Pflugers Arch – Eur J Physiol (2004) 449: 257–264
DOI 10.1007/s00424-004-1337-7

ENDOCRINOLOGY

Carlos Villalobos · Lucía Núñez · Javier García-Sancho

Phenotypic characterization of multi-functional somatotropes, mammotropes and gonadotropes of the mouse anterior pituitary

Received: 19 June 2004 / Revised: 3 August 2004 / Accepted: 16 August 2004 / Published online: 16 September 2004

Abstract The existence of bihormonal anterior pituitary (AP) cells co-storing growth hormone and either prolactin (mammosomatotrope) or gonadotropins (somatogonadotrope) has been described. These cells have been proposed to be involved in "paradoxical" secretion [secretion of an AP hormone induced by a non-related hypothalamic releasing factor (HRH) and transdifferentiation (a phenotypic switch between different cell types without cell division]. Here we combine calcium imaging (to assess HRH responsiveness) and multiple sequential immunoassay of the six AP hormones to perform a single-cell phenotypic study of multifunctional somatotropes, mammotropes and gonadotropes in the normal male and female mouse pituitaries. AP cell phenotypes differed from the classic view, showing multiple HRH-receptor expression and/or hormone storage. Mammosomatotropes represented only 5–6% of somatotropes and were poorly responsive to HRHs, suggesting that their contribution to paradoxical secretion should be very limited. Somatogonadotropes were present only in females and contained adrenocorticotropic hormone. They responded to growth hormone-releasing hormone but failed to respond to gonadotropin-releasing hormone (LHRH). Other polyhormonal cells identified include (1) gonadocorticotropes, restricted to females, where they make up more than 50% of all the gonadotropes and contain other AP hormones; (2) gonadomammotropes, which are present preferentially in female cells and respond to LHRH; and (3) gonadothyrotropes, which are present similarly in male and female pituitaries.

C. Villalobos · L. Núñez · J. García-Sancho (✉)
Departamento de Fisiología y Bioquímica,
Facultad de Medicina, Instituto de Biología y Genética Molecular (IBGM), Universidad de Valladolid and Consejo Superior de Investigaciones Científicas (CSIC), Ramón y Cajal 7,
47005 Valladolid, Spain
E-mail: jgsancho@ibgm.uva.es
Tel.: +34-983-423085
Fax: +34-983-423588

Keywords Anterior pituitary · Hypothalamic releasing hormones · Intracellular calcium · Paradoxical secretion · Transdifferentiation

Introduction

The anterior pituitary (AP) contains five main cell types, somatotropes, mammotropes, corticotropes, thyrotropes and gonadotropes, which secrete growth hormone (GH), prolactin (PRL), adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH) and gonadotropins [follicle-stimulating hormone (FSH) and luteinizing hormone (LH)], respectively. According to the classic view, each cell type stores a single hormone, whose secretion is specifically regulated by a particular hypothalamic releasing hormone (HRH). However, the existence of AP cell subpopulations differing from this pattern has been repeatedly reported, although their contribution to the physiology of the gland is obscure. For example, mammosomatotropes store and release GH and PRL [13]. Polyhormonal corticotropes, [4, 5, 9], somatogonadotropes [6, 7, 8] and cells storing and releasing LH and PRL [14] have also been reported. In addition, some GH cells become thyrosomatotropes after a thyroidectomy [19] or protracted primary hypothyroidism [33]. Consistently, recent single-cell RT-PCR studies revealed that a large pool of AP cells expressed mRNAs for multiple AP hormones [17, 27, 28, 29, 30]. The origin of polyhormonal cells remains obscure. Although there is yet not direct evidence, multihormonal cells have been regarded as an intermediate stage in the conversion of one cell type (e.g. somatotropes) into another (e.g. mammotropes), a phenotypic switch between mature cell types without cell division called transdifferentiation [13, 34] that may happen in situations of high hormone demand (e.g. lactation).

In addition, a large fraction of rat AP cells expresses multiple functional HRH receptors (multi-responsive cells) [20, 35, 36,] and stimulation with different HRHs stimulates paradoxical PRL secretion [36]. Gonado-

tropes may express growth hormone-releasing hormone (GHRH) receptors [10], and somatotropes may transiently express gonadotropin-releasing hormone (LHRH) receptors. In addition, LHRH may stimulate GH secretion [6]. Taken together, the above-mentioned data indicate that the AP contains multi-functional (multi-responsive and/or polyhormonal) cells, whose stimulation may give rise to so-called paradoxical secretion. Paradoxical secretory responses are common in non-normal pituitaries, especially in pituitary tumours [3, 11, 23, 25, 32], but they have been also sporadically reported in normal pituitaries, both in vitro and in vivo, including healthy humans [2, 12, 16].

In a recent study, we attempted a complete phenotypic characterisation of individual AP cells by the hormones they store and the HRH receptors they express [26]. Multi-functional cells were identified within all the five AP-cell subpopulations. Here we analyse the phenotypic complexity of multi-functional somatotropes, mammotropes and gonadotropes, in terms of both hormonal contents and expression of HRH receptors, and discuss their possible contribution to the gland's physiology.

