

C H A P T E R

2

c0002

# The Molecular Cell Biology of Anterior Pituitary Cells

*Arturo E. Gonzalez-Iglesias and Richard Bertram*

Florida State University, Tallahassee, FL, USA

## INTRODUCTION

s0010

AU:1

“Do not stop to question whether these ideas are new or old, but ask, more properly, whether they harmonize with nature.” *Marcello Malpighi*

p0015

The dawn of neuroendocrinology is coincident with the beginning of the tale of a remarkable gland: the pituitary gland. Around AD 170 Galen postulated that the “pituita” (from the Greek *ptuo*, “to spit,” and the Latin *pituita*, “mucus”) secreted waste products (phlegm, one of the four humors of the body) from the brain into the nasal cavities. Nineteen centuries later, we have found not only that Galen’s concept was far from the truth but also that a fascinating – yet still primitive – understanding of the gland has emerged.

p0020

The pituitary is one of the two elements that make up the hypothalamo–hypophysial unit, the joint anatomical structure comprising hormone-producing neurons and cells by which the brain regulates the vital functions of the body. Indeed, a key regulator of body homeostasis during development, stress, and other

physiological processes, the pituitary gland acts as a double interpreter, mediating the talk between the brain and the peripheral organs, and integrating their respective cues as well as those of its own (local autocrine and paracrine factors). Being functionally and anatomically connected to the hypothalamus by the median eminence via the infundibular stalk,<sup>1</sup> the pituitary has two embryologically and functionally distinct divisions: the neurohypophysis (or neural lobe) and the adenohypophysis (anterior pituitary and intermediate lobes). The remarkable molecular and cellular aspects of the biology of the cells that constitute the anterior pituitary is the focus of this chapter.

## ANTERIOR PITUITARY: ORGANIZATION, CELL TYPES, HORMONES AND FUNCTIONS

s0015

p0025

The anterior pituitary is an endocrine gland responsible for secreting hormones that regulate a wide range of functions. These hormones

are synthesized and released by distinct groups of polygonal endocrine cells that are organized as interlacing cords and lined up on an anastomosing web of capillary vessels (the secondary capillary plexus) derived from the hypophysial artery. The cytoplasm of these cells contains granules of stored hormone that are released by exocytosis. The endothelial cells of the capillaries are fenestrated to facilitate the exchange of molecules between the endocrine cells and the blood, which not only bring in the hypothalamic and peripheral factors (through the long portal vessels and hypophysial arteries, respectively) that regulate the activity of the gland but also carry the released pituitary hormones away into the general circulation.<sup>1</sup> In addition, an extensive web of interconnected folliculostellate (FS) cells surround the endocrine cells. These cells regulate both the interaction of neighboring endocrine cells and the exchange of molecules between them and the capillaries. FS cells represent about 5–10% of the anterior pituitary cell population.

p0030 The traditional view of the pituitary holds that there are five endocrine cell types that are responsible for synthesizing six anterior pituitary hormones (Table 2.1). For each cell type, several immortalized cell lines have been developed, characterized and used extensively.<sup>2</sup>

p0035 Somatotrophs, which synthesize and release growth hormone (GH), are the major endocrine cell type in the anterior pituitary and constitute 40–50% of its cell population. They are localized predominantly to the lateral portions of the anterior lobe. Somatotroph function is primarily regulated by hypothalamic factors: growth hormone-releasing hormone (GHRH) produced by neurons in the arcuate nucleus is stimulatory, whereas somatostatin (STT) produced by neurons in the periventricular nucleus is inhibitory. STT suppresses both basal and GHRH-induced GH release, having no effect on GH synthesis. GH production and secretion also receives inhibitory feedback from the major target of GH, insulin-like

growth factor-I from the liver. Somatotrophs express receptors for many other regulators of GH synthesis and release, including ghrelin, pituitary adenylate cyclase-activating peptide, thyroid hormone, glucocorticoids, insulin and endothelins. GH is secreted from the somatotrophs at a pulse frequency of about 1–2 h with a half-life that ranges between 6–20 min, and the pattern exhibits gender differences. In the case of males, the pulses are much larger early at night, whereas in females the pattern is more irregular and the pulses tend to be more uniform throughout the day. The pattern of GH release appears to be driven by the rate at which GHRH is released from the arcuate nucleus neurons.

GH is also called somatotropin (*soma*, p0040 “body”) because of its profound and widespread anabolic effects throughout the body. In its absence, growth is stunted. Although virtually every tissue responds to some degree, skeletal muscle cells, liver, and chondrocytes (cartilage cells) are particularly sensitive to GH levels. Though the metabolic effects are direct actions of GH, it is now apparent that most, if not all, of the anabolic effects of GH are mediated by the production of a family of peptide hormone intermediaries known as insulin-like growth factors (IGFs) which are secreted by the liver, cartilage, muscle, and other tissues where they can act locally in a paracrine or autocrine fashion. GH, acting through the IGFs, stimulates protein synthesis, cell growth and a positive nitrogen balance, leading to an increase in lean body mass and a decrease in body fat. Many hours must elapse after administration of GH before its anabolic, growth-promoting effects become evident.

Thyrotrophs comprise approximately 5% of p0045 the anterior endocrine cell population and are typically spread over the anteriomedial and lateral portions of the gland. Thyrotrophs synthesize and secrete thyroid-stimulating hormone (TSH), also known as thyrotropin, which is controlled by central and peripheral

TABLE 2.1 Hypothalamic Neurohormones and Anterior Pituitary Endocrine Cell Types and Hormones

Hypothalamic Releasing or Inhibiting Neurohormone	Target Cell in Anterior Pituitary Gland	Anterior Pituitary Hormone Release Stimulated or Inhibited	Target Anterior Pituitary Hormone
Growth hormone releasing hormone (GHRH)	Somatotroph	Growth hormone (GH)	Multiple somatic tissues
Somatostatin (STT)		GH	Multiple somatic tissues
Thyrotropin releasing hormone (TRH)	Thyrotroph	Thyroid stimulating hormone (TSH)	Stimulates synthesis and release of thyroid hormone by thyroid gland
Corticotropin releasing hormone (CRH)	Corticotroph	Adrenocorticotrophic hormone (ACTH)	Stimulates synthesis and release of steroids by adrenal cortex
Gonadotropin releasing hormone (GnRH)	Gonadotroph	Follicle stimulating hormone (FSH) and luteinizing hormone (LH)	Stimulates synthesis and release of gonadal steroids, gametogenesis, ovulation
Dopamine	Lactotroph	Prolactin	Milk synthesis and secretion by the mammary gland

regulators. The dominant stimulatory control of thyrotroph function and TSH secretion is exerted by the hypothalamic neurohormone thyrotropin-releasing hormone (TRH) released by neurons of the paraventricular nucleus. TSH is secreted in pulses lasting for 2–3 h with a nocturnal surge before sleep. Once the sleep phase begins, TSH release is curtailed. The half-life of the hormone in blood is approximately of 1 h.

TSH is a glycoprotein with a molecular weight of 28,000 and consists of a heterodimer of two subunits ( $\alpha$  and  $\beta$ ) that are tightly associated by noncovalent forces and encoded by separate genes. Although both subunits are required for receptor binding and hormone action, the  $\beta$  subunit confers biological specificity to the TSH molecule, as the  $\alpha$  subunit is also a component of the anterior pituitary gonadotropin hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The essential actions of TSH are those exerted on the thyroid gland, where it promotes growth and differentiation of the gland and stimulates all steps in the secretion of the thyroid hormones

thyroxine (T4) and triiodothyronine (T3). These steps include glandular uptake of iodide, its organification, the completion of thyroid hormone synthesis, and the subsequent release of thyroid gland products. T3 and T4 act at the hypothalamic and pituitary levels to block the secretion of TSH via feedback inhibition. Fasting decreases thyrotroph responsiveness to TRH, while exposure to cold increases it.

Corticotrophs constitute about 15% of the adenohypophysial endocrine cell population and are scattered throughout the anterior lobe in adult animals, but are primarily found in the anteromedial part of the gland. They synthesize proopiomelanocortin (POMC) and release its proteolytic derivatives, adrenocorticotropin hormone (ACTH),  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH), lipotropic hormone (LPH) and endorphins. The main releasing factors for are the hypothalamic corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP), which acts in synergy with CRH to potentiate hormone release. Glucocorticoids, secreted by the adrenal cortex, are the major physiological inhibitor. ACTH levels in plasma

exhibit a circadian rhythm, with a peak in the early morning followed by a gradual decline during the night, and is controlled by clock neurons in the suprachiasmatic nucleus that synapse with CRH-producing neurons in the hypothalamic paraventricular nucleus. ACTH also exhibits faster, ultradian and hourly rhythms due to feedback between corticotrophs and adrenal cells.<sup>3</sup>

p0060 As its name implies, one of the major actions of ACTH is the promotion of growth of adrenal cortex cells. ACTH effects on these cells are also necessary for both basal and stress-induced secretion of glucocorticoids and aldosterone. Its half-life of 10 min allows for rapid adjustments of circulating levels of glucocorticoids. ACTH, CRH, and glucocorticoids are the main effector molecules of the hypothalamo-pituitary-adrenal (HPA) axis, the system that activates to help the organism adapt and cope with different stress cues (hypoglycemia, anesthesia, surgery, trauma, hemorrhage, infection, pyrogens) and psychiatric disorders like anxiety or depression.

p0065 Gonadotrophs constitute about 10–15% of the anterior pituitary endocrine cells and are localized throughout the pars distalis and most of the pars tuberalis of the anterior lobe. They form intimate contacts with lactotroph cells, with which they have extensive cell-to-cell (paracrine) interactions. Gonadotrophs synthesize and release two hormones essential to the growth and function of the gonads in both genders (hence their common designation as gonadotropins): luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Synthesis and release of both hormones are stimulated by gonadotropin-releasing hormone (GnRH), which is secreted in a pulsatile manner by neurons that are dispersed within the mediobasal hypothalamus and preoptic areas. In addition, other hypothalamic factors such as gonadotropin-inhibiting hormone, vasopressin, substance P, as well as feedback of gonadal factors (estrogens, progestogens, androgens

and inhibin) contribute to the regulation of gonadotropins. The release of LH and FSH occurs in phase with pulses of GnRH, with intervals that range from 30 to 60 min depending on the species and, for females, the stage of the ovarian cycle. During the ovarian cycle, the levels of LH and FSH in plasma correlate strongly with the gonadotropin content of the pituitary, and both are highest just before ovulation.

