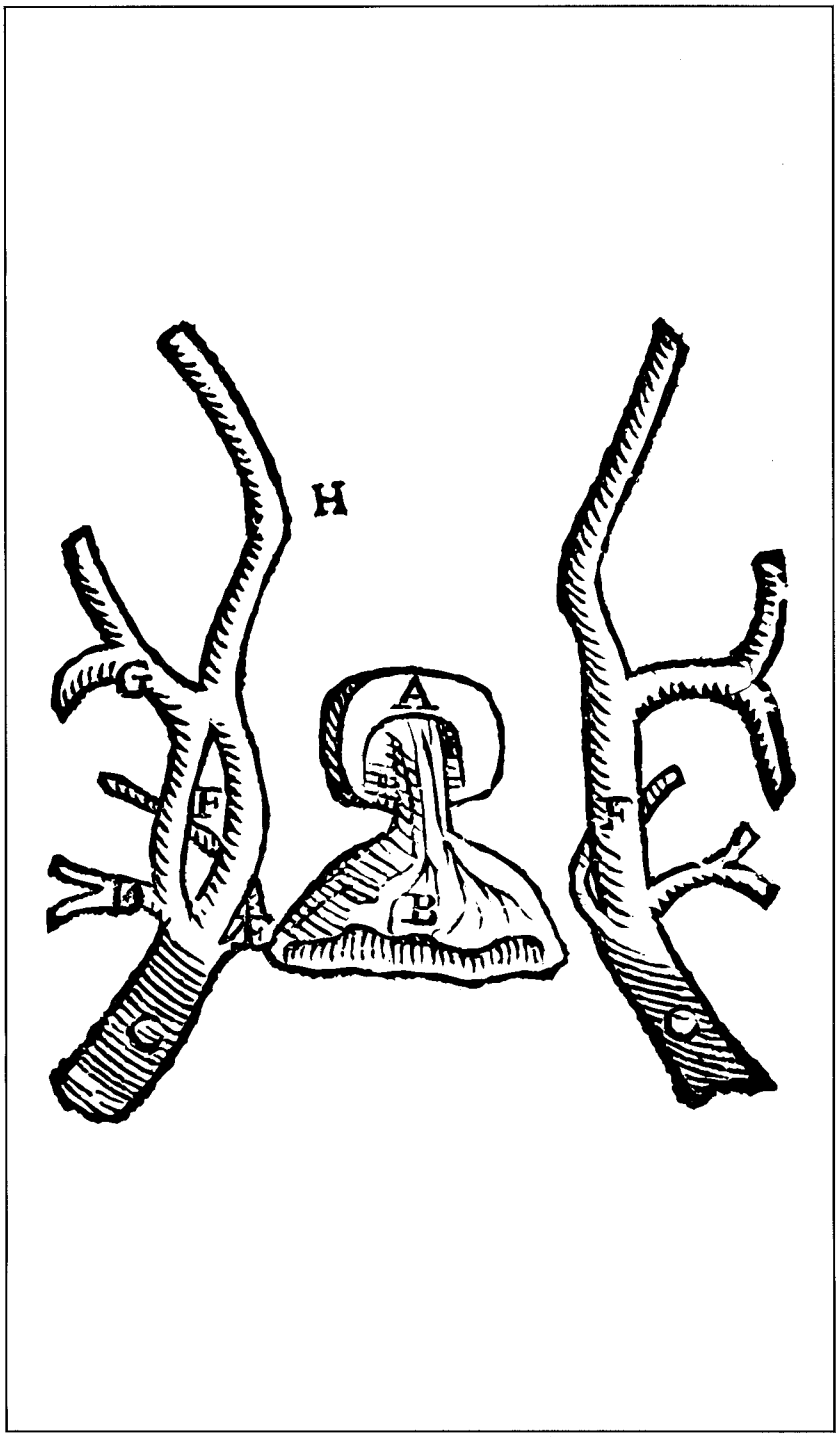
CHAPTER 65



Disorders of the Pituitary Gonadotroph

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Normal function of the gonadotrophs requires both anatomic and functional integrity of the hypothalamic–pituitary axis. Excessive secretion of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and/or their corresponding subunits may occur as a result of gonadotroph cell adenoma. This seemingly uncommon condition is gaining increasing attention because it appears to represent a significant percentage of all null cell (nonsecreting) pituitary adenomas. This unique clinical entity of a gonadotropin-secreting pituitary adenoma and the molecular basis for its pathogenesis are discussed at the beginning of this chapter.

Primary (pituitary) or secondary (hypothalamic) gonadotropin deficiency may occur as a result of numerous disorders involving the pituitary–hypothalamic unit and the CNS. These anatomic, biochemical, and functional derangements are listed in Table 1 and are discussed in this chapter. Primary gonadotropin deficiency is most commonly a result of pituitary infarction or a large pituitary adenoma and therefore is commonly associated with other pituitary hormone defects. Secondary pituitary insufficiency, including gonadotropin deficiency, is also mostly associated with other pituitary hormone deficiencies. An exception is isolated gonadotropin deficiency, which may result from mutant gonadotropins or, more commonly, is associated with anosmia, as described half a century ago by Kallmann and associates. The pathogenesis of the syndrome, failure of gonadotropin-releasing hormone (GnRH) neurons to migrate into the hypothalamus, has only recently been described.

During the last decade, the rapidly expanding field of molecular biology and genetics has allowed us to understand better the molecular and genetic basis of increasing numbers of endocrine disorders, including those resulting in altered gonadotropin secretion. To keep up with contemporary molecular endocrinology, a brief description of the structure of the gonadotropins and the genes encoding them as well as of the regulation of gonadotropin gene expression and secretion is provided. Finally, this chapter also presents a summary of the current knowledge of the molecular and genetic basis for some old or newly described disorders of the gonadotrophs (e.g., hypogonadism caused by LH-β gene mutation).

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GONADOTROPH CELL ADENOMA

Gonadotropin-secreting pituitary adenomas are probably an underdiagnosed clinical entity, mainly because their clinical and hormonal manifestations are often minimal. Commonly, these tumors are erroneously diagnosed as null cell (nonsecreting) pituitary adenomas, especially when the tumor occurs in the postmenopausal patient, in whom elevated gonadotropin levels are normally expected. Recent studies have shown, however, that most of these null cell adenomas secrete LH, FSH, and/or α subunit *in vitro*. Small quantities of other hormones [thyroid-stimulating hormone (TSH), growth hormone (GH), prolactin, and corticotropin] are also secreted. Based on morphologic characteristics, null cell adenomas can be differentiated from pure gonadotroph cell adenomas, although their histologic and ultrastructural features are indistinguishable in some cases. As much as 25% of autopsies of persons who were not suspected of having pituitary disease revealed pituitary adenomas, many of which were gonadotroph adenomas. The first reports describing this distinct clinical entity were published less than 20 years ago (1,2). By now, it is clear that these tumors account for a significant number of all pituitary adenomas in men and women (3).

DEFINITIONS

cDNA—complementary DNA sequence to a given mRNA molecule that codes for production of a specific protein.

Dynamic testing—diagnostic tests in which use is made of the pattern of hormonal secretion to a given stimulus.

Monoclonal cells—a cell line derived from a single cell. Pituitary adenomas are believed to arise from a single cell that underwent a tumorigenic transformation.

Null cell pituitary adenoma—in the frame of a functional classification of pituitary adenomas, these tumors apparently do not secrete any hormonal product.

Etiology

As the cause of gonadotroph adenoma is not known, possible mechanisms have been suggested for the tumoral transformation of the gonadotropin-secreting cells. Longstanding primary hypogonadism may give rise to pituitary enlargement (4), mimicking the clinical and hormonal manifestations of gonadotroph adenoma (elevated FSH and low testosterone levels). Clearly, these conditions are different clinical entities. Patients with gonadotroph adenoma have a history of normal puberty and fertility and normal sexual habitus. It has also been suggested that nonsecreting pituitary adenomas physically suppress LH-secreting cells, leaving FSH-secreting cells intact. This explanation seems unlikely because it is clear that these tumors secrete supranormal amounts of FSH both *in vivo* and *in vitro* (5).

Hypothalamic-releasing hormones are known to possess pituitary growth-promoting properties. Disordered hypothalamic GnRH secretion may therefore lead to unrestrained gonadotroph cell hyperplasia. Pituitary tissue surrounding the adenoma is not hyperplastic, however, suggesting no hypothalamic stimulation of gonadotroph cell growth. It is evident now that gonadotroph cell adenomas are comparable to other pituitary adenomas and may arise *de novo* as a clonal expansion of genomically altered cells independent of hypothalamic influence. The clonal composition of pituitary adenoma has been studied with restriction fragment–length polymorphism analysis and has been found to be monoclonal in nature (6,7), whereas normal pituitary tissue or mixed hormonal adenomas are polyclonal. It seems unlikely, therefore, that hypothalamic hormones play a role in promoting the growth of these tumors. Rather, functional pituitary adenomas, like most human neoplasias, originate from a single cell line that has undergone a somatic mutation, giving rise to abnormal expansion (8). This etiology, somatic cell mutation, is further supported by the following observations:

1. Hormone secretion is autonomous and independent of hypothalamic control.
2. The adenomas are surrounded by normal pituitary tissue, suggesting normal hypothalamic function.
3. Surgical removal of the adenomas results in prompt endocrinologic cure.

Signs and Symptoms

The typical clinical presentation is of a middle-age man with normal gonadal function complaining of visual impairments, cranial nerve palsies, nausea, and headaches. According to clinical reports the majority of patients are men, probably reflecting the difficulty in diagnosing a gonadotropin-secreting adenoma in a postmenopausal woman. Lack of clinical

Table 1. Etiologies of Gonadotropin Deficiency

PRIMARY PITUITARY LESIONS	HYPOTHALAMIC-PITUITARY LESIONS	OTHER
Pituitary adenomas and other tumors	Isolated GnRH deficiency (Kallmann's syndrome)	Systemic diseases (renal failure, cirrhosis, β-thalassemia)
Pituitary infarction (Sheehan's syndrome, pituitary apoplexy, sickle cell anemia)	Extrapituitary: craniopharyngioma, glioma, meningioma, germinoma, endodermal sinus tumor, midline dermoid cyst and teratoma, metastatic tumors	Psychiatric disorders (anxiety, pseudocyesis, anorexia nervosa)
After pituitary surgery or radiation therapy	Granulomas (sarcoidosis, histiocytosis X, tuberculosis)	Functional states (exercise, simple weight loss, obesity)
Empty sella syndrome	Infections (basal meningoencephalitis, syphilis)	Eutopic and ectopic hCG secretion (gestational trophoblastic disease, nontrophoblastic tumors)
Lymphocytic hypophysitis	Vascular malformations, head trauma, irradiation	Aging

manifestations due to excessive hormonal production explains the fact that by the time of diagnosis the tumors attain large volumes, causing extrasellar compression of adjacent structures (3). The distinction between a gonadotroph cell adenoma and a nonsecreting adenoma may not be possible clinically or endocrinologically and may require electron microscopic or immunocytochemical studies of excised tumor.