Materials and methods

Pituitary glands were obtained from male and randomly cycling female mice (strain Balb/c, 12 weeks-old). Animal experimentation was conducted in accord with accepted standards of human animal care and the Valladolid University School of Medicine Ethical Committee. Pituitary glands were removed and digested with trypsin (from bovine pancreas, 1 mg/ml) for 30 min at 37°C, as described elsewhere [26]. Cells were plated on poly-L-lysine-coated coverslips and used for the experiments within 2–4 h. We have shown before that responses to the HRHs by these freshly isolated cells are larger and more frequent than those maintained in primary culture for 1–3 days [35, 36]. Single-cell responsiveness to the four HRHs (10 nM) were assessed from the changes of cytosolic free calcium concentration ($[Ca^{2+}]_c$), which were measured in fura-2-loaded cells by digital imaging fluorescence microscopy (Fig. 1) as described previously [26, 35, 36]. Briefly, cells were incubated with fura-2/AM (4 μM) for

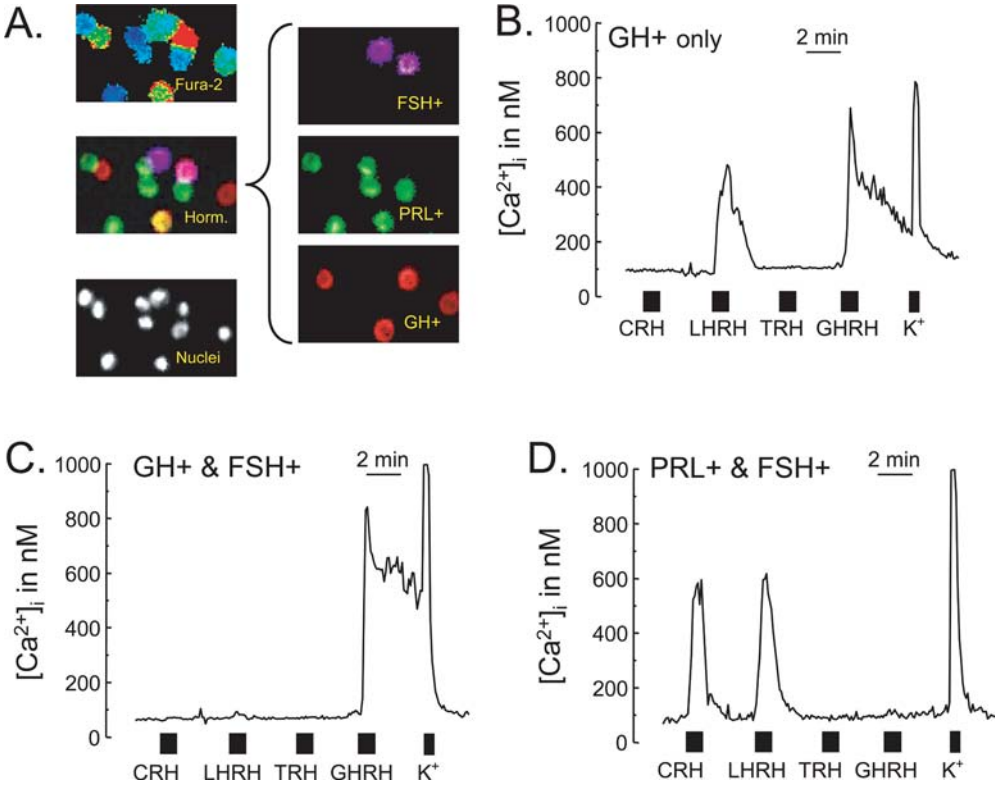

Fig. 1a–d Combination of calcium imaging and multiple immunocytochemistry for characterisation of mouse AP cells. **a** Mouse anterior pituitary (AP) cells were loaded with fura-2, and responsiveness to sequential stimulation with the four HRHs was recorded [fura-2 image, taken during stimulation with gonadotropin-releasing hormone (LHRH)]. At the end of the experiment, the cells were fixed, and multiple hormone storage was determined (see "Materials and methods"). The panel *Horm.* shows the merged image of individual immunocytochemistries for follicle-stimulating hormone (*FSH*), prolactin (*PRL*) and growth hormone (*GH*), shown on the *right*. Immunocytochemistry for the other hormones is not shown for simplicity. The *bottom panel* shows nuclei, stained at the end of the procedure (see Materials and methods). **b** A somatotrope storing only GH and responding to growth hormone-releasing hormone (*GHRH*) and LHRH. **c** A somatotrope storing also gonadotropins together with GH, but responding only to GHRH. **d** A mammogonadotrope responding to corticotropin-releasing hormone (CRH) and LHRH. Multiple immunocytochemistry performed on cells after calcium imaging. Nuclear staining after immunocytochemistry was done to reveal the number of cells

about 1 h at room temperature in standard medium containing (mM): NaCl (145); KCl (5); $MgCl_2$ (1); $CaCl_2$ (1); HEPES (10), pH 7.4; and glucose (10). Then, cells were washed with the same medium, placed in the thermostatically controlled (37°C) stage of an inverted microscope (Nikon Diaphot) and perfused with standard medium (pre-warmed at 37°C). Cells were epi-illuminated alternately at 340 nm 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 $[Ca^{2+}]_c$ was estimated from these ratios by comparison with fura-2 standards. Cells were sequentially stimulated with the four different HRHs (10 nM) for 30 s. Cells were considered responsive to each HRH when a $[Ca^{2+}]_c$ increase larger than 50 nM was recorded after each stimulation. At the end of the experiment, cells were stimulated with high K^+ (75 mM)-containing medium. Cells not responding to this stimulus (usually less than 5%) were removed from the analysis.