LH, with a molecular weight of 28,000, and FSH, with a molecular weight of 33,000, are glycoproteins with similar structures. Each is composed of the common pituitary hormone  $\alpha$  subunit, also found in TSH, and a unique  $\beta$  subunit that confers them hormonal specificity. The carbohydrate moiety of the latter subunit also enhances their half-life (1 h for LH and about 3 h for FSH) and is critical for receptor binding and biological responses, and its modification allows considerable variation of the bioactivity of secreted LH and FSH molecules in different physiological conditions. LH stimulates the interstitial cell lines of male and female gonads (Leydig and thecal cells) mainly to secrete androgens (particularly testosterone), whereas FSH stimulates testicular Sertoli and ovarian granulosa cells to secrete estrogens (particularly estradiol) and a variety of protein products essential to spermatogenesis and oogenesis, respectively. During the initiation of the reproductive cycle of females, FSH acts on primary follicles to stimulate growth of the granulosa cells. Once the follicular phase is advanced and draws near to ovulation, LH also acts in female granulosa cells to promote progesterone production.

Lactotrophs make up about 15–25% of the adenohypophysial endocrine cell population and are a particularly non-homogeneous group of endocrine cells scattered throughout the anterior pituitary. A significant number are also found in the posterior medial portion of the gland. They synthesize and release prolactin (PRL), a 198 amino-acid protein that owes AU:3

its name to its role in milk production during lactation in mammals. PRL has structural similarity and a comparable half-life (20 min) to GH. Like somatotrophs, lactotrophs inherently have high secretory activity due to their spontaneous electrical activity, though the former cells are not as active as lactotrophs in terms of their secretory activity. Thus, unlike all other anterior pituitary hormones, the physiological control of PRL is predominantly via tonic hypothalamic inhibition mediated by dopaminergic neurons located primarily in the arcuate nucleus. Therefore, a drop in the levels of dopamine (DA) in portal plasma often translates into an increase of the PRL-releasing activity of these cells. Hormone release by lactotrophs can also be stimulated by an extensive range of factors such as TRH, oxytocin, vasoactive intestinal peptide (VIP), angiotensin II, endothelin-1, serotonin and estrogens, but none of them have been established as a physiologically relevant PRL-releasing hormone. Like other trophic hormones, PRL secretion rises at night, and release occurs in episodic pulses.

AU:4

p0080

AU:5

In addition to its roles in the growth of alveolar breast cells and milk production, prolactin inhibits GnRH-induced release of gonadotropins in the anterior pituitary as well as the actions of gonadotropins on the gonads. This may be the mechanism by which it prevents ovulation in lactating women and normal sperm production in males. Indeed, PRL facilitates the release of DA from the ME and thus acts in a negative feedback loop to inhibit its own secretion. Many other roles have been described for prolactin, including control of sexual behavior, induction of maternal behavior in pregnancy, and support of the corpora lutea during pregnancy and pseudopregnancy (in rodents). Similarly to ACTH, PRL release is stimulated by different stressor cues, including insulin-induced hypoglycemia, infection, surgery, anesthesia, and fear. Exercise and stimulation of the nipples both stimulate prolactin

secretion, an effect that is thought to be mediated by oxytocin.

## DISEASES OF THE ANTERIOR PITUITARY

s0020

Diseases of the pituitary can be divided into two major categories of pituitary disturbances, namely, hyperactivity (termed hyperpituitarism) and hypoactivity (hypopituitarism).<sup>4</sup> We focus on those that arise from disorders of the anterior pituitary. Because the function of the anterior pituitary is based on the specialized action (and interaction) of its different endocrine cells, the type of clinical response to each pituitary condition varies with the type of cell that is affected.

p0085

### Hypopituitarism

s0025

With an annual estimated incidence (number of new cases per population in a given time period) of 4.2 per 100,000 and prevalence (proportion of the total number of cases to the total population) of 45.5 per 100,000, hypopituitarism might be caused by either an inability of the gland itself to produce hormones or an insufficient supply of hypothalamic-releasing hormones. It is causally associated with pituitary tumors (61%), non-pituitary lesions (9%), and non-cancerous causes (30%), including perinatal insults, genetic causes, trauma and idiopathic cases. Often, mutations in genes encoding single hormones (or the receptor for their cell-specific hypothalamic releasing factor) result in single pituitary hormone deficiency. Unless successfully treated, hypopituitarism is chronic and lifelong, and in cases of shortage of ACTH or TSH it can cause life-threatening events and lead to increased mortality. If there is decreased secretion of most pituitary hormones, the term panhypopituitarism is used.

p0090

s0040 p0095 **ACTH DEFICIENCY** This causes adrenal atrophy and ACTH-receptor downregulation, low blood pressure, low blood sugar level, fatigue, weight loss, and low tolerance for stress.

s0045 p0100 **TSH DEFICIENCY** Features of TSH deficit include underactive thyroid, fatigue, cold intolerance, weight gain, constipation, hair loss, dry skin and cognitive slowing.

s0050 p0105 **LH, FSH DEFICIENCIES** In premenopausal women, absent or infrequent menstrual cycles, infertility, vaginal dryness, dyspareunia (painful sexual intercourse), loss of libido, and loss of some female characteristics occur. In men, these deficiencies are associated with impotence, shriveling of testes, decreased sperm production, infertility, loss of libido, and loss of some male characteristics. In childhood, deficiencies result in delayed or missing onset of puberty.

s0055 p0110 **GH DEFICIENCY** The hallmarks of insufficient GH release are decreased muscle mass and strength, visceral obesity, fatigue, decreased quality of life, and impairment of attention and memory; there is stunted growth and dwarfism in children.

s0060 p0115 **PRL DEFICIENCY** Inability to produce breast milk after childbirth occurs in some women. However, elevated prolactin concentrations sometimes occur in hypopituitarism because of disruption of inhibitory signals by the hypothalamus, causing lactation, tenderness of the breast, and suppression of gonadotropins, leading to symptoms of hypogonadism.

## s0065 **Hyperpituitarism**

p0120 Hyperpituitarism is the primary hypersecretion of pituitary hormones. It typically results from a pituitary tumor or adenoma, which represent from 10–25% of all intracranial

neoplasms. Most pituitary adenomas are benign, functional, and secrete a hormone that produces clear symptoms characteristic of their condition. The four most common types of adenoma-related hyperpituitarism are prolactinoma, corticotropinoma (Cushing's disease), somatotropinoma (gigantism), and null cell adenomas that do not secrete hormones. Since the enlargement of the anterior pituitary gland can damage the optic nerves and compress the hypothalamus, some of its common symptoms are headache, loss of side or peripheral vision, and hyposalivation of neighboring anterior pituitary hormones. Clinically active pituitary adenomas affect approximately one in 1000 of the general population but are rare in children.

**PROLACTINOMA** This is an adenoma of lactotroph cells and the most common type of pituitary tumor (30% of pituitary adenomas). In women, high blood levels of prolactin often cause changes in menstruation (periods may become irregular or disappear) and those who are not pregnant or nursing may begin producing breast milk (galactorrhea). Because of the hyperprolactinemia-induced inhibition of the gonadotropin axis, women with hyperprolactinemia also exhibit symptoms of gonadotropin deficit, such as loss of libido, dyspareunia, vaginal dryness and hypogonadism. In males, the most common symptom of prolactinoma is impotence, loss of libido, hypogonadism, oligospermia, and diminished ejaculate volume. p01250080

**CORTICOTROPINOMA** Also known as Cushing's disease, this is a tumor of corticotroph cells that accounts for 20% of pituitary adenomas. The symptoms reflect the presence of excess cortisol or ACTH and include weight gain, high blood pressure, poor short-term memory, irritability, extra fat around the neck, a round and ruddy face, fatigue, and poor concentration. Women also exhibit menstrual irregularity and hirsutism (abnormal hair growth). p01300085

s0090 p0135 **SOMATOTROPINOMA** This is a tumor of somatotroph cells that represents 15% of pituitary adenomas. The resulting hypersecretion of GH in adults causes acromegaly (an overgrowth of the terminal parts of the skeleton such as the nose, mandible, hands and feet). When it presents in children and adolescents it causes gigantism; disruption of sexual maturation is common, either because of hormone hypersecretion or because of manifestations caused by compression of hypothalamic connections by the adenoma.

s0095 p0140 **NULL CELL ADENOMAS** These are dysfunctional tumors that account for 25% of pituitary adenomas. Because they do not secrete hormones, symptoms are restricted to headache, increased intracranial pressure and visual field defects.