Although, as discussed earlier, it is generally accepted that these tumors arise spontaneously, clinical association with hypogonadism has been reported. Nicolis et al (9) described a 62-year-old man with gonadotroph adenoma 35 years after castration. Similarly, Kovacs et al (10) reported a 57-year-old woman with longstanding hypogonadism secondary to irradiation of the ovaries who was found to have a gonadotroph adenoma. Given the monoclonal nature of these tumors, the association with primary hypogonadism in these cases seems to be coincidental.

Constant stimulation of the seminiferous tubules by high FSH levels can cause palpable testicular enlargement (11) followed by the necessary diagnostic evaluation to rule out gonadotroph adenoma. Inhibin levels are usually elevated, reflecting the autonomous nature of FSH secretion.

Incidence

The incidence of gonadotroph adenomas is not known with certainty and varies according to the diagnostic criteria used by different investigators. In a series of 139 men with untreated macroadenomas, 24% had gonadotroph cell adenomas, 17% had elevated blood FSH levels either alone or in combination with its subunits, and 7% had hypersecretion of only the α subunit (3). In surgical specimens, gonadotroph cell adenomas were present in 3.5% to 8.0% of cases (8,11–14).

In recent years, immunohistochemical, ultrastructural, and molecular biology techniques have been used to show clearly that, in a significant number of patients who were considered to have nonsecreting pituitary macroadenomas, the tumors were of gonadotroph origin. A major difficulty lies in the diagnosis of gonadotroph adenoma in postmenopausal women because high levels of gonadotropins are normal in this group of patients. *In vivo* study using a thyrotropin-releasing hormone (TRH) stimulation test (15; discussed later) has shown that, of 16 patients who had nonsecreting pituitary adenomas, in 11 the tumor arose from gonadotroph cells. Moreover, *in vitro* studies confirm that nearly all apparently nonsecreting adenomas secrete some combination of the gonadotropins or their subunits (16,17). Similarly, nearly 80% of such adenomas were found to have mRNA for the specific β subunit of the gonadotropins (18). Oligonucleotide DNA probes complementary for the mRNA encoding the hormone subunits represent the technique used in demonstrating tumoral FSH or LH production in a resected tumor and for identifying a nonsecreting tumor as a gonadotroph cell adenoma. The close relationship between null cell adenomas and gonadotroph adenomas with respect to gene expression of glycoprotein hormones is now well established (19). In fact, because of the morphologic and biologic similarities between null cell adenomas and gonadotroph cell adenomas, it is possible that the former are actually a variant of gonadotroph cell adenoma. Because nearly 40% of all pituitary adenomas are considered nonsecreting (3), this seemingly rare condition of gonadotroph cell adenoma appears to represent a significant percentage of all pituitary adenomas.

Pathology

On routine light microscopy specimens, gonadotroph adenomas are chromophobic or slightly acidophilic and, like most other pituitary adenomas, have the same light microscopic appearance. A more accurate evaluation requires immunocytologic studies and electron microscopy. Immunospecific stains with antisera to FSH, LH, and their subunits will correspond to the glycoprotein secreted by the tumor *in vivo*.

Using electron microscopy, Kontogeorgos et al. (14) studied 145 gonadotroph adenomas. The tumors that were removed from men had uncharacteristic features similar to those of null cell adenomas. In contrast, tumors of female patients had a diagnostic marker characterized as honeycomb Golgi, which is a distinctive vesicular dilation of the Golgi complex (Figs. 1 and 2). The investigators speculated that this sex-related dichotomy may reflect differences in the final processing of hormones, including their packaging into secretory granules.

Diagnosis

Diagnosis of a pituitary adenoma relies on imaging of the pituitary anatomy, which can be accomplished by high-resolution computed tomography (CT), particularly if thin cuts are used. Combining CT with magnetic resonance imaging improves small tumor imaging, yielding more information regarding the tumor itself as well as its local effect on surrounding tissues.

Elevated levels of intact FSH are found in nearly all patients with gonadotroph adenoma. In normal subjects the concentration of free α and β subunits is low. In a patient with gonadotroph adenoma, in addition to intact dimers, free subunit concentrations may be high as well. To avoid cross-reactivity with its subunits in regular radioimmunoassays, the nature of the FSH molecule secreted was studied using techniques such as gel filtration chromatography and column isoelectric focusing (20) and was found to be normal. Similarly, the granulosa cell aromatase bioassay was used to show that the FSH secreted by the tumors is biologically active (21). Hypersecretion of intact LH is less frequent, and again to avoid cross-reactivity with the free subunits gel filtration chromatography is needed for precise identification of the secreted glycoproteins. When intact LH is secreted, elevated testosterone levels are seen. In the majority of the patients, however, intact LH is not hypersecreted, and the testosterone levels are normal or subnormal. In these cases

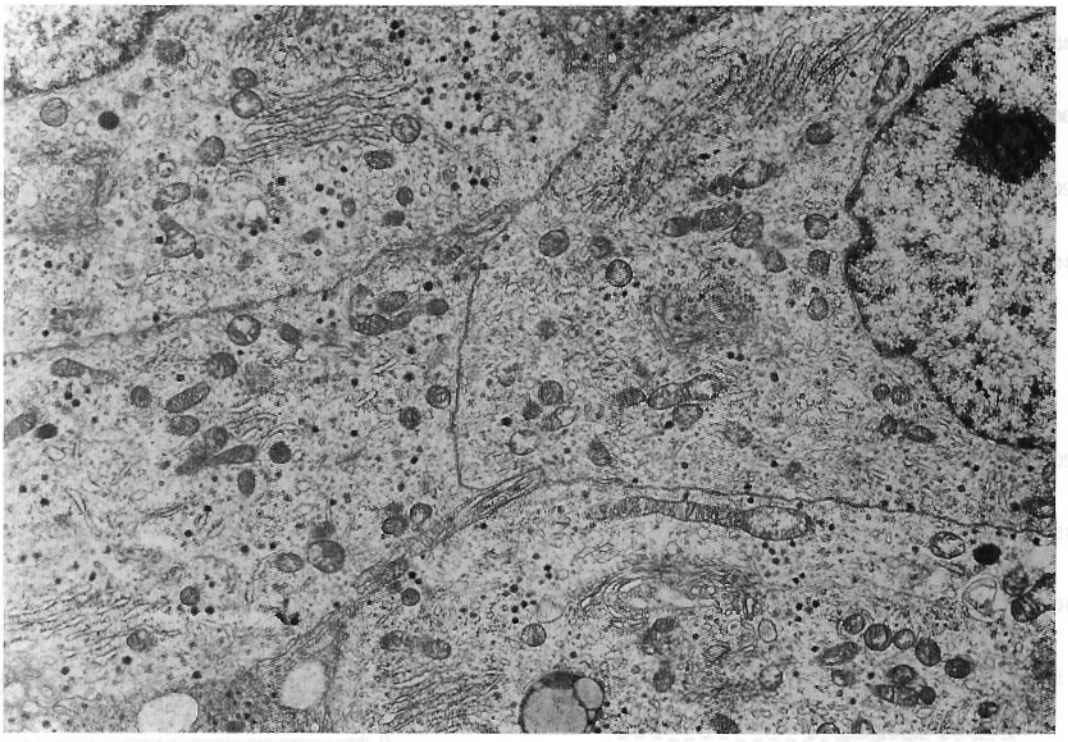

FIG. 1. Gonadotroph adenoma, male type, featuring prominent, slightly dilated rough endoplasmic reticulum, well-developed Golgi complex with regular features, and small secretory granules, ×7700. (From ref. 14.)

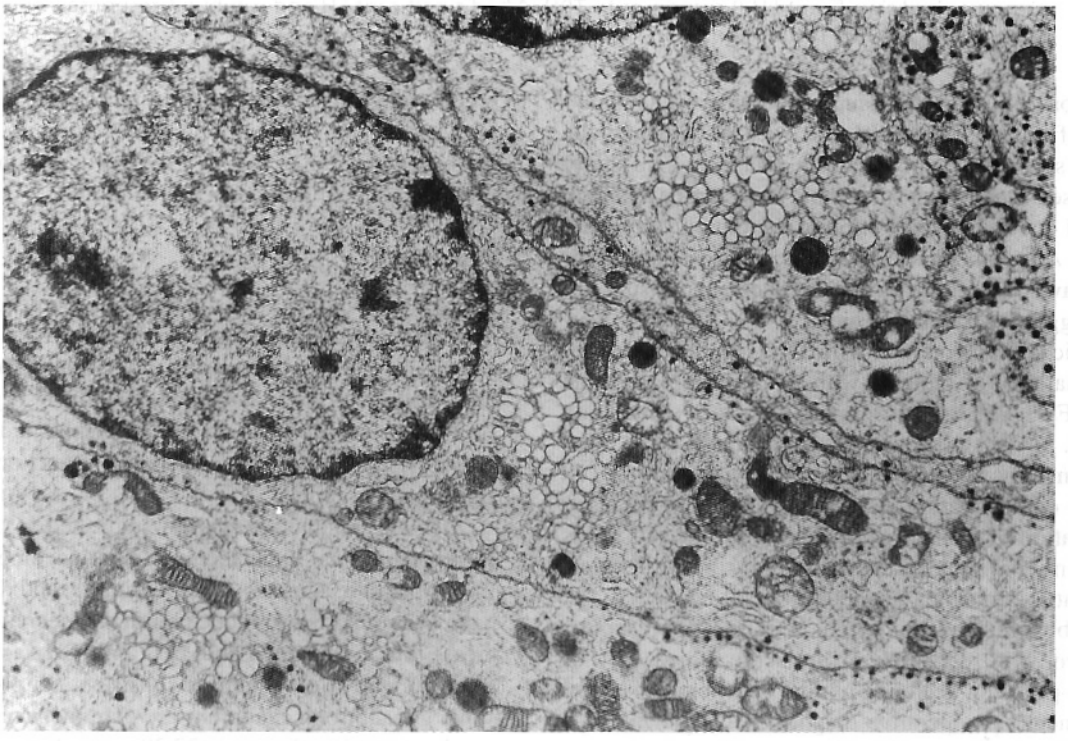

FIG. 2. Gonadotroph adenoma in a woman containing honeycomb Golgi complexes, the diagnostic marker of the female type tumor. Note the difference in granularity between the nuclear poles and cytoplasmic process *(upper right)*, ×7700. (From ref. 14.)