At the end of the $[Ca^{2+}]_c$ measurements, the hormonal contents of the cells were typed by multiple sequential primary immunofluorescence, combining three different fluorescent labels and image processing to resolve the five AP hormones (TSH, LH, PRL, GH and ACTH) within about 2 h (Fig. 1). Briefly, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min, permeabilised with 0.3% Triton X-100 in the above solution for 3 min and washed with PBS for 5 min. Then, 10% goat serum in PBS was added. After 5 min, antibodies against three AP hormones (TSH, FSH and LH) labelled with Oregon Green 488, Cascade Yellow and Alexa Fluor 350, respectively, were added, and the incubation was continued for 30 min. After washing, specific fluorescence images corresponding to each fluorophore were captured to reveal stained cells. This step enables to type cells storing TSH, LH or FSH as well as the ones co-storing combinations of these AP hormones. After capturing the first series of images, cells were washed and incubated again with antibodies against GH, PRL and ACTH, labelled with Oregon Green 488 (PRL), Cascade Yellow (GH) and Alexa Fluor 350 (ACTH), and the incubation was continued for 30 min. After washing, three new fluorescence images were taken with the same fluorescence settings described above. This new series of images revealed cells stained by the second set of antibodies in addition to those stained with the first set. To reveal the specific staining by the second set of antibodies, the first series of fluorescence images were subtracted from the second ones. This procedure enabled detection of cells storing single or multiple AP hormones (Fig. 1). This procedure allowed typing of 90–93% of the cells present in the microscope field (701 male and 558 female cells studied, the total number of cells determined by nuclear staining with Hoeschst 33258) [26]. (See references [1] and [26] for a more detailed description of the procedure.)

Antisera against mouse PRL (no. AFP131078Rb), rat β-TSH (no. AFP1274789), rat GH (no. AFP411S), rat β-FSH (no. AFPHSFSH6Rb), rat β-LH (no. AFP571292393R) and rat ACTH (no. AFP71111591GP) were generous gifts from Dr. A.F. Parlow. The anti-rat reagents work in mouse just as well as in rat (NHPP and Dr. Parlow, personal communication). Fluorescent antibodies were prepared by labelling with Oregon Green 488, Cascade Yellow or Alexa Fluor 350 and purified over a protein A–Sepharose column. Fura-2/AM, Oregon Green 488-isothiocyanate and the succinimidyl esters of Cascade Yellow and Alexa Fluor 350 were purchased from Molecular Probes Europe. The HRHs and trypsin (from bovine pancreas, T4665) were obtained from Sigma.

Results

The combination of calcium imaging and multiple immunocytochemistry in the same cells enabled characterisation of somatotropes, mammotropes and gonadotropes for the expression of functional HRH receptors and storage of different AP hormones. Somatotropes are defined here as cells that either store only GH (monohormonal somatotropes) or GH plus additional hormones (polyhormonal somatotropes). The same naming criteria were used for gonadotropes and mammotropes. Figure 1 illustrates this phenotypic characterisation. The changes of $[Ca^{2+}]_c$ on stimulation with different HRHs were followed in fura-2-loaded cells, which were typed for hormone content at the end of the experiment by multiple quantitative immunofluorescence (Fig. 1a). We found somatotropes bearing multiple HRH receptors (Fig. 1b) or multiple hormones (Fig. 1c) as well as cells that simultaneously bear multiple HRH receptors and hormones (Fig. 1d). In an overall characterisation of mouse AP cells, we found that a large population of cells presented a multifunctional phenotype [26]. Here we report in detail the phenotypic characterisation of somatotropes, mammotropes and gonadotropes from male and female mouse.

Somatotropes and mammotropes represent about 80% of all the cells of mouse AP (32–46% each type). Most of these cells are monohormonal (82–95%, Fig. 2a, b). Polyhormonal cells are two to three times more frequent in female than in male (12.4% vs 4.5% for GH-containing cells and 17.9% vs 11.3% for PRL-containing cells). There is not a well-defined preference for the co-stored hormones (Fig. 2A and 2B). Mammosomatrotropes are a quite rare cell kind (3–5% of somatotropes and 5–7% of mammotropes). All the identified mammosomatotropes were bihormonal, containing no other hormones in addition to GH and PRL. The frequency of cells co-storing GH with AP hormones other than PRL was comparable to that of mammosomatotropes. Cells containing more than two hormones (8 out of 411, often storing three and four hormones) were most frequent among the pools co-storing ACTH

