multiple HRH receptors, but otherwise they were very heterogeneous.

Phenotypic analysis of nonfunctioning adenomas

We also studied four nonfunctioning macroadenomas (tumors 9–12; Table 1). Figure 4 shows the phenotypic profiles of three of them. As expected, some tumors (9 and 12) stored no hormones. However, cells from tumor 10 stored ACTH, GH, and/or PRL, and nearly all the cells from tumor 11 stored both PRL and GH. Regarding responsiveness, most of the cells from all the four tumors showed striking responses to all the four HRHs. Specifically, responses to TRH and CRH were very prominent (70–100% for TRH and 45–71% for CRH). Responses to LHRH and GHRH were very variable (15–70% for LHRH and 5–76% for GHRH). Thus, ironically, the nonfunctioning tumors contained many multiresponsive cells (61–86%) specifically sensitive to TRH and CRH (Fig. 4). In addition, nonfunctioning pituitary adenoma cells may store one or multiple AP hormones despite excess hormone secretion that was not clinically evident (Table 1).

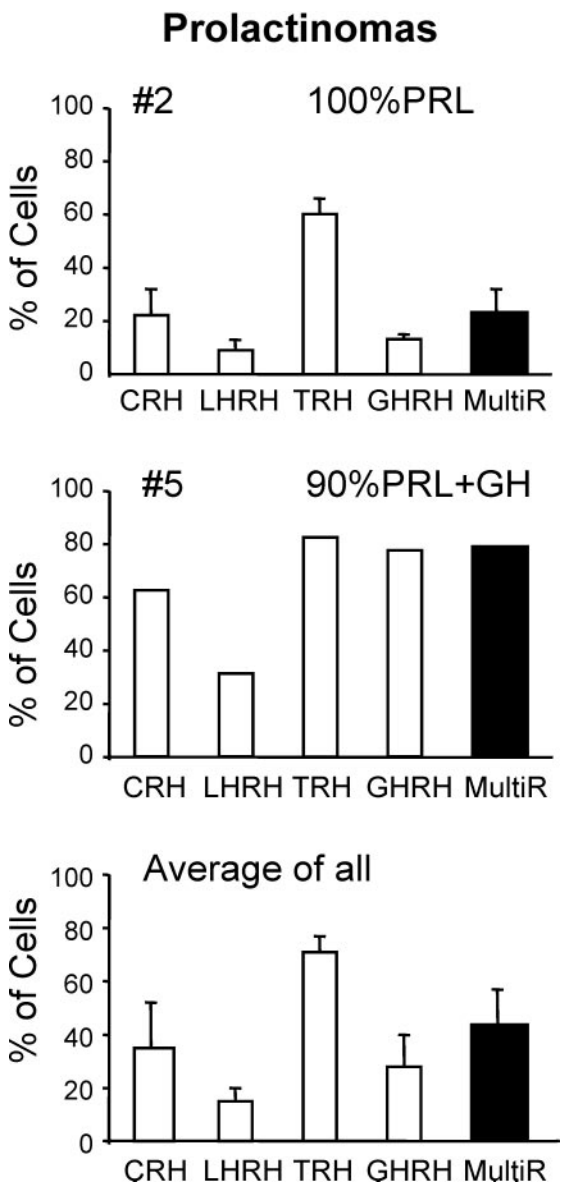

FIG. 2. Phenotypic characterization of prolactinomas. The analysis of two representative prolactinomas (2 and 5) and the average of all the five tumors studied (average of all) are shown. The percentages of cells responding to each HRH (with a calcium rise larger than 50 nM, mean ± SEM) are shown. The *solid bar* at *right* shows the percentage of multiresponsive cells (those showing responses to >1 HRH). The hormonal content of the cells is given in *upper right* corner of each panel. All the cells for prolactinoma 2 stored just PRL, but 90% of cells from tumor 5 stored both GH and PRL. Clinical and pathological data of these tumors are summarized in Table 1. See text for details about these and the other prolactinomas. Data derive from 19 cells (one experiment, tumor 1), 209 cells (three experiments, tumor 2), 142 cells (four experiments, tumor 3), 41 cells (two experiments, tumor 4), and 74 cells (two experiments, tumor 5). Average data derive from 485 cells studied in 12 independent experiments.