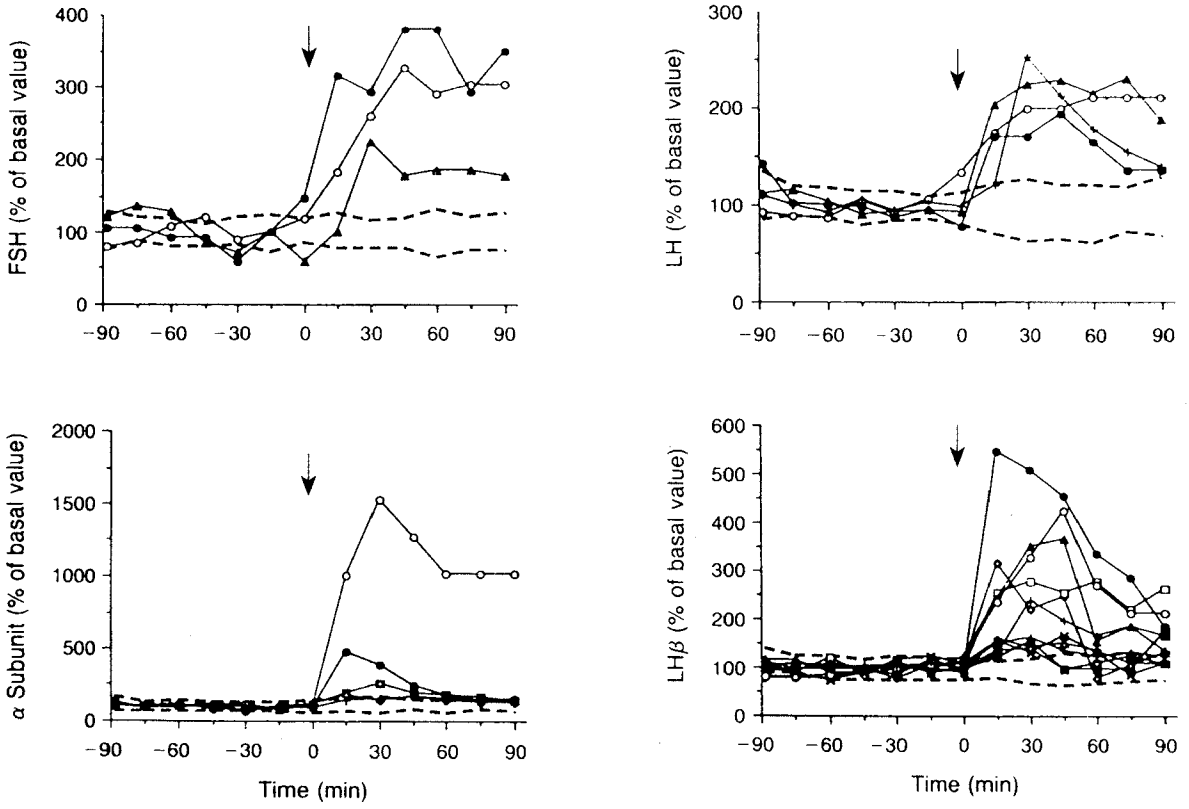

FIG. 3. Responses of serum FSH, LH, LH-β, and α subunit to the intravenous administration of 400 μg of TRH in 16 women with nonsecreting pituitary adenomas. Eleven women with nonsecreting adenomas had significant LH-β responses to TRH, 4 had significant LH and α-subunit responses, and 3 had significant FSH responses. (From ref. 15.)

administration of human chorionic gonadotropin (hCG) will cause a significant rise in testosterone levels, establishing the diagnosis of secondary hypogonadism. Diagnostic difficulty may also arise in cases with subnormal testosterone and high levels of LH due to hypersecretion of the biologically inactive subunit. An erroneous diagnosis of primary hypogonadism can be avoided if a history of headache and visual impairment suggests a pituitary lesion.

Diagnostic stimulation tests with TRH can be helpful because the majority of patients with gonadotroph adenoma will secrete FSH, LH, or their subunits after such stimulation (15,16) (Fig. 3). This preoperative diagnostic test will identify approximately 80% of patients with gonadotroph cell adenoma by demonstrating elevated levels of LH, FSH, or any combination of their subunits. This response to TRH is not found in normal subjects and is presently the best clinical evidence that a tumor is a gonadotroph cell adenoma. The mechanism by which adenoma cells acquire TRH receptors is not known. Mild elevation of prolactin, which may be noted in non-prolactin-secreting pituitary adenoma, may also lead to erroneous preoperative diagnosis of a gonadotroph adenoma as a prolactinoma (22). Women with prolactinomas have an intact reserve capacity of the gonadotrophs and often will demonstrate an enhanced response to GnRH (23). The precise diagnosis of a pituitary tumor as a gonadotroph cell adenoma is clinically important for future use of the glycoprotein secreted in excess as a reliable tumor marker and for possible medical approaches to therapy.

Treatment

Surgery and adjuvant radiation therapy are necessary to control tumor growth and expansion. In most cases, gonadotroph adenomas are first recognized by visual disturbances. By then, the tumors are so large that immediate surgery is necessary to restore normal vision. Transsphenoid surgery improves vision in most patients and corrects hormone-related signs and symptoms.

Medical treatment has been suggested in an attempt to control hormonal hypersecretion pharmacologically (Table 2). Dopamine agonists (bromocriptine and CV 205-502) have been reported to reduce hormonal secretion and to decrease tumor volume in both LH- and FSH-secreting tumors (24–26). The effect is neither consistent nor predictable, but some gonadotroph adenomas are extremely sensitive to dopamine agonist therapy (27,28), resulting in sustained suppression of the gonadotropin levels and tumor volume reduction (Fig. 4).

Table 2. Treatment of Gonadotroph Cell Adenoma

SURGERY	MEDICAL TREATMENT
Transsphenoid excision	Dopamine agonists (bromocriptine) (24–26)
Adjuvant radiation	Somatostatin analog (octreotide acetate) (29–31)
	GnRH antagonist (36)

Somatostatin receptors have been identified on the cell membranes of clinically nonfunctioning pituitary adenomas, most of which secrete LH, FSH, and their separate subunits. The presence of these receptors opens a new therapeutic possibility: treatment with a somatostatin synthetic analog (octreotide acetate) that inhibits gonadotropin secretion *in vitro* and *in vivo*. Preliminary clinical experience with this agent is encouraging (29–31), but tumor volume reduction and correction of visual field abnormalities are not predictable and do not always parallel serum hormone levels (32). This mode of therapy warrants further clinical evaluation to define the subset of patients who may benefit maximally from it.

GnRH agonists and antagonists have also been used to inhibit gonadotroph adenomas. The effect of GnRH agonist is unpredictable. Although successful outcome has been reported (33), it appears that the typical response to GnRH is stimulation of gonadotropin release from the tumor (34,35), reflecting a defective GnRH desensitization mechanism by the tumor (GnRH receptor downregulation). Another possibility is that the tumor cells lose their GnRH receptors, but the fact that they respond to GnRH antagonist makes this explanation unlikely. *In vitro*, Asa et al (17) demonstrated that all but 3 of 53 null cell adenomas showed marked and persistent increases in the release of LH, FSH, and α subunit in response to GnRH in short- and long-duration experiments, suggesting, that although the desensitization mechanism may be defective, the GnRH receptors are functionally intact.

Given the stimulatory effect of GnRH on gonadotroph adenomas, a GnRH antagonist is the natural choice to inhibit hormone secretion. Preliminary experience with GnRH antagonist seems to be promising because in one study a significant decrease in FSH levels was achieved after 7 days of GnRH antagonist administration in four of five patients with gonadotroph adenomas (36) (Fig. 5). The response to a single-dose treatment was poor and did not predict the response to repetitive administration. It is expected that the introduction of new, potent GnRH antagonists with minimal histamine-releasing properties (37) will lead to their clinical application in patients with gonadotropin-secreting adenomas.

Prognosis

Although surgery corrects vision and hormonal abnormalities in most patients, as much as 20% of these tumors recur after surgical treatment (38). Persistent symptoms require adjuvant radiation therapy, which in itself does not prevent recurrence and is associated with significant morbidity (nausea, easy fatiguability, and hypopituitarism). Because the

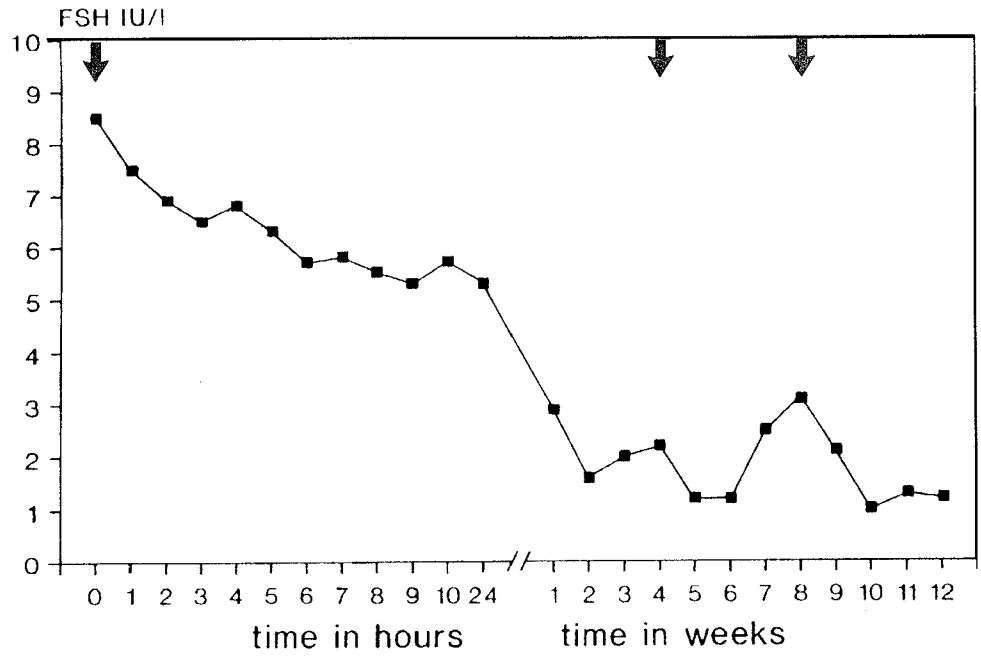

FIG 4. Serum FSH levels during treatment with long-acting bromocriptine (Parlodel-LAR). Each intramuscular administration is indicated by an *arrow*. (From ref. 28.)