s0100 p0145 **GONADOTROPHIC ADENOMAS** These are tumors of gonadotroph cells that account for 10% of pituitary adenomas. Although functional, they are usually clinically silent.

s0105 p0150 **THYROTROPHIC ADENOMAS** These are rare (less than 1%) but have the particular characteristic of being plurihormonal, since they produce the common glycoprotein  $\alpha$ -subunit, prolactin, and the specific  $\beta$ -subunits of LH and FSH.

s0110 **PHENOTYPIC PROFILE OF ANTERIOR PITUITARY CELL TYPES: DIFFERENT, YET SOMEWHAT SIMILAR**

p0155 The anterior pituitary endocrine cell types are classically defined on the basis of the expression of a specific hormone and its corresponding mRNA, and this is determined by various lineage-specific transcription factors.<sup>5</sup> Although two-thirds of the population of each anterior pituitary cell type exclusively express

their respective hormone at both mRNA and protein levels, the remainder coexpress the mRNAs of two to four different hormones.<sup>6</sup> For example, a GH cell can also express the mRNAs of PRL, TSH- $\beta$ , LH- $\beta$  and POMC. In addition, a significant fraction of the non-hormone producing cells contain multiple hormone mRNAs that fail to be translated into the mature protein. Thus, at the mRNA level, the various anterior pituitary hormones are shared by a fraction of cells that express multilineage phenotypes. These multiple mRNA-containing (or polyhormonal) cells are thought to be progenitor cells or "reserve" cells that upon appropriate signals will terminally differentiate depending on developmental and/or physiological needs. The origin and biological relevance of these cells are currently a matter of intense investigation and debate.

The current paradigm for hypothalamic control of anterior pituitary hormone secretion holds that each hypothalamic-releasing hormone modulates the release of a single pituitary hormone by acting on a single cell type. However, when attempts were made to characterize cell phenotypes on the basis of whether their intracellular calcium concentration ( $[Ca^{2+}]_i$ ) responded to a specific hypothalamic releasing hormone, many cells were found to respond to two or more of these agents. Furthermore, hormone release assays in single living cells revealed that multi-responsiveness also exists with respect to hormone release. Such "paradoxical" hormone release by a noncorresponding hypothalamic releasing hormone had, in fact, been observed in a number of *in vitro* and *in vivo* studies using normal and pathological cells. Indeed, a growing body of evidence suggests that 30–40% of normal pituitary cells remain in a multipotential state, able to respond to as many as four different hypothalamic secretagogues.<sup>7</sup> In some pituitary adenomas, these multi-responsive cells can constitute up to 80% of the endocrine cells. Moreover, much like the

polyhormonal cells described above, multi-responsive cells are not restricted to any particular cell type. Thus, different cell types can also share receptors to allegedly cell-specific hypothalamic neurohormones. In fact, the mRNA of the receptor for the classic hypothalamic GH inhibitory hormone, somatostatin, is found among all five major cell types of the anterior pituitary. The type 1 CRH receptor (CRH-R1) is not exclusively expressed by corticotrophs; subsets of lactotrophs, gonadotrophs and thyrotrophs also express it in different degrees. It has also been shown that about 35% of somatotrophs bind biotinylated GnRH, the stimulating hormone for gonadotrophs. It has been hypothesized that estrogens and possibly activin stimulate the expression of GnRH receptors in pre-existing GH cells at midcycle, rendering them capable of fully responding as a gonadotroph to help support the GnRH-mediated proestrus surge of LH and FSH release. Additionally, retention of GHRH receptors would allow supporting the GH needs of the reproductive system at this time.<sup>8</sup>

p0165 Whether these multi-responsive cells derive from an independent multipotential subset or from pre-existing differentiated cells (termed *transdifferentiation*), they provide a cellular basis for anterior pituitary plasticity. Two observations are consistent with this. The first one is that multi-responsive cells are maximally abundant at puberty, a time signed by huge endocrine and physiological transformation. Secondly, multifunctional cells seem to be more abundant in females than in males, suggesting not only that the female pituitary may be more plastic than the male pituitary, but also that the hormonal changes associated with the female reproductive cycle may promote transdifferentiation.

p0170 Unstimulated anterior pituitary cells can also be classified by their biophysical and biochemical properties, more specifically, on their patterns of electrical activity and intracellular

Ca<sup>2+</sup> dynamics.<sup>9,10</sup> A general feature of cultured anterior pituitary cells is that their membrane potential ( $V_m$ ) oscillates between potentials of  $-60$  to  $-50$  mV. When  $V_m$  reaches the threshold level, pituitary cells fire action potentials (APs), a feature called spontaneous electrical activity and is observed in 15–80% of the cells, depending on the cell type and of cultural and/or recording conditions. The firing of APs causes transients of intracellular Ca<sup>2+</sup> concentration ( $[Ca^{2+}]_i$ ) that in turn reflect the pattern of electrical activity. Two patterns of electrical activity are typically observed in anterior pituitary cells. The first, termed axonal-type AP spiking, is typically found in gonadotrophs,<sup>11</sup> thyrotrophs,<sup>12</sup> and largely in corticotrophs,<sup>13</sup> and is characterized by sharp single APs that are short in duration (less than 100 ms), with spike frequencies of about 0.7 Hz and amplitudes of more than 60 mV. Axonal-type AP spiking is associated with small-amplitude  $[Ca^{2+}]_i$  transients that range from 20 nM to 70 nM and low basal hormonal release in these cells. The second pattern, termed pseudo-plateau bursting and characteristic of cultured somatotrophs<sup>14</sup> and lactotrophs,<sup>15</sup> exhibits broader  $V_m$  oscillations in the form of a depolarizing plateau with superimposed bursts of small amplitude APs that usually do not reach 0 mV. Such bursts have a longer duration (several seconds) than gonadotroph APs, so that the burst frequency is significantly lower (about 0.3 Hz), and result in an oscillatory increase in  $[Ca^{2+}]_i$  of high amplitude that ranges from 0.3 to 1.2  $\mu$ M and is sufficient to maintain high and steady hormonal (GH and prolactin) release. Although the typical patterns of spontaneous electrical activity are useful for the identification of the different cell populations of the anterior pituitary, they should not be used as the sole criteria, since the electrical activity of these cells often experience spontaneous reversible transitions between the two modes.

s0115 **HETEROGENEITY WITHIN  
ANTERIOR PITUITARY CELL  
TYPES: SIMILAR, YET SOMEWHAT  
DIFFERENT**

p0175 Although different cell types have similarities, it is also apparent that similar cells (that is, cells within a cell type population) may present striking differences. It has been long recognized that each of the different types of anterior pituitary cells are functionally heterogeneous, which is thought to be related to structural heterogeneity. One of the best known examples is found among gonadotrophs,<sup>16,17</sup> which are heterogeneous in size (cell area ranging from 30–170  $\mu\text{m}^2$ ), morphology (large rounded, small oval and angular stellate cells), physical density (allowing their separation using sedimentation techniques at unit gravity), ultrastructural characteristics (type I gonadotrophs characterized by dilated rough ER (RER) cisternae and secretory granules of 200 nm and 300–700 nm in diameter, type II gonadotrophs by flattened RER cisternae and 200–250 nm secretory granules, type III gonadotrophs by a stellate shape and secretory granules of 220–250 nm), hormone content (small gonadotrophs seem to store only one of the gonadotropins, whereas most of the larger cells either store both LH and FSH, or FSH alone) and responsiveness (variable capacity to bind and respond to GnRH). These gonadotroph subpopulations may account for the differential regulation of LH and FSH secretion *in vivo*.

p0180 The other anterior pituitary cell types exhibit heterogeneity of these cellular features as well. In the porcine pituitary, somatotrophs comprise two morphologically distinct subpopulations of low- (LD) and high-density (HD) cells, separable by Percoll gradient, that respond differently to hypothalamic regulators. In LD somatotrophs, somatostatin inhibits GHRH-induced GH secretion, whereas somatostatin alone stimulates GH release from HD somatotrophs.