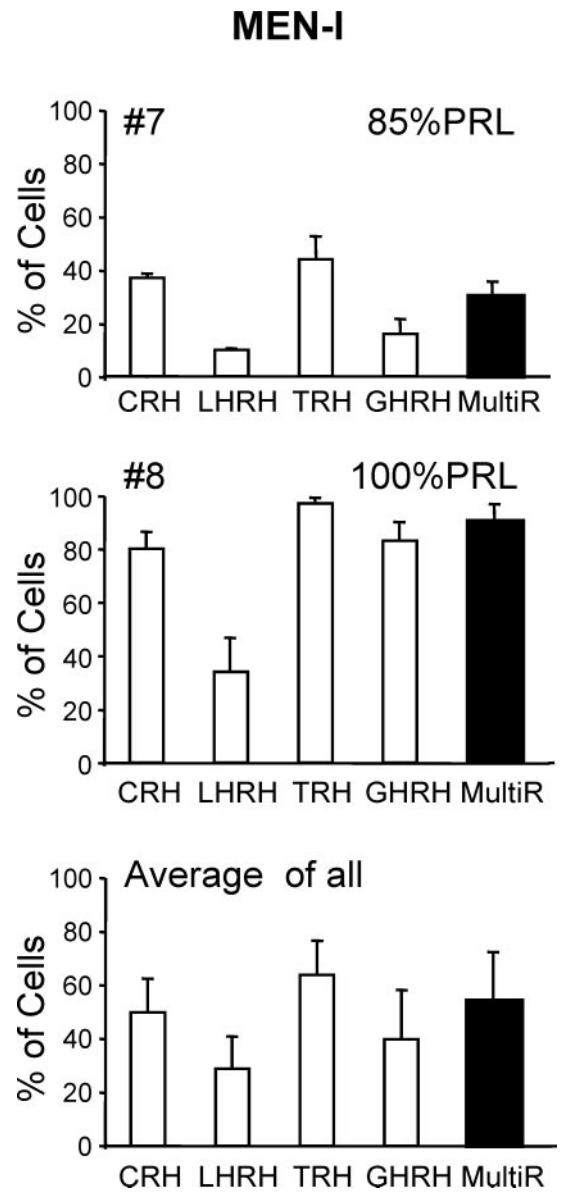

FIG. 3. Phenotypic characterization of pituitary adenomas from MEN-I patients. Data from two representative tumors (7 and 8), and the average of all the three studied are shown. Data derived from 121 cells (three experiments, tumor 6A), 122 cells (three experiments, tumor 7), and 351 cells (five experiments, tumor 8). Average data are representative of 594 cells studied in 11 independent experiments. Other details as in Fig. 2.

Phenotypic analysis of pituitary Cushing's disease adenomas

We studied three AP adenomas obtained from patients suffering pituitary Cushing's disease (Table 1). Figure 5 summarizes this analysis. Cells from tumor 13 showed an interesting phenotype. Most of the cells (>95%) stored ACTH, LH, FSH, and TSH, and a large fraction also contained GH (71%) and/or PRL (43%). Paradoxically, cells from this adenoma were quite responsive to TRH and LHRH but not to CRH. Nearly 50% of the cells were multiresponsive. The second tumor (14) was very different. Most cells stored only ACTH and responded to CRH (82%). Responses to TRH were significant (37%), and cells responding to LHRH (15%) or GHRH (22%) were less abundant. Multiresponsive cells amounted 40% of the cells. The third tumor (15) displayed still a different pattern. We found cells storing PRL (33%) and/or GH (30%) and minor subpopulations containing each of the remaining AP hormones. This cell distribution resem-