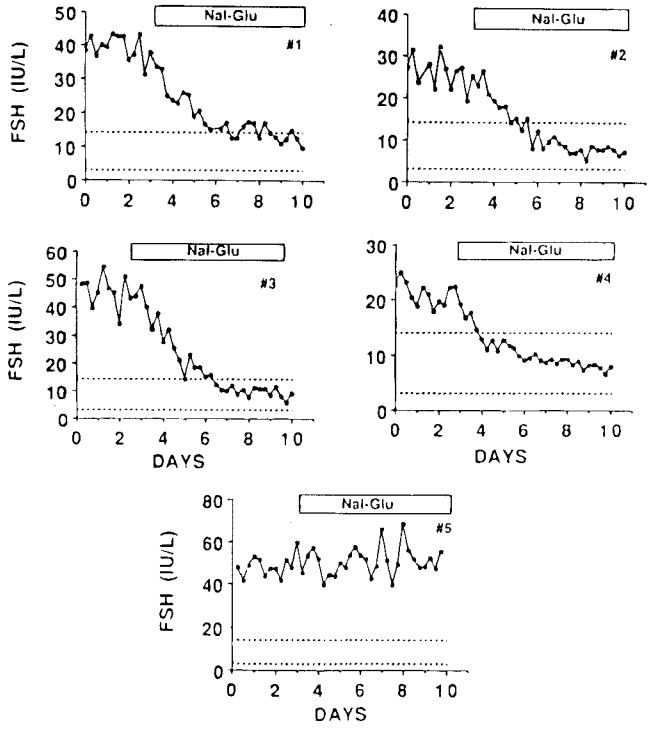

FIG. 5. Serum FSH concentrations of five patients with gonadotroph adenomas for the 3 days before and 7 days during subcutaneous administration of a GnRH antagonist (Nal-Glu GnRH, 5 mg per 12 hours). (From ref. 36.)

effect of the different medial approaches is not fully elucidated, their prognostic impact must await further clinical experience. Given their mechanism of action, positive preliminary clinical results, and future development, however, it seems likely that their use will improve prognosis.

GONADOTROPIN DEFICIENCY

Intact function of the gonadotrophs requires anatomic and functional integrity of the hypothalamic–pituitary unit. Altered gonadotropin secretion, manifesting as either a monohormonal or multihormonal defect, can be traced to anatomic and biochemical derangement in a given region of the hypothalamic–pituitary axis. In addition, systemic diseases, psychoneuroendocrine disorders, or functional states can also affect gonadotropin secretion by the gonadotrophs through alterations of CNS–hypothalamic interaction (Table 1).

Primary Pituitary Lesions

Primary pituitary insufficiency, including gonadotropin deficiency, may occur as a result of a large pituitary adenoma, therapeutic hypophysectomy, pituitary infarction, and autoimmune or granulomatous disease affecting the pituitary gland.

Pituitary Adenomas

Adenohypophyseal cells of all types can form adenomatous lesions. Hypersecretion of all five pituitary trophic hormones and their corresponding subunits has been reported. Pituitary adenomas may produce local endocrine and neurologic effects as well as systemic metabolic complications as a result of hormonal hypersecretion. A detailed discussion of disorders of the somatotropic and thyrotropic axis, hyperprolactinemic states, and their relevance to reproductive dysfunctions is presented elsewhere in this book.

About 20% of pituitary adenomas are not associated with any clinical or biochemical evidence of pituitary hormone hypersecretion (3). Recent studies employing immunohistochemistry, electron microscopy, cell cultures, and molecular biology technologies suggest that these apparently null cell (nonsecreting) adenomas are capable of hormone, most often gonadotropin biosynthesis. Because the capacity of hormone production by the adenoma cells may be limited, circulating hormone levels in such patients are often low and are not associated with clinical evidence of hypersecretion. Consequently, these tumors are large when discovered and commonly are associated with visual disturbances, such as bitemporal hemianopsia secondary to suprasellar extension and chiasmatic compression. Variable degrees of hypopituitarism secondary to the destructing effect of adjacent functional adenohypophyseal cells by the progressive growth of the adenoma are also common. The somatotrophs and the gonadotrophs appear to be the most sensitive. Amenorrhea occurs in approximately 50% of women with null cell adenoma. Hypogonadotropic hypogonadism should always warrant consideration of a pituitary tumor.

Pituitary Infarction (Sheehan's Syndrome)

Infarction and necrosis of the pituitary constitute a rare condition. Their occurrence secondary to postpartum hemor-

rhage and shock was described by Sheehan and Murdoch in 1938 (39). Although Sheehan's syndrome is still the most common cause of panhypopituitarism in women of childbearing age, the number of patients with this syndrome seems to be decreasing because of advances in peripartum care. Pituitary infarction can also occur in association with a pituitary tumor, lymphocytic hypophysitis, and diabetes and in patients with homozygous sickle cell anemia (40).

During pregnancy the anterior pituitary gland almost doubles in size, primarily as a result of hypertrophy of the lactotrophs. The hypertrophied gland is susceptible to ischemia because the oxygen supply is dependent not only on an intact arterial blood supply but also on the low-pressure portal vein system. This explains the relative vulnerability of the gland to hypotension resulting from peripartum hemorrhage and shock (41). Arterial spasm and intravascular coagulation have also been suggested as the pathophysiologic basis for the ischemic insult. Because of improved maternal care during labor and delivery and prompt recognition and modern treatment of intrapartum and postpartum hemorrhage, the classic clinical presentation of Sheehan's syndrome is rare.

The classic panhypopituitarism is characterized by failure of puerperal lactation, breast involution, loss of axillary and pubic hair, amenorrhea, and thyroid and adrenal insufficiency. A direct correlation between the severity of the postpartum hemorrhage and the occurrence of Sheehan's syndrome does not always exist. This may complicate the diagnosis and lead to delayed treatment. Furthermore, 70% of the pituitary gland may be destroyed before hypopituitarism is clinically apparent. Failure to establish the diagnosis and to institute replacement therapy promptly may have later consequences. Late-onset myxedema and adrenal insufficiency resulting in death have been reported. Most cases, however, present with partial or atypical hypopituitarism involving the loss of only a single trophic hormone. GH seems to be the most vulnerable hormone; secretory impairment of the gonadotrophs is less frequent (42). Thus the divergent response to GnRH stimulation is predictable (43).

In a review of pituitary function in patients with pituitary infarction due to various causes, GH deficiency appeared to occur in 88% of patients, hypogonadism in 85%, corticotropin deficiency in 66%, and hypothyroidism due to TSH deficiency in 42% (44). The neurohypophysis is usually involved in Sheehan's syndrome, and plasma vasopressin response to osmotic stimulus is frequently impaired, but overt diabetes insipidus is uncommon in these patients.

Spontaneous subsequent pregnancy depends on the extent of the primary injury. Lesser degrees of pituitary destruction may be associated with atypical clinical presentations and variable degrees of pituitary insufficiency. Under these circumstances, gonadotropin secretion as well as fertility may be preserved. With modern replacement therapy pregnancy is likely, even if the endocrine capacity of the gonadotrophs is lacking.

Hypopituitarism and Pregnancy

Many cases of successful pregnancy in women diagnosed as having Sheehan's syndrome have been reported. Early diagnosis with subsequent replacement of the deficient hormone is essential. In a study involving 39 patients with Sheehan's syndrome, the perinatal outcomes were good in those patients with proper replacement therapy, resulting in 87% live births and 13% spontaneous abortions (45). Failure to establish the diagnosis of hypopituitarism may have lethal consequences for both the mother and the fetus.

Occasionally, the diagnosis of hypopituitarism is made for the first time during pregnancy. The diagnosis of hypopituiarism is difficult to establish during pregnancy because symptoms of nausea, vomiting, fatigue, and weakness are common in normal pregnancy. Moreover, the physiologic changes that normally occur during pregnancy make the interpretation of hormonal tests more difficult. Maintenance of normal adrenal and thyroid function is of primary concern. Extensive diagnostic testing of all pituitary hormones is usually unnecessary, and in some cases not recommended, during pregnancy. Hypoadrenalism is suspected when hypotension, symptomatic hypoglycemia, or unexplained nausea, vomiting, or abdominal pain is noted. Hypoglycemia may occur because of a combination of corticotropin and GH deficiency superimposed on the often observed pregnancy-induced hypoglycemia. Dynamic tests of pituitary corticotropin reserve are usually not recommended during pregnancy because of the confounding changes in pituitary responsiveness to corticotropin and the possibility of harmful effects of insulin-induced hypoglycemia and metyrapone testing on the fetus. Assessment of hypoadrenalism in pregnancy is further complicated by the occurrence of elevated levels of plasma cortisol in normal pregnancy. Plasma levels of cortisol in patients suspected of having hypopituitarism should be compared with those of normal pregnant women.

The diagnosis of hypothyroidism secondary to deficient pituitary TSH secretion is suspected whenever serum levels of thyroid hormone are low and serum TSH levels are low or normal. The changes in the pituitary–thyroid axis that normally occur during pregnancy should be taken into account when thyroid function is being assessed during pregnancy.

During pregnancy and the puerperium, the patient should be closely observed for evidence of inadequate or excessive corticosteroids or the need for thyroid hormone replacement. Corticosteroid dosage need not be higher than in the nonpregnant state except during times of stress, such as labor, delivery, systemic illness, or a surgical procedure. Stressful complications should be recognized and treated promptly, especially when associated with conditions that might lead to shock, such as blood loss or sepsis.

Although rare, spontaneous pituitary necrosis and hypopituitarism in relation to delivery, hemorrhage, or hypotension may occur in pregnant diabetic patients (46,47). The cause is unknown. The unique susceptibility of the pregnant

diabetic patient, however, is probably related to the combination of diabetic vascular changes and the general susceptibility of the anterior pituitary gland to ischemia during pregnancy. Pituitary infarction is about 10 times more common in diabetics than in the general population. It is manifested by severe, deep midline headaches and vomiting lasting 1 to 10 days followed by decreasing insulin requirements and the development of hypoglycemia. Early recognition and management of this syndrome are critical (47).

Pituitary Apoplexy

Pituitary apoplexy is the sudden enlargement of a pituitary tumor related to infarction, necrosis, and hemorrhage within the tumor; it results in rapid expansion of the lesion and acute compression of adjacent structures (48,49). Pituitary apoplexy may occur spontaneously in a number of pituitary tumors (most commonly in silent tumors) or during pregnancy in patients with prolactinomas. Dynamic tests of anterior pituitary function, TRH, GnRH, insulin, or glucagon can be the trigger for the apoplectic episode (50). This is an uncommon but life-threatening disorder that requires prompt recognition and treatment to avoid visual or neurologic impairment or death. The event is usually characterized by the sudden onset of an excruciating retroorbital or retrofrontal headache with nausea and vomiting. Patients may lose consciousness shortly after the appearance of the first symptoms and may deteriorate rapidly. Compression on the optic chiasm and the cranial nerves (third, fourth, fifth, and sixth) results in visual field defects, impaired visual acuity, diplopia, and ophthalmoplegia. Major destruction of the anterior pituitary gland may result in life-threatening pituitary insufficiency. Rapid suprasellar expansion may lead to hypothalamic aberrations, including abnormalities in thermal regulation and disorders of the satiety center.