Functional heterogeneity of corticotrophs displays striking sexual dimorphism. In males, the corticotrophs are of the orthodox phenotype, that is, monohormonal (storing only ACTH) and monoreceptorial (responding only to CRH). Their female counterparts are made of about equal parts of orthodox and multifunctional cells. Sexual dimorphism and functional heterogeneity are even more striking in thyrotrophs, which are mostly polyhormonal in both genders, but only female thyrotrophs co-store GH and/or ACTH in addition to prolactin and/or LH. Among lactotrophs, three morphological types in rodents have been defined by electron microscopy. Type I “classical” lactotrophs contain large irregular-shaped electron-dense secretory granules (diameter 300–700 nm), type II cells contain numerous medium sized spherical electron-dense granules (diameter 100–250 nm) and type III cells contain small (<100 nm) spherical granules. Though thought to represent different stages of cell maturity, these morphologically-defined lactotroph subpopulations have functional differences, as these subtypes are differentially sensitive to hypothalamic and local regulators of prolactin secretion.<sup>18</sup>

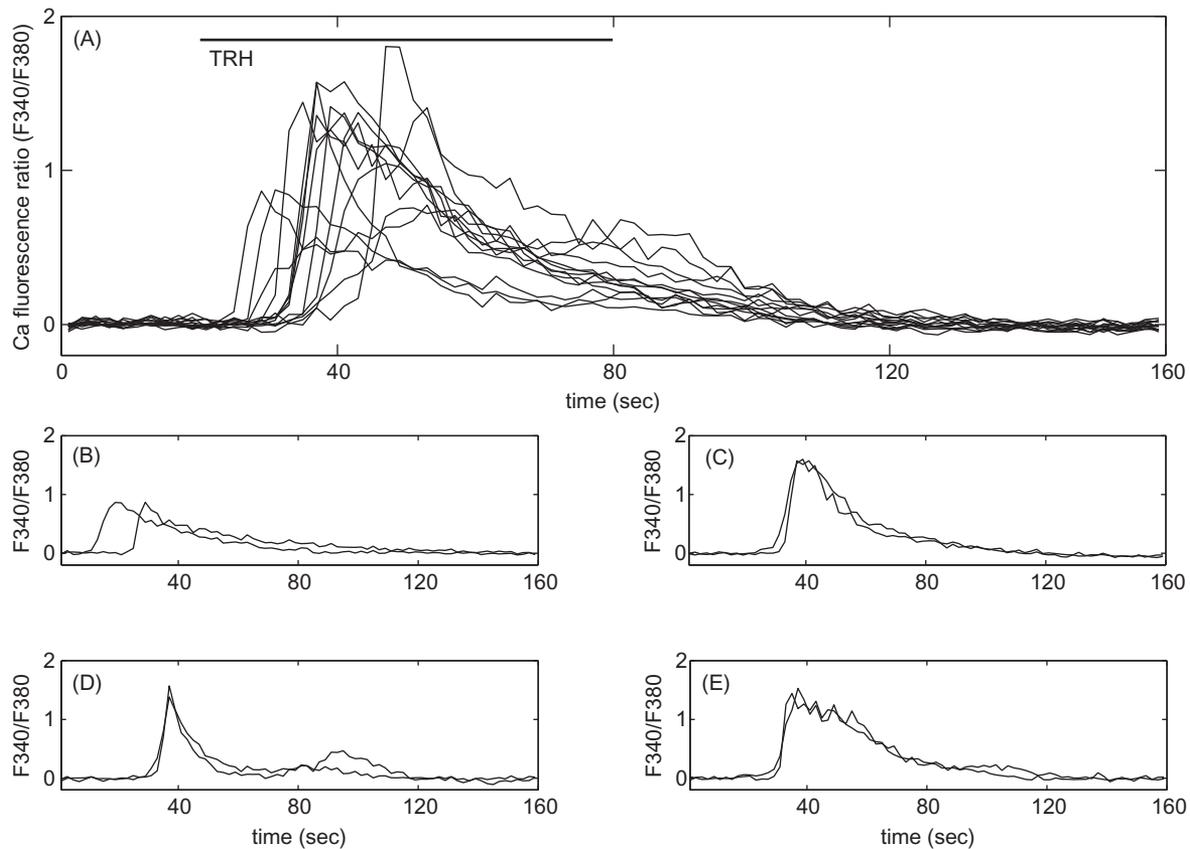
Several substates of a particular pattern of electrical activity can be found within a given anterior pituitary cell type. In somatotrophs, the burst period of somatotrophs ranges from 2 to 10 s, with longer active phase duration associated with the slower bursting, which results in increased  $\text{Ca}^{2+}$  influx through voltage-gated  $\text{Ca}^{2+}$  channels (VGCC) and higher amplitude  $\text{Ca}^{2+}$  oscillations. An explanation of the heterogeneity of the active phase duration has been recently provided.<sup>14,19</sup>

Lactotrophs also display considerable variability in their spontaneous and receptor-controlled patterns of electrical activity and  $\text{Ca}^{2+}$  dynamics. The authors have recently addressed the heterogeneity in the  $[\text{Ca}^{2+}]_i$  responses of lactotrophs to TRH.<sup>20</sup> Responses were evaluated in

the absence of extracellular  $\text{Ca}^{2+}$  to prevent  $\text{Ca}^{2+}$  influx during the agonist challenge and remove one potential source of heterogeneity. Figure 2.1A shows thirteen  $\text{Ca}^{2+}$  traces from individual lactotrophs responding to the same TRH application, exhibiting considerable variability. In contrast, Figure 2.1B–E shows traces from four different lactotroph cells subjected to two consecutive TRH applications. In each cell, the response to the second TRH pulse was very similar to that of the first pulse. Thus, during the time course of the observations, heterogeneity in

the  $\text{Ca}^{2+}$  response to TRH could be observed among lactotroph cells, with uniformity of response within single cells to multiple TRH applications. Of the many potential variables that could result in the observed heterogeneity of the  $\text{Ca}^{2+}$  responses to TRH, the authors have found that variability in the rate of  $\text{Ca}^{2+}$  extrusion through the plasma membrane ( $k_{\text{PMCA}}$ ) largely accounts for it. Since TRH may modulate the activity of the plasma membrane  $\text{Ca}^{2+}$  adenosine triphosphate (ATP)ase pump, it might also contribute to the observed  $k_{\text{PMCA}}$  heterogeneity.

AU:7



f0010 **FIGURE 2.1** Heterogeneity of the  $\text{Ca}^{2+}$  response to thyrotropin-releasing hormone (TRH) in pituitary lactotrophs. In all panels, the  $x$ -axis shows time in seconds, and the  $y$ -axis shows the  $\text{Ca}^{2+}$  fluorescence ratio ( $F_{340}/F_{380}$ ). (A) Thirteen individual  $\text{Ca}^{2+}$  traces from the same experiment. Extensive variation exists in the peak, area, latency of response and decay rate. (B–E) Examples of single cell  $\text{Ca}^{2+}$  traces from the same experiment showing very similar responses to two successive challenges of TRH, 30 min apart. Original figure published in ref.<sup>20</sup>

We also found that variability in the  $[Ca^{2+}]$  in the endoplasmic reticulum ( $[Ca^{2+}]_{ER}$ ) is likely a key element of heterogeneity. In addition, our results did not show a positive correlation between the peak and decay rate of the intracellular  $Ca^{2+}$  transients, suggesting that variations in the  $G_q/IP_3$  signaling pathway are not the main source of heterogeneity.

## PATTERNS OF ANTERIOR PITUITARY CELL SIGNALING AND SECRETION

### Molecular Mechanisms Underlying Excitability and Basal Secretion

In excitable anterior pituitary cells, the resting membrane potential is controlled by classical inward rectifier  $K^+$  ( $K_{ir}$ ), ether-a-go-go-related gene (ERG) and TWIK-related (TREK-1)  $K^+$ -conducting channels. Because the  $V_m$  of anterior pituitary cells ranges between  $-50$  to  $-60$  mV, other depolarizing conductances must contribute to maintaining the resting potential. Recent findings indicate that tetrodotoxin (TTX)-insensitive  $Na^+$ -conducting channels, along with low-voltage-activated transient (T)-type voltage-gated  $Ca^{2+}$  channels, not only participate in the control of the resting potential but also act as pacemaking currents underlying the spontaneous activity frequently observed in isolated cells.<sup>10</sup> In physiological (*in vivo*) conditions, TTX-sensitive voltage-gated  $Na^+$  channels may play a role in the production of action potentials in hyperpolarized cells. However, high voltage-activated, dihydropyridine-sensitive long lasting (L)-type  $Ca^{2+}$  channels account for the bulk of the conductance during spike depolarization as removal of extracellular  $Ca^{2+}$  and addition of VGCC blockers abolish electrical activity in the majority of the endocrine pituitary cells without affecting their resting membrane potential. Finally, the concerted activity of delayed-rectifying  $K^+$  channels with both BK (large conductance) and SK

(small conductance) types of  $Ca^{2+}$ -activated  $K^+$  channels repolarize the  $V_m$  during the downstroke of an AP. In bursting cells, BK channels are also functionally important.

The distinctive patterns of electrical activity and  $Ca^{2+}$  dynamics exhibited by different types of pituitary cells are thought to arise from differences in the expression levels of ion channels such as those involved in bursting and repolarization. For example, BK channels are expressed at higher levels in somatotrophs and lactotrophs (which exhibit pseudo-plateau bursting activity) than in gonadotrophs (which display continuous, axonal-type AP spiking).<sup>21</sup> This correlation between BK channel expression levels and pattern of electrical activity suggests that larger BK conductance favors the generation of bursting activity and global  $Ca^{2+}$  signals. Indeed, blockade of BK channels in pituitary somatotrophs can switch the activity pattern of these cells from bursting to spiking, greatly reducing the amplitude of  $[Ca^{2+}]_i$  oscillations.<sup>22</sup> In other words, it is possible to convert the firing phenotype of somatotrophs to that of gonadotrophs by reducing their BK conductance. The reciprocal conversion is also true: addition of an artificial BK conductance to cultured gonadotrophs through the dynamic clamp technique changes their activity pattern from spiking to bursting.<sup>19</sup> Mathematical modeling predicted that the activation time constant of the BK conductance is important. BK activation must be fast to promote bursting; if too slow, then the BK current does not promote bursting and instead takes on its traditional inhibitory role on electrical activity.<sup>19</sup> Faster BK activation kinetics limit spike amplitude, preventing full activation of the repolarizing delayed rectifier  $K^+$  current, so membrane potential weakly oscillates around a depolarized level<sup>14,22,23</sup> before falling back toward rest, which results in bursting, increased  $Ca^{2+}$  influx and global  $Ca^{2+}$  signals.