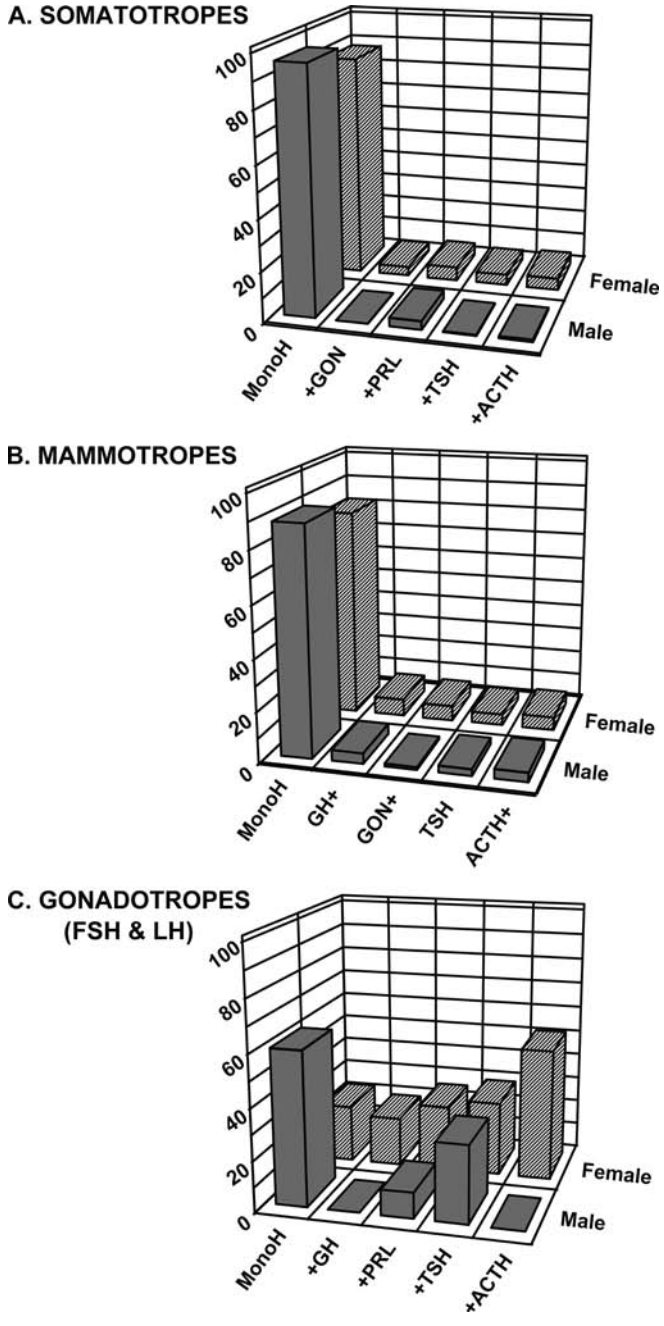

Fig. 2 Frequency distribution of GH-, PRL- and gonadotropin-containing [follicle-stimulating hormone (*FSH*) and luteinizing hormone (*LH*)] cells in male and female mouse pituitary. Data are expressed as per cent of all the cells. *MonoH* Monohormonal cells. Co-storage with other AP hormones is as shown. Data are from analysis of 633 male and 520 female AP cells in 11 different experiments (six males and five females). The percentages of each cell type (male/female) were somatropes (38%/44%), lactotropes (37%/34%), and gonadotropes (3.5%/6.5%)

(seven cells), gonadotropins (six cells) and TSH (four cells), and most of them were found in females. Similar rules were followed for PRL-containing, polyhormonal cells.

Gonadotropes (cells containing both FSH and LH) are an infrequent cell type (4% and 9% of all the mouse AP cells in male and female, respectively). Cells containing either FSH or LH alone are much less frequent and have been excluded from the analyses performed here. The co-storage of other hormones with gonadotropins is quite usual, especially in females (Fig. 2c). Interestingly, the pattern of hormone storage is very different in male and female. In the male, polyhormonal cells represent 40% of all the gonadotropes, and the dominant co-stored hormones are TSH (30%) and PRL (10%). None of the 20 cells studied stored either GH or ACTH. In the female, 77% of the gonadotropes were polyhormonal. In contrast with the male pituitary, in the female gland the most frequently co-stored hormone was ACTH (52% of the gonadotropes), followed by TSH (29%), PRL (26%) and GH (19%). A large fraction (38%) of female gonadotropes contained four or more hormones (including FSH and LH). Therefore, the gonadosomatotrope is the less frequent cell type among polyhormonal gonadotropes in the female mice and was not found in males.

The amount of hormone stored per cell, quantified from the fluorescence emitted by the staining antibody, was very similar in monohormonal and polyhormonal cells (data not shown). For example, mammosomatotropes contained 60% as much GH and 86% as much PRL as the monohormonal somatotropes and mammotropes, respectively. Regarding somatogonadotropes, the relative contents of GH, FSH and LH were 175, 94 and 65% as much as in the corresponding monohormonal cells, respectively.

Stimulation of HRH receptors induces an increase of $[Ca^{2+}]_c$ and hormone release [15, 21, 24, 31]. In order to quantify the responses to the HRHs, we measured the changes of $[Ca^{2+}]_c$ induced in the different cell types. Figure 3 compares the responses of monohormonal and polyhormonal somatotropes to the different HRHs. In Fig. 3a results are shown as $\Delta[Ca^{2+}]_c$ and in Fig. 3b as percentages of the cells responding to the HRH. Male and female cells have been pooled, as the behaviour was similar in both sexes. GHRH elicited the largest Ca^{2+} responses in both monohormonal and polyhormonal cells (Fig. 3a). Monohormonal cells and somatogonadotropes were the most responsive to GHRH, closely followed by the ACTH-, PRL- and TSH-containing cells. The remaining HRHs [LHRH, corticotropin-releasing hormone and thyrotropin-releasing hormone (TRH)] produced very small responses. Although 50% of the somatogonadotropes responded to LHRH (Fig. 3b), the $\Delta[Ca^{2+}]_c$ values were very small. When the six somatogonadotropes identified here were examined individually, only one of them showed a large Ca^{2+} response to LHRH ($\Delta[Ca^{2+}]_c$, 205 nM). The monohormonal gonadotropes of the same experiments responded to LHRH more often (six out of seven female cells) and especially more strongly ($\Delta[Ca^{2+}]_c$, mean ± SEM: 463 ± 99 nM). On the other hand, the monohormonal gonadotropes showed a small response to GHRH ($\Delta[Ca^{2+}]_c$, 186 ± 50 nM). Therefore, the behaviour of somatogonadotropes more closely resembles that of