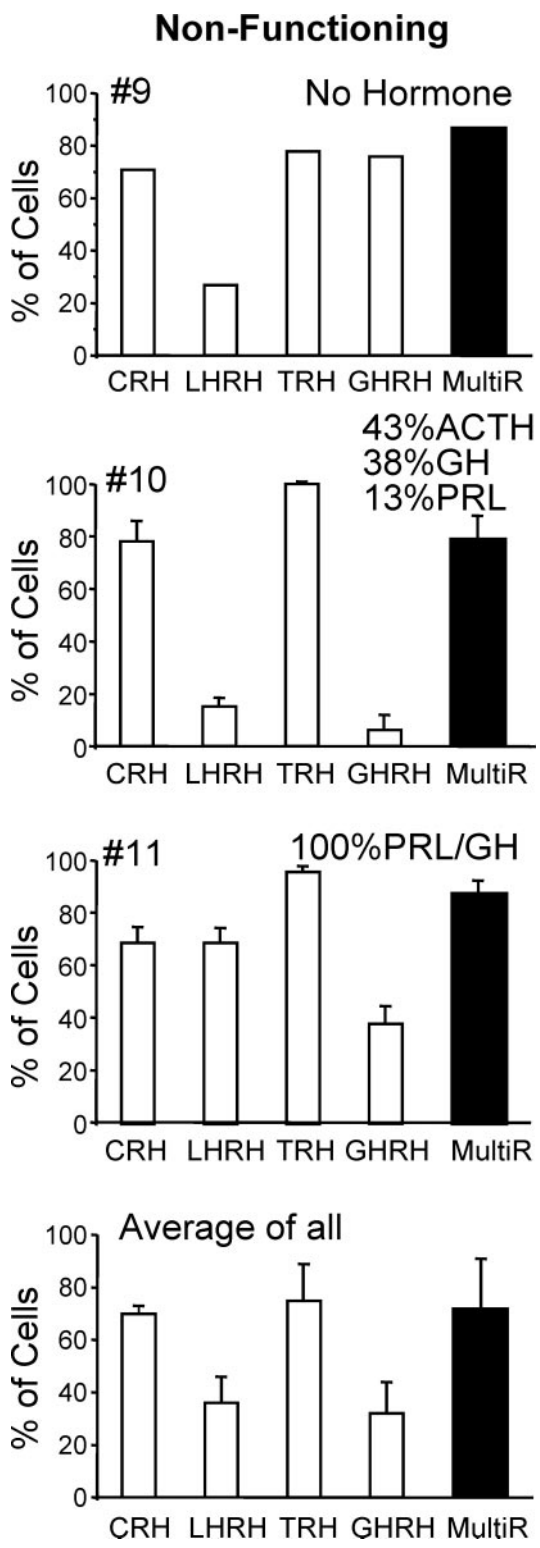

FIG. 4. Phenotypic characterization of nonfunctioning adenomas. Results from three individual tumors (9–11) and the average of all four studied (average of all) are shown. Data derived from 43 cells (two experiments, tumor 9), 207 cells (three experiments, tumor 10), 333 cells (four experiments, tumor 11), and 108 cells (two experiments, tumor 12). Average data are representative of 691 cells studied in 11 independent experiments. Other details as in Fig. 2.