Partial destruction of the pituitary gland may result in various degrees of hypopituitarism. Transient dysfunction of the neurohypophysis may result in temporary diabetes insipidus in patients recovering from pituitary apoplexy (49). Plain films of the skull usually reveal enlargement of the pituitary fossa with erosion of the sella turcica. A CT scan of the sellar and parasellar regions may reveal increased attenuation corresponding to hemorrhage within the tumor (51). An arteriogram is recommended when there is difficulty in distinguishing pituitary apoplexy from a ruptured aneurysm.

The treatment of pituitary apoplexy includes high-dose corticosteroid therapy to provide coverage for imminent, acute hypocortisolism and to alleviate intracranial swelling. Vigorous supportive measures should be initiated without delay. Early transsphenoid surgical decompression is advocated in most cases. Conservative management may be considered in patients who retain consciousness and experience no further visual or neurologic deterioration. Rapid worsening of visual acuity resulting from acute compression of the optic chiasm is an absolute indication for surgery.

Empty Sella Syndrome

In its primary or idiopathic form, this syndrome is characterized by intrasellar herniation of the subarachnoid space through an incomplete diaphragmatic sella. Elevation of CSF pressure in the setting of benign intracranial hypertension probably predisposes to the development of empty sella syndrome. The pituitary fossa becomes filled with CSF, and the pituitary gland is flattened or displaced. In its secondary form, empty sella syndrome results from infarction of a pituitary tumor, surgery, or irradiation therapy in the sellar region.

The syndrome is clinically important because the presence of an enlarged sella requires further evaluation to exclude a pituitary tumor (52). Among 75 patients presenting with a radiographically enlarged sella turcica and no visual symptoms, empty sella syndrome was diagnosed in 25, emphasizing the relative frequency of the syndrome (53).

The presenting symptoms of patients with empty sella syndrome are usually nonspecific, and headache is the most common complaint. Primary empty sella syndrome is typically found in middle-age women. Many patients are evaluated because of sellar enlargement seen on skull films obtained after incidental trauma. On plain films the sella turcica is usually enlarged symmetrically without cortical bone erosion, giving a balloonlike appearance. Lateral plain films usually reveal a double floor. A CT scan is needed to confirm the diagnosis. Radiologic studies with intrathecal contrast media might be needed in patients with visual symptoms to demonstrate the intrasellar herniation of the optic chiasm. Although most patients with primary empty sella syndrome lack evidence of endocrine abnormalities, some have signs of pituitary hormone abnormalities such as amenorrhea-galactorrhea and acromegaly (52). In the latter instances, the hypersecretion of prolactin and GH is likely to be due to the presence of microadenomas. Rarely, patients may manifest spontaneous CSF rhinorrhea, sinusitis, and papilledema, usually in association with benign intracranial hypertension. Patients with primary empty sella syndrome without associated abnormalities can conceive and sustain pregnancy to term.

Lymphocytic Hypophysitis

Lymphocytic hypophysitis is a rare autoimmune disease involving extensive lymphocyte and plasma cell infiltration of the anterior pituitary; it is most associated with pregnancy and the puerperium. It presents as a mass lesion of the sella turcica and clinically mimics a pituitary adenoma. Patients may present during pregnancy or in the postpartum period with signs and symptoms of pituitary insufficiency or with visual or neurologic abnormalities related to suprasellar expansion of the lesion. Only 39 cases have been reported to date (54,55). Most cases have been associated with multiglandular endocrine autoimmune phenomena such as Hashimoto's thyroiditis, parathyroiditis, pernicious anemia, and diabetes mellitus. In all cases, the diagnosis was made in

women with apparent pituitary tumors who were found to have lymphocytic hypophysitis on biopsy. Light microscopic findings showed extensive infiltration of lymphocytes and plasma cells in the anterior pituitary. Ultrastructural features resembled those seen in autoimmune thyroiditis. Experimental hypophysitis with similar histologic features can be produced in pregnant rats by injecting homogenates of whole anterior pituitary glands.

The detection of organ-specific antimitochondrial and antinuclear antibodies gives further support to the concept of an endocrine autoimmune phenomenon. Lymphocytic hypophysitis should be considered in the differential diagnosis of a pituitary adenoma during and after pregnancy. Definitive diagnosis is only possible by histopathologic examination of pituitary biopsy material. Surgery is indicated only in patients with visual or neurologic abnormalities secondary to suprasellar expansion of the lesion.

Evaluation and Treatment of Hypopituitarism

Dynamic testing designed to evaluate pituitary secretory reserve should be performed whenever partial or complete hypopituitarism is suspected because normal hormone levels in the basal state do not exclude the diagnosis. Furthermore, the institution of replacement therapy in the absence of documented hormonal deficiency is unjustified. The results obtained from such testing should be interpreted with caution, with consideration being given to the clinical presentation of the patient and the fact that patients with hypopituitarism may respond normally to provocative stimuli (56).

Thorough testing of the pituitary–adrenal, pituitary–thyroid, and pituitary–gonadal axes ordinarily requires several dynamic tests on several separate days (57). The cost of these tests can be reduced by using a combined injection of several stimulatory agents; this results in neither impairment of reliability nor increased risk to the patient. For example, a sequential stimulation test consisting of insulin-induced hypoglycemia (intravenous insulin, 0.2 U/kg), during which GH, prolactin, and cortisol are measured, followed 2 hours later by GnRH and TRH (intravenous GnRH, 150 μg + TRH, 500 μg) stimulation, during which LH, FSH, TSH, and prolactin are measured, has been used successfully in diagnosing pituitary insufficiency in patients with Sheehan's syndrome (58). Patients with pituitary insufficiency failed to develop a normal increase in serum GH, prolactin, and cortisol after insulin-induced hypoglycemia and showed a blunted LH, FSH, and TSH response.

More recently, combined anterior pituitary function tests using four hypothalamic-releasing hormones [GnRH, corticotropin-releasing factor (CRF), GH-releasing hormone, and TRH] have been used in normal subjects and in patients studied after pituitary surgery (59,60). Combined administration of these hormones caused no apparent inhibition or synergism of individual hormonal responses in 26 normal subjects (14 men and 12 women (59) (Fig. 6). In another study, however, the plasma TSH and prolactin responses were significantly higher after the combined test in 10 normal men (60). In 8 patients studied after surgery for pituitary adenoma, combined administration of insulin, TRH, and GnRH provided results comparable to those obtained after separate administration of these agents (60). To increase the cost effectiveness of the combined tests, Sheldon et al. (59) proposed a shortened schedule for assaying hormone level (Table 3), reducing the total number of assays from 70 to 23 and the time from 120 to 90 minutes. Cohen et al. (60) suggested adding a later sampling at 120 minutes for patients with disease of hypothalamic origin, in whom the responses to GnRH, TRH, and CRF might be delayed.

The treatment of hypopituitarism consists of replacing the deficient hormones of the end-organ glands. Documentation of overt hypoadrenalism requires maintenance doses of cortisol given as cortisone acetate (20 to 25 mg in the morning and 10.0 to 12.5 mg in the evening) or an equivalent dose of a synthetic corticosteroid (eg, dexamethasone, 0.5 mg). Milder degrees of pituitary hypofunction usually do not require routine replacement therapy except during times of stress. Deficient pituitary corticotropin reserve is characterized by the secretion of sufficient corticotropin to maintain normal basal levels of cortisol but insufficient corticotropin to maintain normal cortisol response to stress- and insulin-induced hypoglycemia.

Normal thyroid function in patients with pituitary hypothyroidism is maintained with L-thyroxine (0.1 to 0.2 mg/day). These patients exhibit low levels of serum thyroid hormones with low or normal TSH levels.

Pituitary deficiency of gonadotropins (FSH and LH) results in hypogonadotropic hypogonadism and hypoestrogenism. Estrogen replacement therapy is recommended for these patients. When fertility is desired, treatment with exogenous gonadotropins is needed to induce ovulation and to achieve pregnancy in most patients with Sheehan's syndrome. During pregnancy, replacement therapy with glucocorticoids and thyroxine results in favorable outcomes. If fever, infection, or a surgical procedure occurs during pregnancy, larger doses of corticosteroids may be required.

Hypothalamic–Pituitary Lesions

Hypothalamic lesions, either congenital [isolated gonadotropin deficiency (Kallmann's syndrome]) or acquired, may cause various degrees of hypopituitarism. Hypothalamic etiologies of hypopituitarism are listed in Table 1. These include suprasellar tumors (craniopharyngioma, glioma, meningioma, germinoma, endodermal sinus tumor, or metastatic tumors), granulomas [sarcoidosis or Hand-Schüller-Christian disease (histiocytosis X)], infections (basal meningoencephalitis, syphilis, or tuberculosis), vascular malformation, postirradiation damage, and head trauma. The clinical features suggesting hypothalamic etiology of hypopituitarism include moderate hyperprolactinemia resulting from