p0205 Unlike classical neuronal synapses, neuroendocrine and endocrine cells require sustained depolarization to trigger the exocytotic pathway. In anterior pituitary cells, VGCC are open only briefly during the short time of a single AP, and the elevated  $[Ca^{2+}]_i$  is localized to the nanodomains that form at the inner mouths of open channels. With longer durations and smaller burst amplitudes, VGCC stay open much longer and significant  $Ca^{2+}$  influx ensues, resulting in the summation of individual  $[Ca^{2+}]_i$  nanodomains that generate a global  $Ca^{2+}$  signal. Therefore, differences in the ability of spontaneous firing patterns to generate global  $[Ca^{2+}]_i$  signals will determine their basal secretory activity. For example, the basal release of GH and prolactin is much higher than that of LH, which is consistent with the pseudo-plateau bursting activity of somatotrophs and lactotrophs that result in high-amplitude (global)  $[Ca^{2+}]_i$  signals and the short-duration APs of gonadotrophs that evoke low-amplitude (local)  $[Ca^{2+}]_i$  signals.

s0130  
AU:8 **Receptor-Modulated Hormone Release and Voltage-Gated  $Ca^{2+}$  Influx through cAMP-Dependent Pathways**

p0210 A number of hypothalamic neurohormones and peripheral hormones control AP-driven  $Ca^{2+}$  dynamics and  $Ca^{2+}$ -dependent hormone release in anterior pituitary cells through modulation of cAMP-dependent pathways. These bind and activate a G protein-coupled receptor (GPCR) to modulate the basal activity of adenylyl cyclase (AC, the enzyme responsible for synthesizing cAMP) and, in turn,  $Ca^{2+}$  entry. Those that promote AP-driven  $Ca^{2+}$  dynamics and  $Ca^{2+}$  dependent secretion bind to GPCRs coupled to the stimulatory class of G proteins ( $G_s$ ) to increase AC activity through the activation of the  $\alpha$  subunit of the  $G_s$  protein, whereas those that reduce  $Ca^{2+}$  signaling and secretion bind to GPCRs coupled to the inhibitory class of G proteins ( $G_{i/o}$ ) to decrease AC activity.

The stimulatory  $G_s$ -signaling pathway is triggered by CRH receptors in corticotrophs, GHRH receptors in somatotrophs and VIP/pituitary adenylate cyclase-activating polypeptide (PACAP) receptors in lactotrophs, somatotrophs and FS cells. Activation of the  $G_s$ -operated GPCRs causes plasma membrane depolarization, increased electrical activity and  $Ca^{2+}$  entry. The type of  $[Ca^{2+}]_i$  response is a plateau elevation of  $[Ca^{2+}]_i$  or an increase in the frequency and/or amplitude of  $[Ca^{2+}]_i$  transients as elevated cAMP levels promote, directly or indirectly, electrical activity and voltage-gated  $Ca^{2+}$  influx (VGCI). The direct pathway consists of the activation of a background  $Na^+$  conductance that results upon binding of cAMP to hyperpolarization-activated and cyclic nucleotide-regulated (HCN) channels. HCN channels are expressed in somatotrophs,<sup>24</sup> lactotrophs,<sup>25</sup> gonadotrophs and thyrotrophs,<sup>12</sup> and probably corticotrophs.<sup>26</sup> Because they are non-selective cation channels, they are likely to play a role in the initiation of the pacemaker depolarization. The indirect pathway consists of the activation of cAMP-dependent kinases (PKAs) that induce phosphorylation-mediated modulation of the function of several plasma membrane ion channels. In corticotrophs, CRH-induced PKA activation inhibits Kir channels to promote slow depolarization and enhanced excitability. In somatotrophs, GHRH-stimulated PKA-mediated phosphorylation results in the opening of not only T-type and L-type  $Ca^{2+}$  channels, but also TTX-insensitive voltage-gated  $Na^+$  channels, leading to the upstroke of a voltage spike.

The inhibitory  $G_{i/o}$ -signaling pathway is triggered in lactotrophs by dopamine D2 and endothelin-1 ETA receptors, and in both lactotrophs and somatotrophs by somatostatin sst1, sst2 and sst5 receptors. Other receptors linked to this pathway and expressed by anterior pituitary cells include those for adenosine,  $\gamma$ -aminobutyric acid (GABA), serotonin, melatonin and neuropeptide Y. Activation of this pathway opposes the actions mediated by the  $G_s$ -

signaling pathway resulting in membrane hyperpolarization, silencing of electrical activity and inhibition of  $\text{Ca}^{2+}$  entry and  $\text{Ca}^{2+}$ -dependent hormone secretion.  $G_{i/o}$ -mediated inhibitory actions, which can be irreversibly blocked by application of pertussis toxin, comprise two major signaling branches. The first one stems from the  $G_{i/o}$   $\alpha$ -subunit-mediated inhibition of AC activity, downregulating all cAMP-stimulated effects on electrical activity,  $\text{Ca}^{2+}$  entry and secretion. The second is due to the activation of  $G_{i/o}$   $\beta\gamma$  dimers that activate Kir3 (also known as G protein-gated inwardly rectifying  $\text{K}^+$  channels, GIRK) and inhibit L-type  $\text{Ca}^{2+}$  channels in a cAMP/PKA-independent fashion, leading to hyperpolarization and cessation of AP firing. Interestingly, three of the four mammalian Kir3 channels are specifically induced by estradiol in lactotrophs in proestrus,<sup>27</sup> underlying dopamine effects that are only observed in this stage of the cycle: strong Kir conductance and robust hyperpolarization, the latter playing a critical role in the prolactin secretory rebound that follows dopamine withdrawal.<sup>28</sup> A novel mechanism has been recently described for endothelin ETA and dopamine D2 receptors in lactotrophs. In addition to the  $G_{i/o}$  class of G proteins, these receptors couple to  $G_z$  proteins, a subfamily of  $G_{i/o}$  proteins that are insensitive to pertussis toxin. Activation of the  $\alpha$  subunit and  $\beta\gamma$  dimers of  $G_z$  by these receptors potently block VGCI and prolactin release by inhibiting AC activity and the exocytotic machinery responsible for secretion.<sup>29,30</sup>

### s0135 Receptor-Modulated Hormone Release through $\text{Ca}^{2+}$ -Mobilizing Pathways

p0225 All anterior pituitary cell types express  $\text{Ca}^{2+}$ -mobilizing GPCRs. Examples in gonadotrophs include the receptors for GnRH, endothelins, PACAP and substance P; in lactotrophs such receptors include those for acetylcholine, angiotensin II, TRH, oxytocin, ATP,

endothelin, serotonin, galanin and substance P; somatotrophs express ghrelin and endothelin receptors; in corticotrophs, AVP and norepinephrine receptors; and in thyrotrophs, TRH (the main thyrotroph secretagogues) and endothelin elicit  $\text{Ca}^{2+}$  mobilization. When activated, these  $\text{Ca}^{2+}$ -mobilizing GPCRs couple to the  $G_q$  protein class of heterotrimeric G proteins, eliciting the dissociation of the  $\alpha_q$  subunit that triggers phospholipase C-mediated phosphoinositide hydrolysis resulting in the formation of inositol 1,4,5 triphosphate ( $\text{IP}_3$ ) and diacylglycerol (DAG) (Figure 2.2).  $\text{IP}_3$  binds to  $\text{IP}_3$  receptors expressed in the membrane of the endoplasmic reticulum (ER), the primary storehouse of  $\text{Ca}^{2+}$  in most cells, causing a mobilization of  $\text{Ca}^{2+}$  out of this compartment and leading to a large and fast increase of  $[\text{Ca}^{2+}]_i$ .<sup>31</sup>

Two different  $\text{Ca}^{2+}$  signaling patterns can be observed after the initial  $[\text{Ca}^{2+}]_i$  spike. The first one, termed "biphasic," is a non-oscillatory pattern found in lactotrophs, somatotrophs and thyrotrophs where the transient  $[\text{Ca}^{2+}]_i$  spike is followed by a slow decline to a plateau level that is above basal. However, some cells exhibit "monophasic" responses where only the spike or the plateau is observed. A key condition of the biphasic  $\text{Ca}^{2+}$  response is that the  $\text{IP}_3$ Rs are opened continuously throughout the time of agonist application. The microdomain of  $\text{Ca}^{2+}$  that forms near the mouth of the  $\text{IP}_3$  receptor during the  $\text{Ca}^{2+}$  spike is high enough to trigger exocytosis. Furthermore, the transient  $[\text{Ca}^{2+}]_i$  surge activates the small-conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels (SK) and hyperpolarizes the plasma membrane, terminating any previous electrical activity.<sup>10,32</sup> As the  $\text{Ca}^{2+}$  is removed from the cytosol, by means of plasma membrane ATPase pumps and  $\text{Na}^+/\text{Ca}^{2+}$  exchangers as well as sarco-endoplasmic reticulum  $\text{Ca}^{2+}$  ATPase (SERCA) pumps, the SK channels close and the membrane depolarizes. Depletion of the ER  $\text{Ca}^{2+}$  store also provides a signal for the activation of transient receptor potential cation channels (TRPCs) and other store-operated  $\text{Ca}^{2+}$  channels