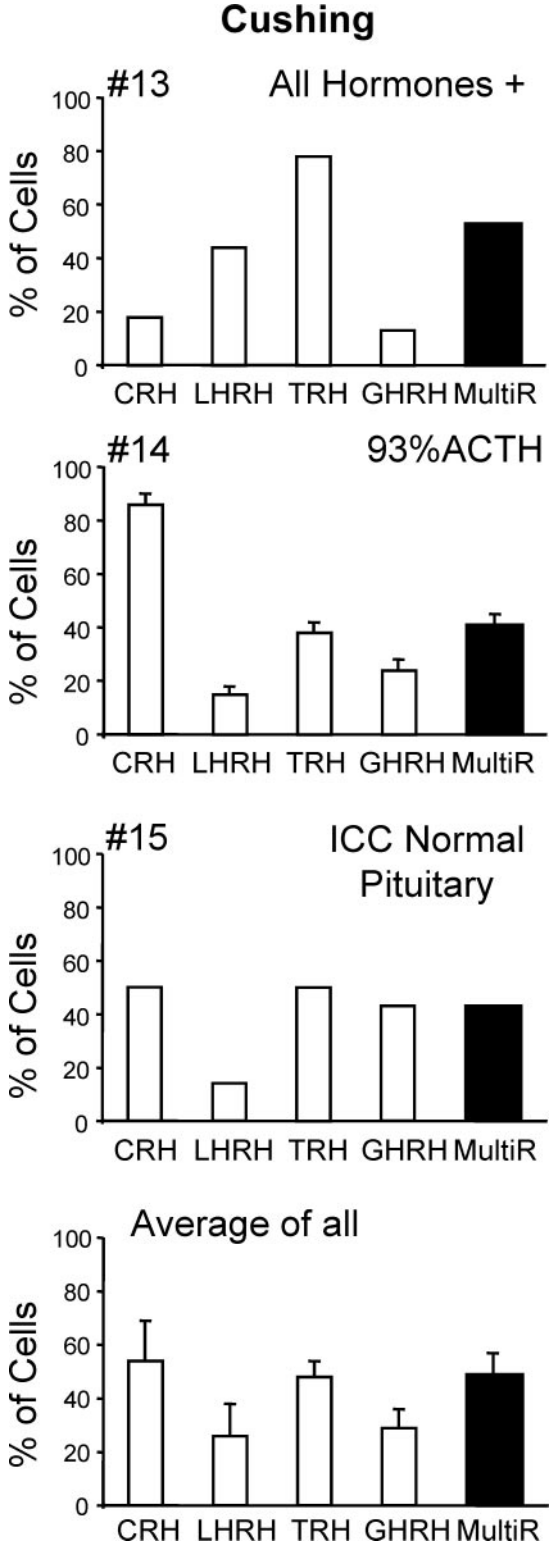

FIG. 5. Phenotypic characterization of pituitary adenomas from Cushing's disease patients. Results from three individual tumors (13–15) and the average of all are shown. Data are representative of 18 cells (two experiments, tumor 13), 60 cells (three experiments, tumor 14), and 14 cells (one experiment, tumor 15). Average data are representative of 92 cells studied in six independent experiments. Other details as in Fig. 2.

bles more a normal pituitary than a tumor, except because all cells also showed slight staining for ACTH. Multiresponsiveness approached 50%. Thus, AP tumor cells from pituitary Cushing's disease patients exhibited very different phenotypic patterns. In all the cases, about half of the cells showed responses to multiple HRHs, especially to CRH and TRH.

Multifunctional cells in AP tumors

It seems clear from the above-described data that human pituitary tumor cells may exhibit very different phenotypes, including mixed phenotypes with cells storing more than one AP hormone (polyhormonal cells) and/or bearing multiple HRH receptors (multiresponsive cells). Figure 6 compares the average relative abundance of multiresponsive cells (*solid bars*) and polyhormonal cells (*empty bar*) within each type of tumor. Multiresponsive cells varied between 40 and 70%, whereas polyhormonal cells were 2–50%. In eight of 15 tumors, more than 80% of the cells were either multiresponsive and/or polyhormonal. On average, 68% of the cells in all tumors presented a multifunctional phenotype.

Discussion

We present here the first complete phenotypic characterization of individual pituitary tumor cells according to the AP hormones they store and the functional HRH receptors they express. Cell phenotypes varied largely among different tumors and also among tumors of the same type. In addition, most tumors contained large subpopulations of cells with mixed phenotypes, expressing multiple HRH receptors and, much less frequently, storing multiple AP hormones. In eight of 15 tumors, more than 80% of the cells were multifunctional