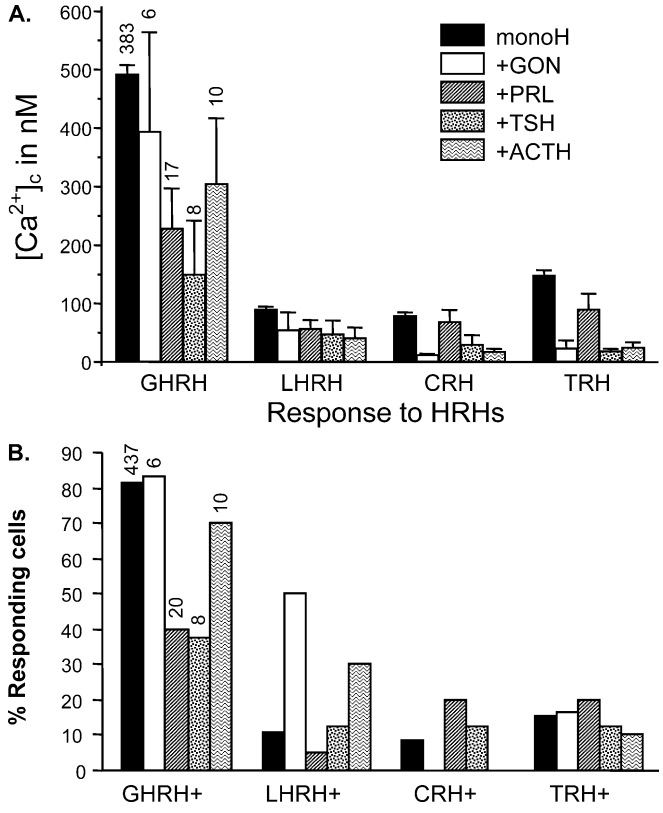

Fig. 3a, b Comparison of the responses of monohormonal and polyhormonal somatotropes to the hypothalamic releasing hormones (*HRHs*). **a** Data are expressed as changes of cytosolic free calcium concentration ($\Delta[Ca^{2+}]_c$) in nanomolar. Bars represent mean ± S.E.M. **b** Data are expressed as the percentage of cells of each type (either monohormonal or polyhormonal, containing additional hormones) that respond to the indicated HRH. The number of cells is given at the top of the *bars*

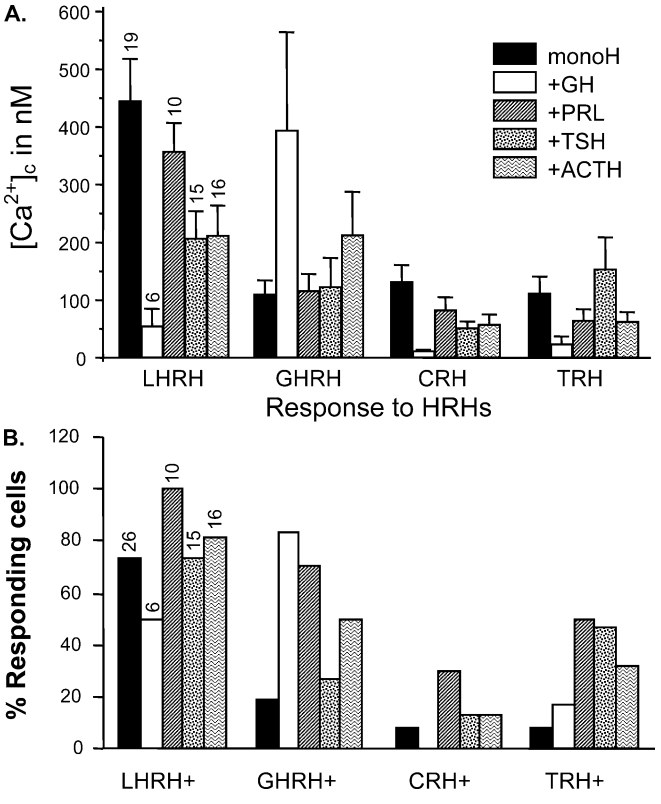

Fig. 4a, b Comparison of the responses of the monohormonal and the polyhormonal gonadotropes to the HRHs. **a** Data are expressed as $\Delta[Ca^{2+}]_c$ in nanomolar. *Bars* represent mean ± SEM. **b** Data are expressed as the percentage of cells of each type (either monohormonal or polyhormonal, containing additional hormones) that respond to the indicated HRH. The number of cells is given at the top of the *bars*

somatotropes than gonadotropes, at least regarding their responses to HRHs.

Figure 4 compares the responses of monohormonal and polyhormonal gonadotropes. Again, the responses in male and female have been pooled for simplicity. The monohormonal gonadotropes and the gonadomammotropes responded strongly to LHRH (Fig. 4a). In contrast, as commented above, somatogonadotropes responded poorly to LHRH but strongly to GHRH. Gonadocorticotropes and gonadothyrotropes also responded well to LHRH. Gonadocorticotropes also responded to GHRH. All these tendencies were similar in male and female cells (not shown). The responses to the other releasing factors were small, except for female gonadothyrotropes, which showed a good response to TRH. The profiles for the percentage of responding cells (Fig. 4b) was consistent with $\Delta[Ca^{2+}]_c$ profile. The only exceptions were somatogonadotropes (commented above) and gonadomammotropes. One half of these cells responded to GHRH, but the $\Delta[Ca^{2+}]_c$ values were small.

Figure 5 compares the responsiveness profiles in monohormonal and polyhormonal mammotropes. Responsiveness profiles corresponding to cells derived from male and female pituitaries were similar, except that female cells were generally somewhat more responsive to all the HRHs than the male cells (more cells responded to each HRH in female than in male-derived pituitaries, not shown). In general, mammotropes showed little selectivity to HRHs, the best responses being generally to GHRH. As noted above, gonadomammotropes exhibited a good response to LHRH. At variance with the rat, where TRH is a potent PRL secretagogue [36], mouse mammotropes responded poorly to TRH (Fig. 5).