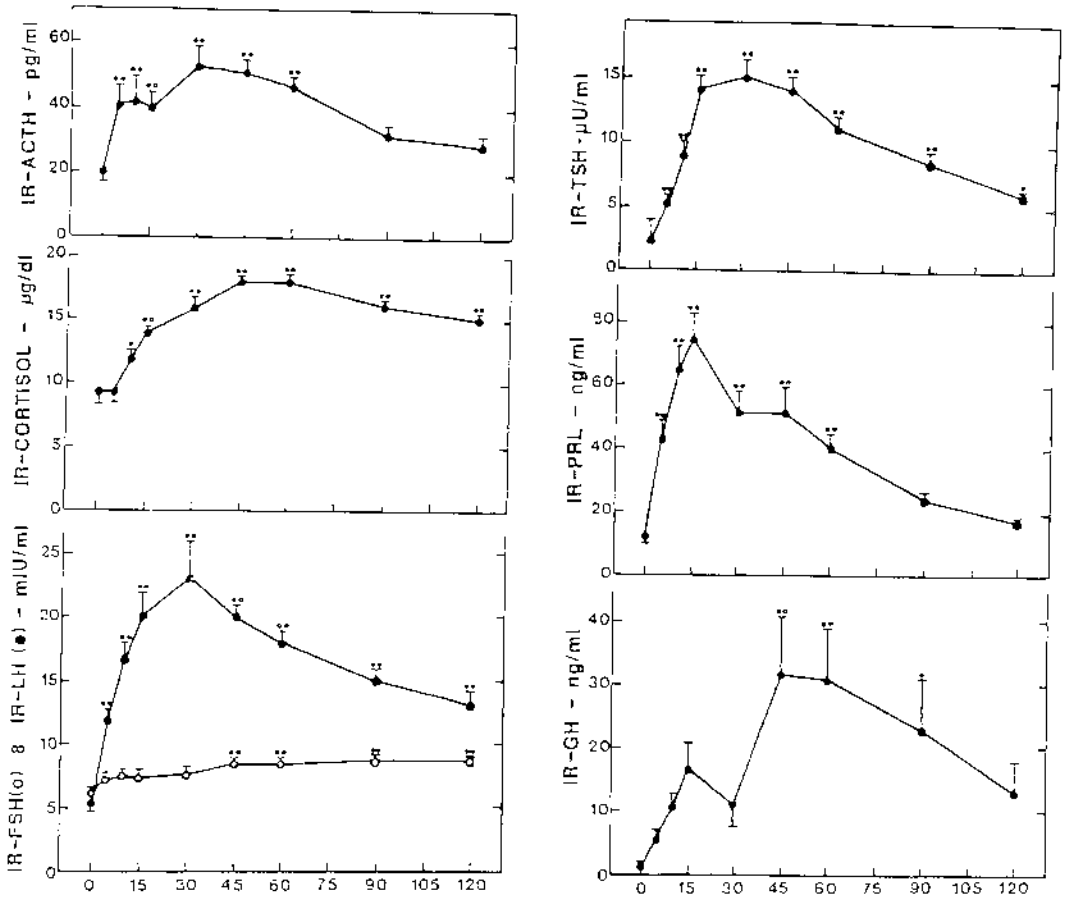

FIG. 6. Mean plasma or serum levels (± standard error) of seven immunoreactive *(IR)* hormones at defined intervals after the administration of four hypothalamic-releasing hormones in 26 normal subjects (14 men, 12 women). $*P < 0.05$, $**P < 0.01$ (compared with baseline values). The releasing hormones were administered as sequential 20-second intravenous infusions in the following order and doses: ovine corticotropin-releasing hormone, 1 µg/kg; GnRH, 100 µg; human GH-releasing hormone, 1 µg/kg; TRH, 200 µg. (From ref. 59.)

Table 3. Recommended Schedule for Assaying Plasma Hormone Levels During Combined Anterior Pituitary Function Testing (TRH, GnRH, CRF)

PLASMA/SERUM HORMONE ASSAYED	TIME AFTER INFUSION (MINUTES)							TOTAL ASSAYS
	0	10	15	30	45	60	90	
Corticotropin	x	x			x	x		4
Cortisol	x				x	x		3
FSH	x				x	x		3
LH	x		x	x				3
TSH	x		x	x				3
Prolactin	x	x	x					3
GH	x				x	x	x	4
Total assays	7	2	3	2	4	4	1	23

From ref. 59.

loss of prolactin-inhibiting factors, the presence of diabetes insipidus, visual disturbances, obesity, somnolence, and psychiatric disturbances. The degree of impairment of pituitary function depends on the extent of hypothalamic or pituitary stalk involvement.

Isolated gonadotropin deficiency as a clinical entity hosts a number of distinct conditions. The ability to delineate the molecular basis of gonadotropin-deficient states will probably add more specific conditions to the list.

Kallmann et al. (61) described in 1944 a rare (frequency is about 1 in 10,000 males and 1 in 50,000 females) syndrome of hypogonadotropic hypogonadism associated with anosmia that is transmitted by several modes of inheritance: autosomal dominant with variable penetrance, autosomal recessive, or X linked (62). This is currently the most common presentation of isolated gonadotropin deficiency. The pathogenesis of the syndrome has been clearly demonstrated in several species, including the human fetus. GnRH neurons of olfactory epithelial origin fail to migrate into the hypothalamus (63). In the male the clinical presentation ranges from complete failure of gonadotropin secretion and sexual infantilism to incomplete forms of GnRH deficiency leading to isolated LH deficiency with normal FSH levels. In these cases, normal spermatogenesis in the face of androgen deficiency was described as the fertile eunuch syndrome. In the female, primary amenorrhea with immature ovaries is common. Ovarian biopsy specimens from untreated women have shown that follicular development past the primordial stage is rare (64). As in male patients, the clinical presentation ranges from absent secondary sex features to nearly normal breast development (65). Other pituitary endocrine axes (eg, prolactin, TSH, and GH) are normal (66).

Non–endocrine-associated symptoms include cleft lip, cleft palate, cardiac abnormalities, renal aplasia, cryptorchidism, skeletal anomalies, obesity, and bleeding disorders (67). Detailed clinical and laboratory studies have been presented by Lieblich and coworkers (68). Neurologic features that have been described in these patients include mirror movements, cerebellar ataxia, seizures, mental retardation, suprasellar Rathke's pouch cyst, right cerebral hemiatrophy, retinitis pigmentosa (69), and visual-spatial attention abnormalities (70). These associated abnormalities suggest that the anatomic location of the lesion is not limited to the hypothalamus and the olfactory bulbs and tracts but involves midline CNS structures, including the corpus callosum and cerebellar vermis.

Numerous descriptions of isolated gonadotropin deficiency states that are not necessarily associated with anosmia have been published. Rabin et al (71) described a 22-year-old patient with normal olfactory acuity and isolated FSH deficiency. Current molecular biology technology allowed precise diagnosis of a mutant LH molecule as a basis for a deficient LH state with elevated serum LH, and a variant form of LH was detected in a healthy woman with deficient serum LH. The molecular basis of these states is discussed later in this chapter.

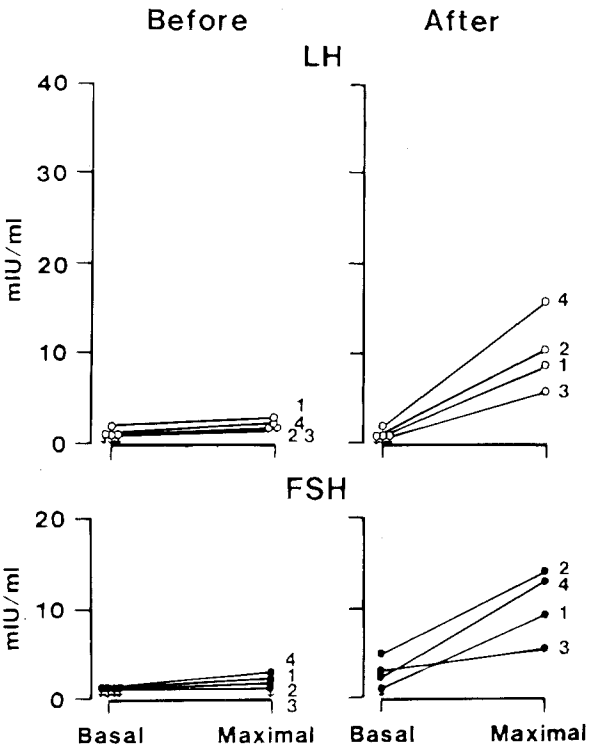

FIG. 7. Acute responses of LH and FSH to the first *(left panel)* and last *(right panel)* injections of GnRH (25 ng/kg intravenously every 2 hours for 4 days) in patients with no evidence of GnRH secretion. (From ref. 73.)

The gonadotroph responsiveness to exogenous GnRH reflects the heterogenous nature of these syndromes and ranges from complete failure of LH and FSH secretion to monohormonal deficiency (71) and a normal rise in LH and FSH. It should be emphasized that patients with hypothalamic hypogonadism do not respond readily to exogenous GnRH with raised levels of gonadotropins. A prolonged pulsatile exposure to GnRH is required for the gonadotrophs to recover from endogenous GnRH deprivation. In one study, 7 days of GnRH treatment were needed before a distinction between a primary pituitary lesion and hypothalamic disease on the basis of response to 250 μg GnRH was possible (72). There is a clear correlation between the pattern of hormone response to pulsatile GnRH therapy and the magnitude of the underlying GnRH secretory defect (73) (Figs. 7 and 8). The extent of GnRH neuron migrational failure determines the degree of the endocrine deficiency. Even if LH levels in these patients are low, α subunit is present in the serum. Because in one study α subunit remained detectable even after L-thyroxine blocked TSH secretion, the gonadotrophs are a probable source of the α subunit (74). A concomitant significant secretion of α subunit from the thyrotrophs in euthyroid patients with hypogonadotropic hypogonadism has been demonstrated (75). Lack of a pulsatile pattern of secretion in these patients suggests that, although LH-β se-

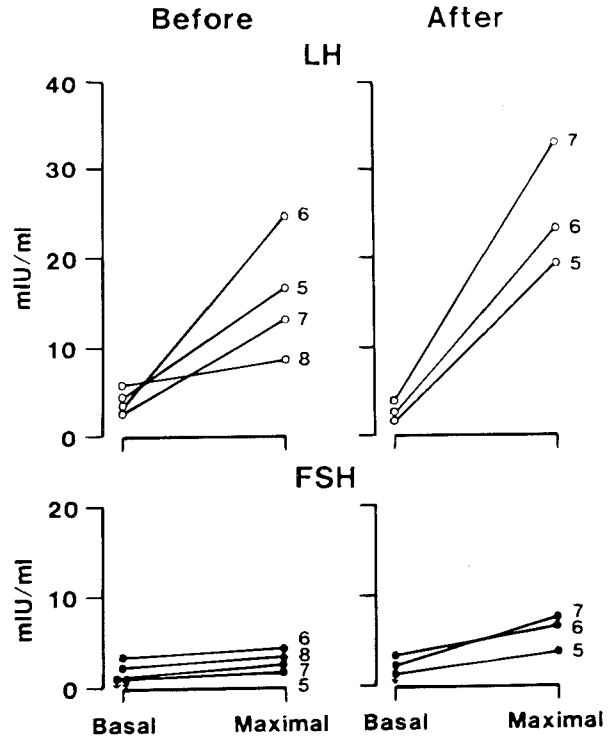

FIG. 8. Acute responses of LH and FSH to the first *(left panel)* and last *(right panel)* injections of GnRH (25 ng/kg intravenously every 2 hours for 4 days) in patients with partial idiopathic gonadotropin deficiency. (From ref. 73.)

cretion is GnRH dependent, other factors besides GnRH regulate α-subunit gene expression in the gonadotrophs. The pulsatility of α-subunit secretion is restored after administration of pulsatile GnRH. When a sensitive monoclonal assay was used to detect LH-α, a 100% concordance between pulses of the intact LH and the subunit was demonstrated. The use of this assay was proposed in GnRH-deficient patients because after administration of GnRH the level of free subunit rises more quickly and sharply than that of either LH-β or FSH (76).

In addition to deficient GnRH secretion, patients with idiopathic hypogonadotropic hypogonadism may have defective Sertoli and Leydig cell function. In one study, when pulsatile exogenous GnRH was administered for a period of time sufficient to raise gonadotropin levels to the midnormal range, inhibin and testosterone levels did not rise to the same extent (77).