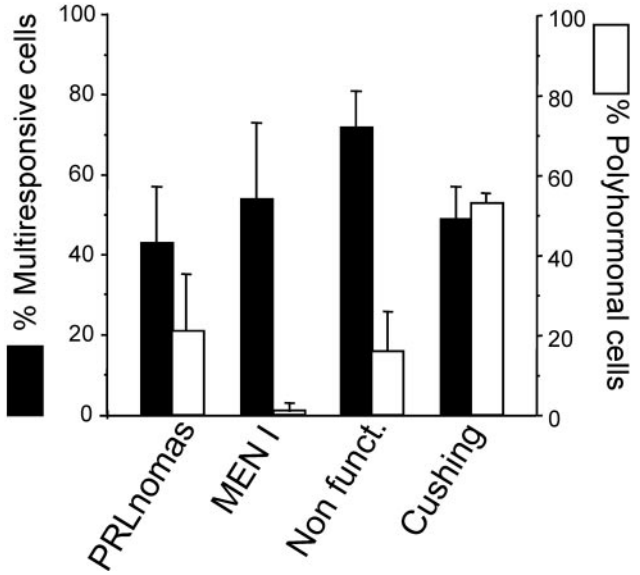

FIG. 6. Percentage of multiresponsive and polyhormonal cells in the different types of tumors. Average (±SEM) of the percentages of multiresponsive cells (*solid bars*) and polyhormonal cells (*empty bars*) for the different tumor types. Data are representative of 1862 cells from 15 tumors (five prolactinomas, three MEN-1 adenomas, four nonfunctioning adenomas, and three Cushing's disease-related adenomas) studied in 40 independent experiments. Other details as in Fig. 2.

(multiresponsive and/or polyhormonal). A similar study has not been performed in normal human pituitaries, but the fraction of multifunctional cells in mice and rat pituitary was smaller, between 20 and 40% (15, 16). The increased fraction of multifunctional cells could explain why paradoxical secretion is not infrequent in pituitary tumor patients (5–10) and much less frequently reported in normal subjects.

It is thought that multifunctional cells are involved in pituitary plasticity. Multifunctional cells seem to be generated by phenotypic switches between mature cell types without cell division, a process called transdifferentiation. This concept was introduced to explain the existence of mammosomatotropes, a cell type that stores and secretes both GH and PRL. Mammosomatotropes are generated by the conversion of somatotropes into mammotropes during situations demanding large amounts of PRL, such as lactation (18). The concept of transdifferentiation was further extended to include paradoxical expression of HRH receptors. Thus, Childs (19) showed that somatotropes can transiently express GnRH receptors and gonadotropins (LH and FSH) and proposed that this process underlies the surge-dependent GH secretion required for ovulation. In addition, somatotropes may transdifferentiate to thyrosomatotropes during protracted hypothyroidism in humans (20). The presence of multifunctional cells has been documented within all the five AP cell types in normal mice (16, 17), suggesting that transdifferentiation can take place in all these cell types under physiological conditions. Thus, pituitary plasticity may be subsidized by phenotypic changes of the individual cells to take care of changing AP hormone demands in different physiological and pathophysiological situations. Now, we find that multifunctional cells are abundant in most of the pituitary adenomas studied, suggesting that pituitary adenomas might arise from transformation and proliferation of multifunctional AP cells.

Multifunctional cells in the normal AP may tend to proliferate more than normal cells, and this behavior may play a role in AP cell plasticity. For example, during lactation, the rate of proliferation of PRL-producing cells increases dramatically (25). It is well known that, in addition to promoting hormone secretion, HRHs stimulate the mitotic activity of their target cells. Thus, for example, excessive GHRH secretion or GHRH overexpression results in dysregulated somatotrope proliferation, leading to hyperplasia and neoplastic transformation (26, 27). Multiresponsive cells are the target of multiple signals for proliferation; therefore, they should proliferate more than normal AP cells. If pituitary adenomas arose from multifunctional cells, then physiological or pathophysiological situations that promote transdifferentiation should favor tumor generation. Hypothyroidism, which induces transdifferentiation of somatotropes into thyrotropes, favors the appearance of pituitary adenomas (28) and estrogens, which promote transdifferentiation of somatotropes into mammotropes that also favor AP tumor generation (29). In addition, mutations of transcription factors involved in pituitary development and cell lineage commitment may also lead to pituitary adenomas (30). Thus, a link between pituitary plasticity and tumorigenesis deserves consideration.

A final point worthy of consideration is the possible therapeutic implications of our results. Because we find that

pituitary adenoma cells contain large populations of cells responsive to both TRH and CRH, antagonists of HRH receptors, especially for the TRH and CRH receptors, should be considered to inhibit or delay AP tumor growth.

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