AU:11

p0230

AU:12



receptors, allowing these messengers to exert a local regulatory function during specific physiological states. Depending on the cellular location of both the signaling molecule and its respective receptor, such messengers may act either on the same cell (autocrine control), an adjacent cell (juxtacrine control), a neighboring cell (paracrine control) or even within the same cell where the signaling molecule is produced without being secreted (intracrine control). Such interactions are highly context-dependent and constitute the cellular basis of locally controlled anterior pituitary plasticity. In general, they either promote or inhibit hormone release and cell proliferation, and are switched on/off when hormonal outputs need to be adapted to changing demands of the organism, such as during mating, pregnancy, lactation, stress, inflammation, immune responses, starvation and circadian rhythms. We will briefly highlight physiologically relevant paracrine and autocrine interactions that have been substantiated with reasonable evidence. Comprehensive reviews on this topic have been recently published.<sup>17,35</sup>

p0245 Well-established autocrine mechanisms in lactotrophs include the stimulatory loops of VIP, galanin and TGF- $\alpha$  as well as the inhibitory loops of TGF- $\beta$ 1 and endothelins. In gonadotrophs, the autocrine loop made up by activin B (stimulatory) and follistatin (inhibitory), along with that of inhibin (inhibitory), constitute one of the fundamental mechanisms for selective regulation of FSH expression and secretion, as changes in GnRH pulse frequency cause changes of expression of these three modulators that in turn change the FSH:LH ratio.<sup>36</sup> Nitric oxide (NO) is also an important autocrine/paracrine modulator of gonadotrophs as both these cells and FS cells express nitric oxide synthase (NOS). NO may play both inhibitory and stimulatory roles, and this might depend on the cellular source of NO, as NO from FS cells seems to inhibit GnRH-stimulated LH release whereas gonadotroph

NO stimulates basal LH and FSH secretion. PACAP also plays autocrine roles in gonadotrophs where it is specifically expressed in proestrus to stimulate LH release through interaction with the gonadotroph NO system and GnRH signaling pathways. The purine ATP has been shown to be costored with hormones in secretory granules and cosecreted in all endocrine cell types of the anterior pituitary,<sup>37</sup> but autocrine roles have only been consistently shown in gonadotrophs where it potently stimulates basal LH release. In corticotrophs, expression of CRH and AVP (which has also been detected in all endocrine cell types except somatotrophs) and their receptors underlies an autocrine mechanism by which CRH and AVP may contribute to the well-established potentiation of ACTH release by hypothalamic CRH and AVP. In somatotrophs, ghrelin has been shown to sensitize the cell to GHRH at early postnatal age (and possibly at puberty).<sup>38</sup> In thyrotrophs, stimulatory and inhibitory autocrine roles for leptin and neurotrophin B, respectively, have been shown to be important in the control of TSH secretion during adaptation to nutritional status.

The majority of the paracrine interactions p0250 reported so far are those of gonadotrophs with different endocrine cell types of the anterior pituitary, particularly lactotrophs, somatotrophs and corticotrophs. Among these, one of the first described is the GnRH-stimulated prolactin release by lactotrophs,<sup>39</sup> an effect that is mediated by the release of a still uncharacterized molecule from immature postnatal gonadotrophs.<sup>40</sup> In immature cells, GnRH also elicits a biphasic hormone release response on somatotrophs that begins with inhibition of growth hormone secretion during GnRH application and is followed by a rapid rebound secretion of GH that slowly returns to basal levels. These interactions might be partially related to developmental roles that GnRH may have on these cell types. Some of the candidate paracrine factors from gonadotrophs thought

to be involved in the development of lactotrophs are the glycoprotein hormone  $\alpha$ -subunit, the growth factor TGF- $\alpha$  and prolactin-releasing peptide (PrRP). Stimulatory paracrine interactions must be counterbalanced with inhibitory ones for the gland to meet and maintain homeostasis throughout different physiological stages; one good example of negative interaction between gonadotrophs and lactotrophs is that mediated by calcitonin (and calcitonin-like peptides). Calcitonin immunoreactivity is primarily located in gonadotrophs that are associated with *cup-shaped* lactotrophs (described later in this section). It inhibits basal and TRH-stimulated prolactin synthesis and release as well as lactotroph mitosis. This paracrine effect is likely mediated by the release of TGF- $\beta$ 1 by lactotrophs, which in turn inhibits lactotroph proliferation as well as prolactin expression and secretion.<sup>41</sup> Consistent with this role, estradiol, a well-known promoter of lactotroph secretory activity and proliferation, negatively regulates calcitonin expression in gonadotrophs. Positive and negative interactions have also been described between gonadotrophs and corticotrophs; calcitonin gene-related peptide accounts for the former whereas the natriuretic peptides (ANP and CNP) and adrenomedullin (a calcitonin peptide family member) account for the latter. Since stress responses are attenuated in pregnancy and lactation, it is likely that the inhibitory tone of natriuretic peptides on corticotroph function is upregulated during these states of elevated estradiol levels in plasma as estrogens are known to upregulate natriuretic peptide expression in heart tissue.

<sup>p0255</sup> Gonadotrophs can also be paracrine targets themselves. Although inhibition of ovulation during lactation is achieved primarily by endorphin-mediated inhibition of hypothalamic GnRH neurons triggered by the suckling stimulus, local inhibitory cues from lactotrophs and corticotrophs to gonadotrophs at the anterior pituitary may contribute

to the suckling-induced negative influence on ovulation. Increased release of  $\beta$ -endorphin and galanin by corticotrophs and lactotrophs, respectively, may contribute to inhibit preovulatory LH secretion. In pregnancy, estrogen-induced high galanin release by lactotrophs underlies increased lactotroph activity and growth as well as decreased LH release. During stress and undernutrition, stress-induced activation of the hypothalamo-pituitary-adrenal (HPA) axis inhibits the hypothalamo-pituitary-gonadal (HPG) axis. At the pituitary level, this negative influence of the HPA onto the HPG axis may be mediated by the negative paracrine signals of corticotroph  $\beta$ -endorphin on GnRH-induced gonadotroph LH release.

AU:13

<sup>p0260</sup> At the heart of the structure and function of the anterior pituitary gland is the key supportive and dynamic role of the FS cells. These cells are also excitable and are thought to coordinate activity of endocrine cells. They form two microanatomical structures that may have a large impact on pituitary cell physiology. Located in the center of the hormonal cell cord, they are often arranged in clusters and form small follicles in rats, but are larger in humans and some other species. In the follicles, numerous microvilli protrude and some cilia are present. Follicle-forming FS cells are polarized. At the apical pole, bordering the follicle, they form tight junctions among each other, although not always fully sealed, and, more laterally, junctions of the "zonula adherens" type (desmosomes). The basolateral side makes contact with the endocrine cells and with other FS cells, and extends processes that end on the basal membrane surrounding the cell cords. The role of follicles remains unclear but the structures are thought to be involved in intercellular transport of metabolic products and ions. A second group of FS cells extends long processes between the hormonal cell types within each glandular cell cord. Although these processes

form intercellular junctions among each other, mostly of the zonula adherens-type, they are also electrotonically coupled through gap junctions as shown by rapid propagation of  $\text{Ca}^{2+}$  currents over long distances in the gland.<sup>42</sup> On this basis, it is hypothesized that these cells coordinate the activity of endocrine cells. In support of this is the finding of a correlation between the number of gap junctions and reproductive maturation in the rat. Interestingly, estradiol seems to increase FS network connectivity, as a steep rise in gap junction number is observed at the end of pregnancy and during lactation. Moreover, in the estrous cycle, connectivity is highest during proestrus and estrus, and it has been recently suggested that this increased connectivity plays a significant role in the preovulatory LH surge.<sup>43</sup> Some FS cells make intimate foot processes with the basal membrane of the extra-vascular spaces at the periphery of the cell cords. In some species FS cells located in the periphery of the cell cords are juxtaposed in a way that they form sinusoid-like spaces. Intercellular lacunae are also often seen between endocrine cells. These lacunae, along with the sinusoid-like spaces surrounded by FS cells and perivascular spaces are thought to form a micro-channel system within the pituitary, through which hormones, local factors, nutrients, ions and waste products can circulate. The three-dimensional architecture of FS cells is under developmental control: in the infant rat, the FS follicles are elongated and participating FS cells have a columnar shape without cellular extensions and displaying very few junctions. At the onset of puberty, they separate into smaller follicular units and start making extensions and junctions, especially tight junctions.

p0265 It is thus reasonable to hypothesize that FS cells play key roles in anterior pituitary adaptation to varying physiological conditions, including immune, nutritional and other stresses in which they may operate as critical

interfaces in homeostatic mechanisms. Prolonged pituitary activation during immune stress, pregnancy, lactation, starvation and other conditions, may lead to excess production of specific hormones that in turn may result in inhibition of essential physiological processes. In such events, it is likely that FS cells provide a mechanism to circumvent this issue through their capability to blunt many stimulated activities in the anterior pituitary. FS cells release NO that in turn may stimulate guanylyl cyclase activity and increase cyclic guanosine monophosphate (cGMP) in different endocrine cell types to inhibit hormone release (Figure 2.3). In addition, FS cells are permissive for the mitogenic effect of estradiol on lactotrophs by releasing fibroblast growth factor-2 (FGF-2), and mediate the stimulatory action of bacterial endotoxin lipopolysaccharide (LPS), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), VIP, PACAP and interleukin-1 (IL-1) on ACTH secretion by releasing IL-6 which act in corticotrophs in a paracrine fashion (Figure 2.3). FS cells may possibly modulate responses to immune stress as they express receptors for epinephrine, acetylcholine, angiotensin II, calcitonin and ATP that are known to regulate immune cell functions. Indeed, FS cells also express glucocorticoid receptors and have been shown to mediate the glucocorticoid fast negative feedback effect on ACTH (and also prolactin and GH) secretion via ATP-binding cassette (ABC) transporter-mediated externalization of annexin 1, which in turn acts on specific binding sites on endocrine cells to inhibit hormone release<sup>44</sup> (Figure 2.3).