After space-occupying lesions of the pituitary hypothalamic system are excluded, replacement therapy can be administered. The distinction of isolated gonadotropin deficiency from delayed puberty is not readily made in cases where characteristic congenital anomalies are lacking. Of the many tests offered [basal levels of testosterone, gonadotropin and prolactin levels, or responses to GnRH (78)], the prolactin response to TRH was the best discriminant and identified 9 of 10 subjects with isolated gonadotropin deficiency (79). These patients had a subnormal increase in prolactin after TRH. The effect of timing of replacement therapy in puberty is controversial, but data reported recently by Dickerman et al (80) suggest that patients achieve normal final height irrespective of the timing of initiation of sex hormone therapy.

When fertility becomes the main concern, exogenous gonadotropin in a female patient can readily induce ovulation. Pulsatile administration of GnRH is a successful alternative. Coexistence of gonadotropin deficiency and ovarian failure, however, has been documented in a patient who failed to respond to exogenous GnRH (10 μg GnRH per 90-minute pulse increasing over 21 days to 30 μg per pulse) or to gonadotropins (81).

In male patients, gonadotropins will in most cases successfully stimulate spermatogenesis. Prior androgen treatment does not adversely influence this response. Initial testicular size (reflecting partial or complete gonadotropin deficiency) may predict the response to hCG therapy without additional FSH. If a patient fails to restore spermatogenesis, however, long-term subcutaneous GnRH has been reported to result in fertility (82–84). To test whether an initial treatment with GnRH would be advantageous in these patients because of its more physiologic action, Liu et al (85) prospectively evaluated two groups of patients for 2 years. Five patients received pulsatile GnRH in doses of 143 to 174 ng/kg every 2 hours, and 11 were treated with hCG (2000 IU) and human menopausal gonadotropin (HMG, 75 IU FSH and 75 IU LH) three times a week. The groups did not differ in the following outcome measures: testicular growth, onset of sperm production, sperm quality, and estradiol concentration. The GnRH-treated group had lower total and free testosterone and lower FSH levels. It is therefore suggested that GnRH is not superior to hCG and HMG as the first line of therapy.

A related syndrome has been reported (86,87) in which patients with cerebellar ataxia and hypogonadotropic hypogonadism failed to respond with a rise in gonadotropin to repeated stimulation with GnRH, implying that hypogonadism in these patients is secondary to a defect in production or release of gonadotropins by the pituitary itself rather than to a hypothalamic lesion. Similarly, patients with hypogonadotropic hypogonadism associated with congenital adrenal hypoplasia fail to respond to GnRH administration with elevated gonadotropins, suggesting abnormal pituitary function (88).

Systemic Disorders and Gonadotropin Deficiency

Severe illness can affect pituitary–hypothalamic unit function, causing a decrease in gonadotropin secretion as well as blunted or absent response to GnRH. In severely ill postmenopausal women, mean levels of LH and FSH are significantly suppressed irrespective of weight loss (89). The hypo-

thalamus is involved in the neurologic process that determines mood, emotion, and behavior. It is therefore not surprising that psychologic disturbances are expressed in altered GnRH secretion and subsequent impaired gonadotropin secretion.

By abnormal secretion of GnRH resulting in altered frequency and amplitude of LH pulses, emotional stress and anxiety can cause secondary amenorrhea (90). The mechanism for abnormal secretion of GnRH at the hypothalamus level is probably unbalanced neuronal activity with increased dopaminergic and β-endorphin neuronal activity resulting in suppression of GnRH neuronal effect (91).

Pseudocyesis is another example of a condition in which a psychogenic disturbance, expressed chemically at the hypothalamus level, leads to an altered gonadotropin secretion pattern. High basal levels and increased pulsatility of LH with normal FSH levels have been reported (92). In this condition, too, abnormal opioid dopamine activity has been postulated as the mechanism for impaired gonadotropin secretion (93).

Hypothalamic dysfunction is also postulated as the mechanism of decreased gonadotropin levels in anorexia nervosa (94). Although such a dysfunction is predicted in the face of extreme weight reduction, gonadotropin secretion abnormalities are found in patients of normal weight with bulimia nervosa. This syndrome is characterized by periods of excessive eating alternating with caloric restriction. When patients with this syndrome who reported amenorrhea were studied, a decreased LH pulsatile secretion was found associated with increased cortisol concentrations (95).

Intensive exercise, especially in women with low body weight, may cause amenorrhea due to abnormal GnRH secretion, leading to low levels of gonadotropins, estradiol, and progesterone. Increased activity of β-endorphin secretion at the hypothalamic level is the proposed mechanism because pituitary response to exogenous GnRH is normal (96).

Extreme changes in body weight, either weight loss or obesity, may cause gonadotropin secretion abnormalities. In the case of simple weight loss, the hormonal profile is similar to that in anorexia nervosa, and GnRH dysfunction has been implicated as the cause for a pubertal LH pulse pattern (97). The significance of eating disorders and exercise as causes of hypothalamic disorders is discussed in other chapters in this book.

Renal failure may cause abnormal hypothalamus-gonadotroph system abnormalities in male and female patients. In men, primary testicular failure is also present because testosterone levels remain low after hCG administration. In addition, LH levels are inappropriately low for the testicular failure. Loss of or abnormal LH pulsatility and exaggerated LH response to exogenous GnRH suggest a combined hypothalamus and pituitary dysfunction. The gonadotropin profile in female patients is similar, showing loss of the midcycle LH surge. In both sexes dialysis fails to restore a normal gonadotropin secretion pattern, but successful renal transplant often does.

A similar hypothalamic–pituitary dysfunction has been described in patients with cirrhosis, primary testicular failure, and inappropriately low levels of gonadotropins. Iron deposition in patients with hemochromatosis may cause pituitary destruction with hypogonadotropic hypogonadism (98). Similarly, gonadotroph iron deposition in β-thalassemia patients due to multiple blood transfusions may result in low LH and FSH levels and reduced response to GnRH. These patients have normal basal serum cortisol, thyroxine, and prolactin as well as normal responses of GH and TSH to stimulation by insulin-induced hypoglycemia and TRH, respectively, suggesting a selective destruction of the gonadotrophs. Once the damage is established, chelation therapy fails to restore normal gonadotropin secretion.

Granulomatous diseases (sarcoidosis, histiocytosis, X and tuberculosis) involving the hypothalamic–pituitary system may result in hypogonadotropic hypogonadism.

Aging in healthy men is considered to cause a primary decrease in testicular function. This conclusion is based on decreased response to hCG, morphologic studies demonstrating a decreased number of Leydig cells, and increased basal immuno-LH levels. Deconvolution analysis of pulsatile patterns of LH release, however, have shown that the LH secretory burst amplitude decreases, that burst duration and frequency increase, and that basal LH secretory rates increase (99). It was suggested that age does not decrease the gonadotroph sensitivity to GnRH but rather influences the hypothalamus, causing a decrease in the amount of GnRH released with each pulse (100).

In healthy women, early menopause is characterized by an increase in gonadotropin levels associated with the gradual decline in estrogen secretion from the ovaries. A study of 680 postmenopausal women showed that this increase was followed by a gradual decrease of LH, FSH, and α subunit from the age of 55 to 75 years (101). The effect is independent of sex steroid or sex hormone–binding globulin concentrations. Because aging has no effect on prolactin secretion, a general decline in pituitary function is unlikely. Hence a direct effect of aging on the hypothalamic–pituitary GnRH–gonadotropin system is suggested.

MOLECULAR ENDOCRINOLOGY AND DISORDERS OF THE GONADOTROPHS

Recent advances in molecular biology technology have made it possible to study the structure of the gonadotropins and the genes that regulate their synthesis and secretion. This section provides an overview of these technologies and describes how they have shed a new light on the molecular basis of diseases involving the endocrine function of the gonadotrophs.

Structure of the Gonadotropins and the Genes Encoding Them

The two glycoprotein molecules, LH and FSH, as well as the placentally derived chorionic gonadotropin share a com-

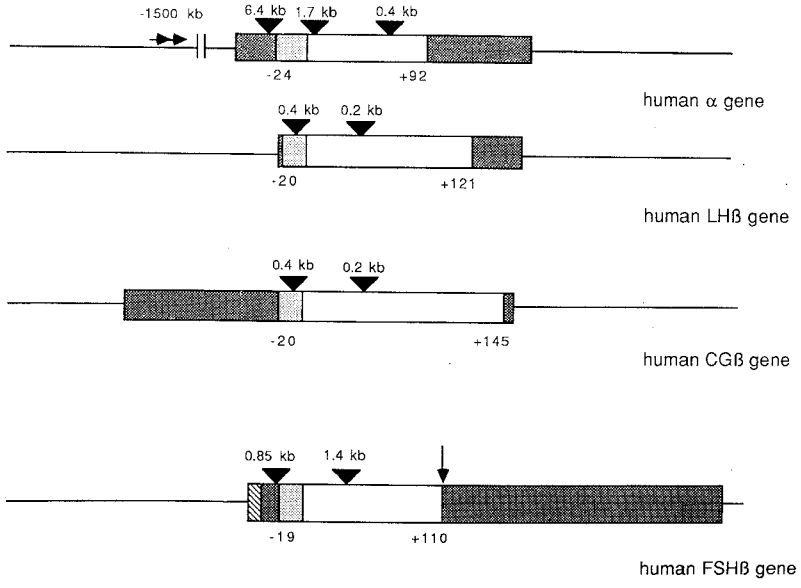

FIG. 9. Structure of the human gonadotropin genes. Schematic diagrams of the human α, LH-β, chorionic gonadotropin β, and FSH-β genes are shown. Untranslated regions are depicted by *dark shaded regions.* Signal peptides and mature apoproteins are represented by *stippled* and *unshaded regions,* respectively. *Solid triangles* demonstrate the position of introns. Intron sizes are marked above these triangles. *Numbers* below each diagram signify amino acid position with respect to the first amino acid of the mature apoprotein. The pair of *horizontal arrows* in the diagram of the α gene denotes the position of the directly repeated cyclic AMP regulatory element *(CRE).* In the diagram of the FSH-β gene, the *hatched region* represents the extended 5′-untranslated region produced by alternative splicing that occurs in 35% of transcripts. The *solid arrow* indicates a polyadenylation site utilized by 20% of the transcripts. (From ref. 107.)

mon α subunit. Their specific biologic activity is determined by different β subunits. Although the β subunits share considerable amino acid sequences, their specificity is mainly determined by three-dimensional structure and by carbohydrate chains that are attached to specific sites. Modulation of these segments causes attenuation of their bioactivity. For example, desialation of hCG and FSH results in rapid *in vivo* clearance (102). GnRH influences the acidity of the secreted hormones and their bioactivity. Clearly, this nonuniformity of molecular structure is hormonally regulated to serve a specific biologic activity (103).