Discussion

We have examined the mixed phenotypes existing in the somatotrope, gonadotrope and mammotrope cell types of mouse AP. We find polyhormonal cells within all these cell types. It should be remarked that the analysis was carried out after sequential stimulation with four secretagogues. This could result in an underestimation of the proportional abundance of polyhormonal cells, as we cannot be positively certain that monohormonal cells were actually monohormonal before stimulation. In most of the cases polyhormonal cells were more frequent

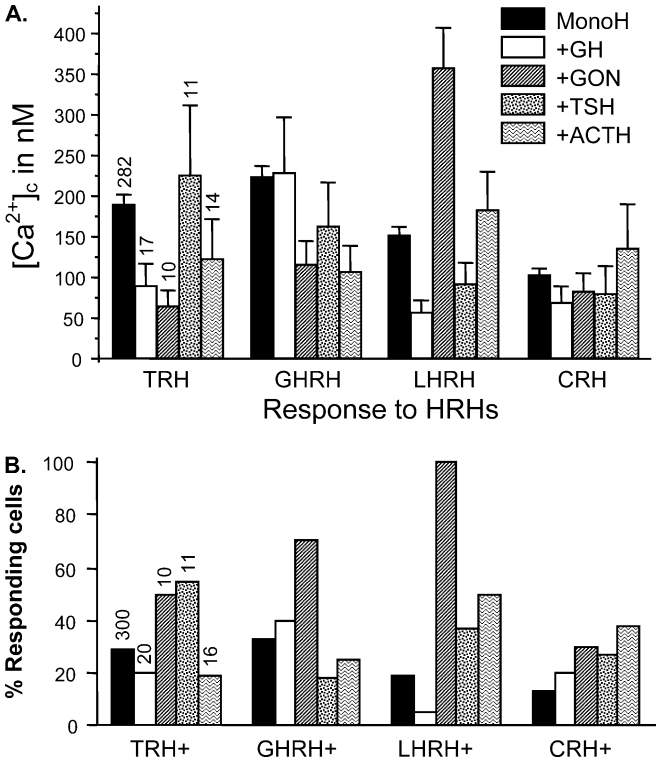

Fig. 5a, b Comparison of the responses of the monohormonal and the polyhormonal mammotropes to the HRHs. **a** Data are expressed as $\Delta[Ca^{2+}]_c$ in nanomolar. *Bars* represent mean ± SEM. **b** Data express the percentage of cells of each type (either monohormonal or polyhormonal, containing additional hormones) that respond to the indicated HRH. The number of cells is given at the top of the *bars*

in the female than in the male, suggesting that their generation is somehow favoured by hormonal changes associated to the oestrus cycle. Curiously, the cell types that had been described more extensively in previous studies, mammosomatotropes [13] and somatogonadotropes [6, 7], are rarer than many of the other combinations found here, although species differences may also apply.

Mammosomatotropes make up about 5% of the total somatotropes or mammotropes. It has been shown before that some of these cells are able to release both GH and PRL [13]. In our hands, they responded poorly to the HRHs. In fact, the responses of monohormonal somatotropes and mammotropes to GHRH and TRH, respectively, were stronger than the ones of mammosomatotropes. Therefore, paradoxical secretion (i.e. GH secretion induced by TRH or PRL secretion induced by GHRH) produced by the monohormonal somatotropes and mammotropes should be quantitatively larger than that of mammosomatotropes, as the former are much more abundant and respond better to the HRHs than the latter. These results suggest that mammosomatotropes should not contribute significantly to paradoxical secretion of either GH or PRL in the mouse. The situation may be different during lactation, where the number of mammosomatotropes has been reported to increase [13]. Mammosomatotropes have been reported to derive from somatotropes by transdifferentiation [13], and the process is favoured by estrogens [18, 22]. The finding here that mammosomatotropes are more frequent in females is consistent with this proposal. Although generation of mammosomatotropes and other cells with mixed phenotypes has been attributed to transdifferentiation, this has not been conclusively proven yet.

Somatogonadotropes have been extensively studied by Childs et al. [8] in female rats. They were proposed to be formed from somatotropes during the oestrus cycle under the influence of estrogens and to acquire LHRH receptors [6]. We find here that 21% of female gonadotropes (6 out of 28) co-store GH, whereas no somatogonadotropes were found in the male. These findings support the proposal of Childs et al. [9] that these cells are formed specifically during the oestrus cycle, but the same restrictions as to the origin of cells with mixed phenotype described above apply. However, we found that all the identified somatogonadotropes (six out of six) contained also ACTH and two of them contained, in addition, TSH. This suggests that the hormonal changes of the sexual cycle promote in somatotropes the expression not only of gonadotropins but also of multiple AP hormones. Childs et al. have proposed that somatogonadotropes may allow collaboration of [paradoxical] GH secretion (GH secretion induced by LHRH) in the control of the gonadal function [6, 7]. Our results may limit this view as (1) somatogonadotropes are only 3% on the total somatotrope pool, and (2) they respond poorly to LHRH (Fig. 4a). Indeed, the responsiveness profile of these cells remains very similar to the one of monohormonal somatotropes. Although 50% of the cells showed meaningful response to LHRH (Fig. 4b), the $\Delta[Ca^{2+}]_c$ was very small. Perhaps further maturation is needed before attaining full sensitivity to the releasing factor that is reached at a late stage of the transdifferentiation process accompanied by the loss of GH. This would explain why we do not observe $[Ca^{2+}]_c$ responses to LHRH in somatogonadotropes.