### PLASTICITY OF ANTERIOR PITUITARY CELL NETWORK ORGANIZATION AND FUNCTION

s0145

The architecture of the anterior lobe is based on interlacing strands of mostly polygonal cells separated by a tremendously developed

p0270

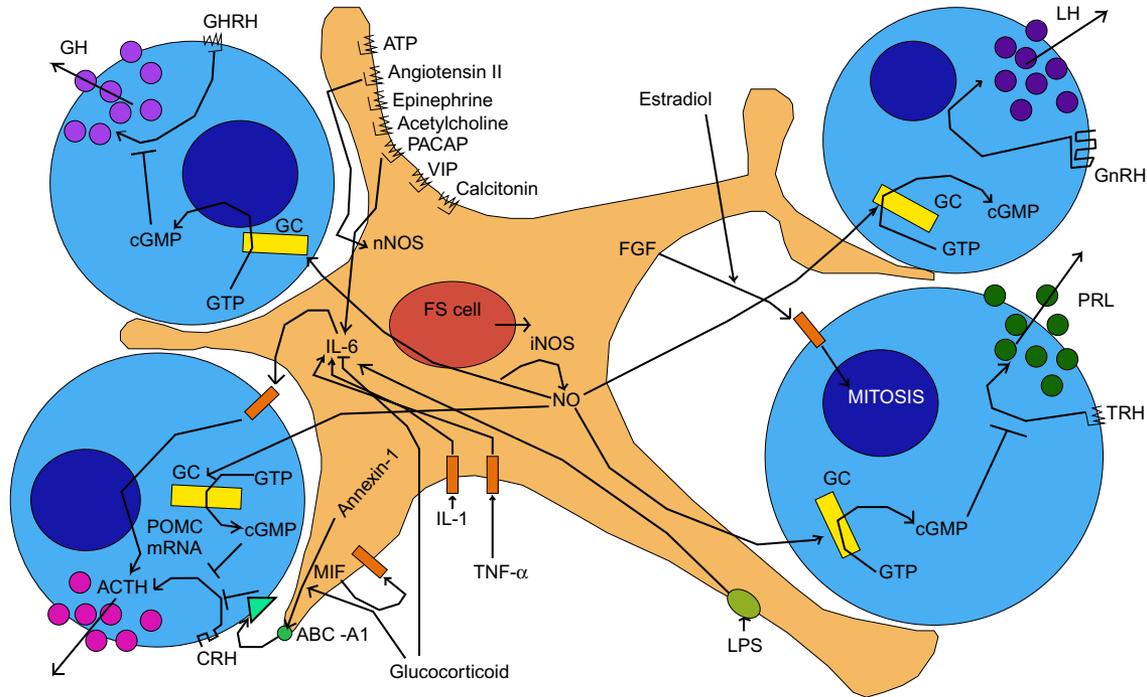


FIGURE 2.3 Schematic representation of some of the paracrine interactions thought to occur between folliculostellate (FS) cells, lactotrophs, somatotrophs, gonadotrophs and corticotrophs. →, stimulatory effect; |, inhibitory effect (see text for details).

array of sinusoidal capillaries. Early descriptions of the microanatomy of the adenohypophysis suggested that its various cell types are intermingled, forming cell networks (reviewed in refs<sup>35,42</sup>). Nakane later made the seminal observation that the different pituitary cell types are not distributed homogeneously over the various areas of the gland and within a specific cell cord.<sup>45</sup> In fact, a recent study using two-photon microscopy imaging of genetically engineered mouse somatotrophs expressing the enhanced green-fluorescent protein (EGFP) showed that GH-producing cells are interconnected via adherens junctions, seeming to form a “homotypic connected 3D cell continuum” that displays coordinated  $Ca^{2+}$  transients.<sup>46</sup> This cell system consists of numerous intercrossing strands of single GH cells, with larger clusters of GH cells

positioned at the intersection of the cords. Consistent with Nakane’s observation, there are differences in the GH cell strand and cluster densities in different regions of the gland. Across the lifespan, the GH system architecture shows plasticity.<sup>46</sup> In prepubertal animals, the patterning of the GH cell system is similar between the lateral and the median zones of the gland. From puberty to adulthood there is a marked increase in the proportion of GH cells in clusters in the lateral portions of the gland than in the median zone surrounding the stalk. Interestingly, the volume-to-surface ratios of the GH cell system returns to prepubertal values in the lateral zones of aged mice, indicating that the plasticity of this cell system continues well into adulthood.<sup>46</sup> More recently, it has been shown that gonadotrophs exhibit significantly different distributions across

physiological states,<sup>47</sup> so it is likely that other cell types share a similar cell system architecture and plasticity. In fact, Nakane had observed close associations between somatotrophs and corticotrophs and between gonadotrophs and lactotrophs. Some of the lactotrophs embraced the oval-shaped gonadotrophs with long cellular processes and Nakane named them “cup-shaped” PRL cells.<sup>45</sup> These cell–cell associations are thought to have functional consequences, not only in the cells involved in the association, but also in the pituitary gland as a whole. That the different cell types are both structurally and functionally interconnected is confirmed by the fact that genetic ablation of GH cells dramatically reduced the pituitary content of all hormones.<sup>48</sup>

p0275 Finally, the ample possibilities brought about by interconnectivity at the cellular network level may offer emergent properties that might be critical to anterior pituitary cells to adapt their physiology to suit the prevailing environmental conditions. As interconnected cell populations bestowed with the role of mounting large-scale responses to maintain homeostasis, experience-dependent plasticity may represent an inherent property of these cells. This concept has been recently illustrated in female mice that underwent one or more lactations to repeatedly and selectively stimulate activity of pituitary lactotrophs.<sup>49</sup> Throughout the lactotroph population, each lactation event induced alterations in functional connectivity through changes in structural connectivity mediated by differential homotypic lactotroph–lactotroph gap junctional contacts. Following weaning, the lactotroph population is able to maintain the pattern of functional connectivity for weeks to months, a hallmark of long-term experience-dependent plasticity that is reminiscent to that observed in neurons. Likewise, the lactotroph network retains functional connectivity through changes in the extent and strength of

cell–cell communication, allowing repeated lactation demands to be met with evolved network dynamics and improved tissue output.<sup>49</sup> Thus, experience-dependent plasticity allows lactotrophs to adapt their hormone releasing activity upon repeated stimulation, a feature that is likely shared by other endocrine cells within the anterior pituitary and, possibly, throughout the body.

## CONCLUDING REMARKS

s0150

Endocrine cells of the anterior pituitary have a remarkably varied signaling toolkit at their disposal which, in combination with GPCR expression profiles, and gap junctional electrical coupling, may allow incoming signals to be processed and propagated from cell to cell in a specific manner. Not only are these features subject to dynamic changes according to physiological needs, but also the morphological arrangement and functional connectivity of the different cell types of this gland can also go through profound remodeling. These fundamental features allow anterior pituitary cells to adapt their responses to environmental challenges and demands and thereby to meet their vital role in homeostatic control.

p0280

## Acknowledgements

s0155

The authors wish to gratefully acknowledge Jose Arias-Cristancho for preparing Figures 2 and 3 of this chapter as well as the support provided by National Institute of Health grant DK-43200.

p0285

## References

1. Gonzalez-Iglesias AE, Freeman ME. Brain control over pituitary gland hormones. In: Pfaff DW, editor. *Neuroscience in the 21st Century*. New York: Springer-Verlag; 2012.
2. Ooi GT, Tawadros N, Escalona RM. Pituitary cell lines and their endocrine applications. *Mol Cell Endocrinol* 2004;228:1–21.