Cells able to secrete both gonadotropins and PRL have been described in rat AP [14]. The size of this gonadomammotrope pool has been reported to reach 2.5–5% of the mammotropes and 15–18% of the gonadotropes [14]. These figures are close to the ones found here for female mice (Fig. 2). In the male mouse, the size of this pool is smaller (1% and 9%, respectively), suggesting that transdifferentiation of mammotropes to gonadotropes could be favoured by hormonal changes associated to the oestrus cycle. Gonadomammotropes responded well to LHRH (Fig. 4). In fact, the $[Ca^{2+}]_c$ response was the highest found among the different mammotropes subgroup (Fig. 5). These results suggest that gonadomammotropes could contribute to paradoxical PRL secretion in response to LHRH in the female. Fifty per cent of the gonadomammotropes contained also ACTH.

Gonadocorticotropes were described and studied extensively by Childs et. al in the rat [4, 5, 9]. In the mouse, we find that the gonadocorticotrope is an intriguing cell type only found in females, suggesting that their generation is favoured by the oestrus cycle. Gonadocorticotropes represented as much as 35% of all the gonadotropes and 31% of all the corticotropes. They responded fairly well to LHRH, suggesting that they could contribute to paradoxical ACTH secretion (ACTH release induced by LHRH) during the sexual cycle. They also showed a reasonably good response to GHRH. Most (75%) of the gonadocorticotropes contained other hormones in addition to gonadotropins and ACTH,PRL (31%), TSH (31%) or GH (25%), so that this type of cell could be considered superpolyhormonal.

Gonadothyrotropes were found in both sexes in about the same proportions (20–25% of all the gonadotropes). This suggests that generation of this mixed phenotype is regulated by factors other than sexual cycle. In terms of the whole thyrotrope population, gonadothyrotropes represent 24% in males and as much as 45% in females. They respond quite well to LHRH, suggesting that they could contribute paradoxical TSH secretion during sexual cycle in females. In males gonadothyrotropes did not contain additional hormones, but in females over half of the cells contained also ACTH, thus overlapping with the gonadocorticotrope group.

This first phenotypic characterisation of multi-functional somatotropes, mammotropes and gonadotropes reveals complex phenotypes for these cell types. Further research will be required to establish the origin of these cells and their contribution to hormone secretion and endocrine regulation under demanding physiologic or pathophysiologic situations.

Acknowledgements We thank the National Hormone and Peptide Program and Dr. A.F. Parlow for providing antisera against AP hormones and J. Fernández for technical help. This work was supported by a grant from the Spanish Ministerio de Ciencia y Tecnología (MCyT; BFI2001–2073) and Fondo de Investigaciones Sanitarias (FIS 01/0769 and 03/1231). C. Villalobos and L. Nuñez are fellows of the Ramón y Cajal Program of MCyT.

References

1. Alarcón P, García-Sancho J (2000) Differential calcium responses to the pituitary adenylate cyclase-activating polypeptide (PACAP) in the five main cell types of rat anterior pituitary. Pflugers Arch 440:685–691
2. Amsterdam JD, Winokur A, Lucki I, Snyder P, Harris RI, Caroff S, Rickels K (1982) Growth hormone, prolactin and thyrotropin responses to gonadotropin-releasing hormone in depressed patients and healthy volunteers. Psychoneuroendocrinology 7:177–184
3. Barlier A, Pellegrini-Bouiller I, Caccavelli L, Gunz G, Morange-Ramos I, Jaquet P, Enjalbert A (1997) Abnormal transduction mechanisms in pituitary adenomas. Horm Res 47:227–34
4. Childs GV (1991) Multipotential pituitary cells that contain ACTH and other pituitary hormones. Trends Endocrinol Metab 2:112–117
5. Childs GV (1992) Structure-function correlates in the corticotropes of the anterior pituitary. Front Neuroendocrinol 13:271–317
6. Childs GV (2000) Growth hormone cells as co-gonadotropes: partners in the regulation of the reproductive system. Trends Endocrinol Metab 11:168–175
7. Childs GV (2002) Development of gonadotropes may involve cyclic transdifferentiation of growth hormone cells. Arch Physiol Biochem 110:42–49
8. Childs GV, Unabia G, Wu P (2000) Differential expression of growth hormone messenger ribonucleic acid by somatotropes and gonadotropes in male and cycling female rats. Endocrinology 141:1560–1570
9. Childs GV, Ellison DG, Ramaley JA (1982) Storage of anterior lobe adrenocorticotropin in corticotropes and a subpopulation of gonadotropes during the stress-nonresponsive period in the neonatal male rat. Endocrinology 110:1676–1692
10. Childs GV, Unabia G, Miller BT, Collins TJ (1999) Differential expression of gonadotropin and prolactin antigens by GHRH target cells from male and female rats. J Endocrinol 162:177–187
11. De Marinis L, Mancini A, Zuppi P, Anile C, Maira G (1990) Paradoxical growth hormone response to thyrotropin-releasing hormone in acromegaly. Clinical correlations and prognostic value. Acta Endocrinol (Copenh) 122:443–449
12. Fischer UG, Wood SH, Bruhn J, Roseff SJ, Mortola J, Rivier JE, Yen SSC (1992) Effect of human corticotropin-releasing hormone on gonadotropin secretion in cycling and postmenopausal women. Fertil Steril 58:1108–1112
13. Frawley LS, Boockfor FR (1991) Mammosomatotropes: presence and functions in normal and neoplastic pituitary tissue. Endocr Rev 12:337–355
14. Fukami K, Tasaka K, Mizuki J, Kasahara K, Masumoto N, Miyake A, Murata Y (1997) Bihormonal cells secreting both prolactin and gonadotropins in normal rat pituitary cells. Endocr J 44:819–826
15. Gershengorn MC (1986) Mechanism of thyrotropin releasing hormone stimulation of pituitary hormone secretion. Annu Rev Physiol 48:515–526
16. Harvey S (1990) Thyrotrophin-releasing hormone: a growth hormone-releasing factor. J Endocrinol 125:345–358
17. Hauspie A, Seuntjens E,Vankelecom H, Denef C (2003). Stimulation of combinatorial expression of prolactin and glycoprotein hormone a subunit genes by gonadotropin-releasing hormone and estradiol-17 b in single rat pituitary cells during aggregate cell culture. Endocrinology 144:388–399
18. Horvath JE, Schally AV (1994) Reciprocal changes in prolactin and growth hormone secretion in vitro after in vivo estrogen treatment. Acta Biol Hung 45:249–262
19. Horvath E, Lloyd RV, Kovacs K (1990) Propylthiouracyl-induced hypothyroidism results in reversible transdifferentiation of somatotrophs into thyroidectomy cells. A morphologic study of the rat pituitary including immunoelectron microscopy. Lab Invest 63:511–520
20. Kasahara K, Tasaka K, Masumoto N, Mizuki J, Tahara M, Miyake A, Tanizawa O (1994) Characterisation of rat pituitary cells by their responses to hypothalamic releasing hormones. Biochem Biophys Res Commun 1999:1436–1441
21. Kato M, Hoyland J, Sikdar SK, Mason WT (1992) Imaging of intracellular calcium in rat anterior pituitary cells in response to growth hormone-eleasing factor. J Physiol (Lond) 447:171–189
22. Kineman RD, Faught WJ, Frawley LS (1992) Steroids can modulate transdifferentiation of prolactin and growth hormone cells in bovine pituitary cultures. Endocrinology 130:3289–3294
23. Losa M, Schopohl J, Müller A, Von Werder K (1985) Growth hormone releasing factor induces prolactin secretion in acromegalic patients but not in normal subjects. Acta Endocrinol 109:467–473
24. Luini A, Lewis D, Guild S, Corda D, Axelrod J (1985) Hormone secretagogues increase cytosolic calcium by increasing cAMP in corticotropin-secreting cells. Proc Natl Acad Sci USA 82:8034–8038

25. Matsukura S, Kakita T, Hirata Y, Yoshimi H, Fukase M (1977) Adenylate cyclase of GH and ACTH producing tumors of human: activation by non-specific hormones and other bioactive substances. J Clin Endocrinol Metab 44:392–397
26. Nuñez L, Villalobos C, Senovilla L, Garcia-Sancho J (2003) Multifunctional cells of mouse anterior pituitary reveal a striking sexual dimorphism. J Physiol (Lond) 549:835–843
27. Okada Y, Fujii Y, Moore JP Jr, Winters SJ (2003) Androgen receptors in gonadotrophs in pituitary cultures from adult male monkeys and rats. Endocrinology 144:267–274
28. Roudbaraki M, Lorsignol A, Langouche L, Callewaert G, Vankelecom H, Denef C (1999) Target cells of γ3-melanocyte-stimulating hormone detected through intracellular Ca^{2+} responses in immature rat pituitary constitute a fraction of all main pituitary cell types, but mostly express multiple hormone phenotypes at the messenger ribonucleic acid level. Refractoriness to melanocortin-3 receptor blockade in the lacto-somatotroph lineage. Endocrinology 140:4874–4885
29. Seuntjens E, Hauspie A, Roudbaraki M, Vankelecom H, Denef C (2002) Combined expression of different hormone genes in single cells of normal rat and mouse pituitary. Arch Physiol Biochem 110:12–15
30. Seuntjens E, Hauspie A, Vankelecom H, Denef C (2002) Ontogeny of plurihormonal cells in the anterior pituitary of the mouse, as studied by means of hormone mRNA detection in single cells. J Neuroendocrinol 14:611–619
31. Shangold GA, Murphy SN, Miller RJ (1988) Gonadotropin-releasing hormone-induced Ca^{2+} transients in single identified gonadotropes require both intracellular Ca^{2+} mobilization and Ca^{2+} influx. Proc Natl Acad Sci USA 85:6566–6570
32. Tabarin A, Corcuff JB, Rashedi M, Angibeau R, Caille JM, Ducassou D, Dufy B, Roger P (1992) Multihormonal response to CRH in inferior petrosal sinus blood of one patient with Cushing's disease: comparison with in vitro secretion of the tumoral corticotropes. Acta Endocrinol 127:284–288
33. Vidal S, Horvath E, Kovacs K, Cohen SM, Lloyd RV, Scheithauer BW (2000) Transdifferentiation of somatotrophs to thyrotrophs in the pituitary of patients with protracted primary hypothyroidism. Virchows Arch 436:43–51
34. Vidal S, Horvath E, Kovacs K, Lloyd RV, Smyth HS (2001) Reversible transdifferentiation: interconversion of somatotrophs and lactotrophs in pituitary hyperplasia. Mod Pathol 14:20–28
35. Villalobos C, Núñez L, García-Sancho J (1996) Functional glutamate receptors in a subpopulation of anterior pituitary cells. FASEB J 10:654–660
36. Villalobos C, Núñez L, Frawley LS, García-Sancho J, Sánchez A (1997) Multi-responsiveness of single anterior pituitary cells to hypothalamic-releasing hormones: A cellular basis for paradoxical secretion. Proc Natl Acad Sci USA 94:14132