3. Walker JJ, Terry JR, Tsaneva-Atanasova K, Armstrong SP, Mc Ardle CA, Lightman SL. Encoding and decoding mechanisms of pulsatile hormone secretion. *J Neuroendocrinol* 2010;**22**:1226–38.
4. Melmed S. Update in pituitary disease. *J Clin Endocrinol Metab* 2008;**93**:331–8.
5. Perez-Castro C, Renner U, Haedo MR, Stalla GK, Arzt E. Cellular and molecular specificity of pituitary gland physiology. *Physiol Rev* 2012;**92**:1–38.
6. Roudbaraki M, Lorsignol A, Langouche L, Callewaert G, Vankelecom H, Denef C. Target cells of gamma3-melanocyte-stimulating hormone detected through intracellular Ca<sup>2+</sup> 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* 1999;**140**:4874–85.
7. Villalobos C, Nuñez L, Frawley S, García-Sancho J, Sánchez A. Multi-responsiveness of single anterior pituitary cells to hypothalamic-releasing hormones: a cellular basis for paradoxical secretion. *Proc Natl Acad Sci USA* 1997;**94**:14132–7.
8. Childs GV. Development of gonadotropes may involve cyclic transdifferentiation of growth hormone cells. *Arch Physiol Biochem* 2002;**110**:42–9.
9. Stojilkovic SS. Molecular mechanisms of pituitary endocrine cell calcium handling. *Cell Calcium* 2012;**51**:212–21.
10. Stojilkovic SS, Tabak J, Bertram R. Ion channels and signaling in the pituitary gland. *Endocr Rev* 2010;**31**:845–915.
11. van Goor F, Zivadinovic D, Martínez-Fuentes AJ, Stojilkovic SS. Dependence of pituitary hormone secretion on the pattern of spontaneous voltage-gated calcium influx. Cell type-specific action potential secretion coupling. *J Biol Chem* 2001;**276**:33840–6.
12. Kretschmannova K, Kucka M, Gonzalez-Iglesias AE, Stojilkovic SS. The expression and role of hyperpolarization-activated and cyclic nucleotide-gated channels in endocrine anterior pituitary cells. *Mol Endocrinol* 2012;**26**:153–64.
13. Liang Z, Chen L, McClafferty H, Lukowski R, MacGregor D, King JT, et al. Control of hypothalamic-pituitary-adrenal stress axis activity by the intermediate conductance calcium-activated potassium channel, SK4. *J Physiol* 2011;**589**:5965–86.
14. Tsaneva-Atanasova K, Sherman A, van Goor F, Stojilkovic SS. Mechanism of spontaneous and receptor-controlled electrical activity in pituitary somatotrophs: experiments and theory. *J Neurophysiol* 2007;**98**:131–44.
15. Gonzalez-Iglesias AE, Jiang Y, Tomic M, Kretschmannova K, Andric SA, Zemkova H, et al. Dependence of electrical activity and calcium influx-controlled prolactin release on adenylyl cyclase signaling pathway in pituitary lactotrophs. *Mol Endocrinol* 2006;**20**:2231–46.
16. Tougaard C, Tixier-Vidal A. Lactotropes and gonadotropes. In: Knobil E, Neill JD, editors. *The physiology of reproduction*. New York: Raven Press Ltd; 1994. p. 1711–47.
17. Childs GV. Gonadotropes and lactotropes. In: Neill JD, editor. *Knobil and neill's physiology of reproduction*. 3rd ed. New York: Raven Press Ltd; 2006. . p. 1483–562..
18. Christian HC, Chapman LP, Morris JF. Thyrotrophin-releasing hormone, vasoactive intestinal peptide, prolactin-releasing peptide and dopamine regulation of prolactin secretion by different lactotroph morphological subtypes in the rat. *J Neuroendocrinol* 2007;**19**:605–13.
19. Tabak J, Tomaiuolo M, Gonzalez-Iglesias AE, Milescu LS, Bertram R. Fast-activating voltage- and calcium-dependent potassium (BK) conductance promotes bursting in pituitary cells: a dynamic clamp study. *J Neurosci* 2011;**31**:16855–63.
20. Tomaiuolo M, Bertram R, Gonzalez-Iglesias AE, Tabak J. Investigating heterogeneity of intracellular calcium dynamics in anterior pituitary lactotrophs usign a combined modelling/experimental approach. *J Neuroendocrinol* 2010;**22**:1279–89.
21. van Goor F, Zivadinovic D, Stojilkovic SS. Differential expression of ionic channels in rat anterior pituitary cells. *Mol Endocrinol* 2001;**15**:1222–36.
22. van Goor F, Li YX, Stojilkovic SS. Paradoxical role of large-conductance calcium-activated K<sup>+</sup> (BK) channels in controlling action potential-driven Ca<sup>2+</sup> entry in anterior pituitary cells. *J Neurosci* 2001;**21**:5902–15.
23. Tabak J, Toporikova N, Freeman ME, Bertram R. Low dose of dopamine may stimulate prolactin secretion by increasing fast potassium currents. *J Comput Neurosci* 2007;**22**:211–22.
24. Simasko SM, Sankaranarayanan S. Characterization of a hyperpolarization-activated cation current in rat pituitary cells. *Am J Physiol – Endocrinol Metab* 1997;**272**:E405–14.
25. Gonzalez-Iglesias AE, Kretschmannova K, Tomic M, Stojilkovic SS. ZD7288 inhibits exocytosis in an HCN-independent manner and downstream of voltage-gated calcium influx in pituitary lactotrophs. *Biochem Biophys Res Commun* 2006;**346**:845–50.
26. Tian L, Shipston MJ. Characterization of hyperpolarization-activated cation currents in mouse anterior pituitary, AtT20 D16:16 corticotropes. *Endocrinology* 2000;**141**:2930–7.

27. Christensen HR, Zeng Q, Murawsky MK, Gregerson KA. Estrogen regulation of the dopamine-activated GIRK channel in pituitary lactotrophs: implications for regulation of prolactin release during the estrous cycle. *Am J Physiol - Regul Integr Comp Physiol* 2011;**301**:R746–56.
28. Gregerson KA. Functional expression of the dopamine-activated K<sup>+</sup> current in lactotrophs during the estrous cycle in female rats. *Endocrine* 2003;**20**:67–74.
29. Andric SA, Zivadinovic D, Gonzalez-Iglesias AE, Lachowicz A, Tomic M, Stojilkovic SS. Endothelin-induced, long lasting, and Ca<sup>2+</sup> influx-independent blockade of intrinsic secretion in pituitary cells by Gz subunits. *J Biol Chem* 2005;**280**:26896–903.
30. Gonzalez-Iglesias AE, Murano T, Li S, Tomic M, Stojilkovic SS. Dopamine inhibits basal prolactin release in pituitary lactotrophs through pertussis toxin-sensitive and -insensitive signaling pathways. *Endocrinology* 2008;**149**:1470–9.
31. Berridge MJ. *Neuronal signalling. Cell signaling biology*. Portland Press Limited; 2012. p. 10.1–10.104
32. Li YX, Rinzel J, Keizer J, Stojilkovic SS. Calcium oscillations in pituitary gonadotrophs: comparison of experiment and theory. *Proc Natl Acad Sci USA* 1994;**91**:58–62.
33. Vergara LA, Stojilkovic SS, Rojas E. GnRH-induced cytosolic calcium oscillations in pituitary gonadotrophs: phase resetting by membrane depolarization. *Biophys J* 1995;**69**:1606–14.
34. Kukuljan M, Vergara L, Stojilkovic SS. Modulation of the kinetics of inositol 1,4,5-trisphosphate-induced [Ca<sup>2+</sup>]<sub>i</sub> oscillations by calcium entry in pituitary gonadotrophs. *Biophys J* 1997;**72**(2 Pt 1):698–707.
35. Deneff C. Paracrine: the story of 30 years of cellular pituitary crosstalk. *J Neuroendocrinol* 2008;**20**:1–70.
36. Kirk SE, Dalkin AC, Yasin M, Haisenleder DJ, Marshall JC. Gonadotropin-releasing hormone pulse frequency regulates expression of pituitary follistatin in folliculostellate cell-enriched primate pituitary cell cultures. *Endocrinology* 1994;**135**:876–80.
37. Stojilkovic SS, He ML, Koshimizu TA, Balik A, Zemkova H. Signaling by purinergic receptors and channels in the pituitary gland. *Mol Cell Endocrinol* 2010;**314**:184–91.
38. Szabo M, Cuttler L. Differential responsiveness of the somatotroph to growth hormone-releasing factor during early neonatal development in the rat. *Endocrinology* 1986;**118**:69–73.
39. Deneff C, Andries M. Evidence for paracrine interaction between gonadotrophs and lactotrophs in pituitary cell aggregates. *Endocrinology* 1983;**112**:813–22.
40. Andries M, Deneff C. Gonadotropin-releasing hormone influences the release of prolactin and growth hormone from intact rat pituitary *in vitro* during a limited period in neonatal life. *Peptides* 1995;**16**:527–32.
41. Sarkar DK, Kim KH, Minami S. Transforming growth factor-beta 1 messenger RNA and protein expression in the pituitary gland: its action on prolactin secretion and lactotropic growth. *Mol Endocrinol* 1992;**6**:1825–33.
42. Hodson DJ, Romano N, Schaeffer M, Fontanaud P, Lafont C, Fiordeliso T, et al. Coordination of calcium signals by pituitary endocrine cells in situ. *Cell Calcium* 2012;**51**:222–30.
43. Lyles D, Tien JH, McCobb DP, Zeeman ML. Pituitary network connectivity as a mechanism for the luteinizing hormone surge. *J Neuroendocrinol* 2010;**22**:1267–78.
44. Morris JF, Christian HC, Chapman LP, Epton MJ, Buckingham JC, Ozawa H, et al. Steroid effects on secretion from subsets of lactotrophs: role of Folliculostellate cells and Annexin 1. *Arch Physiol Biochem* 2002;**110**:54–61.
45. Nakane PK. Classifications of anterior pituitary cell types with immuno-enzyme histochemistry. *J Histochem Cytochem* 1970;**18**:9–20.
46. Bonnefont X, Lacampagne A, Sanchez-Hormigo A, Fino E, Creff A, Mathieu MN, et al. Revealing the large-scale network organization of growth hormone-secreting cells. *Proc Natl Acad Sci USA* 2005;**102**:16880–5.
47. Alim Z, Hartshorn C, Mai O, Stitt I, Clay C, Tobet S, et al. Gonadotrope plasticity at cellular and population levels. *Endocrinology* 2012;**153**:4729–39.
48. Waite E, Lafont C, Carmignac D, Chauvet N, Coutry N, Christian H, et al. Different degrees of somatotroph ablation compromise pituitary growth hormone cell network structure and other pituitary endocrine cell types. *Endocrinology* 2010;**151**:234–43.
49. Hodson DJ, Schaeffer M, Romano N, Fontanaud P, Lafont C, Birkenstock J, et al. Existence of long-lasting experience-dependent plasticity in endocrine cell networks. *Nat Commun* 2012;**3**:605.