The gonadotropins are compact globular glycosylated proteins; their molecular weights are 28,000, 29,000, and 37,000 for LH, FSH, and hCG, respectively. The α and β subunits of the gonadotropins are each encoded by a single gene (104). The gene for the α subunit is located on chromosome 7, and that for the β subunit is on chromosome 19. The hCG β subunit, which is synthesized by the syncytiotrophoblast, is encoded by several genes located also on chromosome 19 (105). Although the two genes for LH and hCG are nearly identical, they have different transcriptional start sites, reflecting the different factors that may regulate these genes (106,107) (Fig. 9).

The α gene contains four exons and three introns (108). Human variants of the gene differ not in their exons but in their intron A. The case for chorionic gonadotropin β is more complicated because seven genes have been identified, and only three of them can be translated into mRNA that will yield a β subunit.

The GnRH receptor plays a key role in reproduction. Pulsatile GnRH, released from the hypothalamus in low concentrations, is trophic to the receptor, whereas high concentrations cause downregulation and diminished biologic effect. A PCR-based strategy was used to identify the mouse GnRH receptor cDNA (109). A functional 1.3-kb cDNA was isolated that encodes a predicted 327–amino acid receptor protein demonstrating seven stretches of highly hydrophobic amino acids. Compared with other G protein–coupled receptors, the GnRH receptor has an unusual structure that is believed to serve its proposed mechanism of action. A gonadotroph cell line (αT3-1) obtained by tumorigenesis in transgenic mice was used for characterization of the GnRH receptor (110). Although this cell line does not express the β subunit and hence does not secrete gonadotropins, it does express the α-subunit gene and its product. This latter expression is GnRH inducible. Receptor activity is linked via a G protein (Gq) and a second messenger (inositol 1,4,5-triphosphate) associated with a rise in intracellular calcium.

Regulation of Gonadotropin Gene Expression and Secretion

Although the α subunits secreted by gonadotrophs, thyrotrophs, and the placenta are identical, on the molecular level each cell demonstrates a unique DNA sequence that mediates selective transcription of its α-subunit gene. The sequence between −500 and −200 is important for pituitary expression, and two regions have been found to be cell specific for gonadotroph expression: −445 to −438 and −337 to −330 (111).

On the hormonal level, gonadotropin secretion is regulated by a number of factors. Pulsatile secretion of GnRH stimulates gonadotropin secretion in a parallel pulsatile fashion. Classically, the end organ–derived sex steroids exert negative feedback on gonadotropin secretion, but the well-orchestrated mechanism of ovulation depends on paradoxic positive feedback of estrogen on LH secretion. Inhibin, activin, and follistatin are also regulatory peptides secreted by the gonads.

Estradiol negatively regulates pituitary subunit mRNA; postovariectomy high levels of mRNA for the gonadotropin subunits can be suppressed to normal levels by exogenous estradiol replacement (112). The site on which estradiol exerts its feedback is controversial. Experimental evidence suggests that the negative effect on gonadotropin subunit mRNA synthesis *in vivo* is mediated via the hypothalamus (113,114). Estrogen was reported to increase the number of GnRH receptors on the gonadotrophs and to augment their gonadotropin stores (115). In contrast, other investigators have shown, using ovariectomized rats treated with estradiol to induce an LH surge, that GnRH concentrations increase in the median eminence (116). In GnRH-deficient mice, estradiol administration did not increase mRNA levels for LH (117), indicating that, at least in this animal, estradiol does not exert its effect directly on the pituitary in the absence of GnRH. This GnRH self-priming effect of estrogen in the human was suggested by the observation that estrogen increased LH release in postmenopausal women who were given serial injections of fixed doses of GnRH (118). Without this priming effect, repetitive GnRH stimuli cause receptor downregulation and decreased gonadotropin release.

How and where progesterone affects the gonadotropins is not clear. Progesterone was demonstrated to affect the pulse frequency of LH secretion. A slower pulse frequency is caused by progesterone during the luteal phase of the cycle (119). Conflicting data were presented by different investigators on how progesterone affects subunit mRNA, probably reflecting the fact that its biologic effect is less pronounced compared with that of estrogen. The direct effect of progesterone at the pituitary level in women was demonstrated in patients with isolated GnRH deficiency (Kallmann's syndrome) given constant-frequency GnRH pulses and exogenous estradiol to keep plasma estradiol levels fixed (120). Under these conditions, progesterone caused a significant increase in both mean plasma LH level and LH pulse amplitude. This stimulatory effect is probably not at the *de novo* LH synthesis level but rather on the releasable LH pool in the gonadotrophs. FSH levels were not affected by progesterone.

Androgens also modulate subunit mRNA levels. In the castrated male rat, mRNA for LH subunits is significantly increased (by up to a factor of 8), whereas mRNA for FSH subunits is only moderately elevated (112). As expected, testosterone replacement in these rats restores subunit mRNA to intact levels. Testosterone, when given to male rats in the absence of endogenous GnRH action, selectively increases FSH-β concentrations.

Clearly, sex steroids play different roles in gonadotrophin regulation in male and female rats. When castrated male rats were given estradiol, a reduction in LH-β and FSH-β but not α mRNA was found. In the ovariectomized female rat, testosterone reduced all three subunit mRNA, although FSH-β remained higher than in intact animals (121).

GnRH has the unique capability of increasing the responsiveness of the gonadotroph to itself. The mechanism of this priming effect is unknown, but it is blocked by protein kinase C inhibitors (122) without altering initial gonadotropin release. GnRH modulates LH secretion by the gonadotrophs by controlling LH-β mRNA levels. Levels of LH-α mRNA are less tightly controlled, explaining the fact that α-subunit levels can be paradoxically high in patients treated with GnRH agonists.

The gonadal peptides that have been characterized, inhibin, activin, and follistatin, have also been shown to affect FSH secretion by regulating mRNA for FSH-β (123). Recent data suggest that, in addition to inhibin, another nonsteroidal factor that is found in bovine follicular fluid can suppress pituitary FSH secretion (124). Insulin is another hormonal peptide that modulates gonadotropin release; in an *in vitro* experiment using rat anterior pituitary cells, the addition of insulin to the serum-free medium resulted in a significant increase in both basal and maximal (GnRH-stimulated) release of LH and FSH (125).

The paracrine and autocrine interactions within the anterior pituitary have been reviewed (126). The gonadotrophs are involved in two paracrine systems: angiotensin II, whose physiologic role as a paracrine factor is not clear, and activin. Follistatin, originally discovered in ovarian follicular fluid, is secreted in the anterior pituitary. It decreases FSH secretion and gene expression and FSH response to GnRH. In addition, follistatin serves as an activin-binding protein, which is an alternative way of modulating FSH expression. Other factors capable of a paracrine effect on the gonadotrophs include interleukin-6 and endothelin, which increase LH and FSH secretion, and galanine, which is produced mainly by the lactotrophs and stimulates LH secretion.

Molecular Biology of Disorders of the Gonadotrophs

Molecular biology techniques can pinpoint the exact molecular expression of a disease state. Weiss et al. (127) described an infertile man whose low testosterone and elevated LH levels suggested primary hypogonadism. Because testosterone secretion in response to exogenous LH or hCG was normal, however, a defect in the β subunit was suspected. PCR amplification of a peripheral blood leukocyte–derived DNA identified a single base substitution in the LH-β gene. This mutation caused substitution of arginine for glutamine in amino acid 54 of the LH-β subunit (Fig. 10). The mutant LH molecule lost its ability to bind to the target organ receptor (Fig. 11). The glutamine in position 54 seems to be crucial because it is conserved in all β subunits of the glycoproteins (128). Although this substitution did not change its immunoreactivity, the mutant LH was rendered biologically inactive. This interesting example illustrates the inherent weakness in using immunoassays as a test to reflect bioactivity. The clinical significance of the described mutation is unclear, as is the question of measuring bioactivity of hormones in certain cases. Because most cases of male infertility remain unexplained, one may speculate that at least some of them are the result of similar mutations. Even so, it is not known how common are such mutations as a cause of

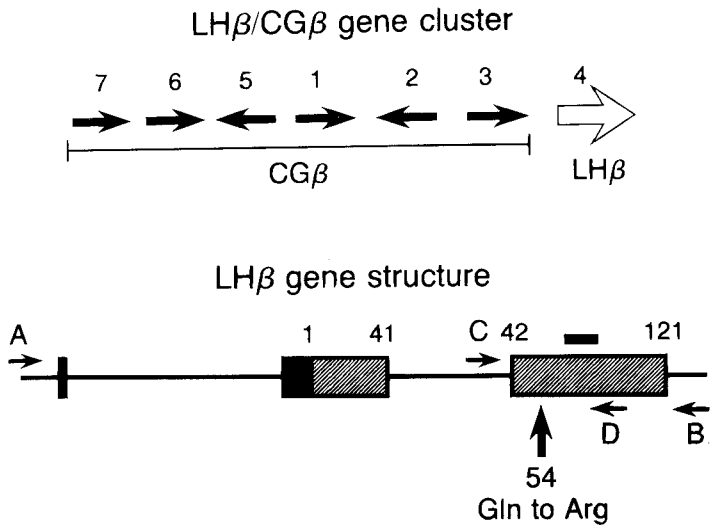

FIG. 10. Arrangement of the LH-β/chorionic gonadotropin β gene cluster on chromosome 19 and the structure of the LH-β gene and the mutation in codon 54 that eliminates LH ability to bind to its receptor. There are six chorionic gonadotropin β genes and pseudogenes *(dark arrows)* and a single LH-β gene *(open arrow)* in the gene cluster, which spans approximately 52 kb. The diagram of the LH-β gene structure also shows the strategy for PCR amplification. The three exons are indicated by *boxes;* the signal peptide sequence is *black,* and the mature LHβ peptide sequence is *hatched.* The positions of the boundaries of the LH-β coding sequence are shown above the exons. The sites of action of the PCR primers are indicated by *arrows* (*A, B, C,* and *D*). The location of the mutation at amino acid 54 (substitution of arginine for glutamine) is indicated. The location of an oligonucleotide probe that distinguishes LH-β and chorionic gonadotropin β sequences is denoted by a *bar* above exon 3. (From ref. 127.)

hypergonadotrophic hypogonadism that can be corrected simply by hCG stimulation.

In contrast, Pettersson et al (129) described a 31-year-old healthy woman, a mother of two children, whose serum LH was undetectable when measured by monoclonal-based immunometric assays. Given her regular ovulatory 28-day menstrual cycle and previous pregnancies, clearly she had a normal serum LH bioactivity. Interestingly, her family analyses suggested an autosomal dominant inheritance of a variant form of LH with normal bioactivity but minimal immunologic activity with the antibodies that were tested. Again, divergent bioactivity and immunoactivity should be suspected in the face of clinically inappropriate levels of gonadotropins.

Kallmann's syndrome is probably caused by an embryonal migrational defect of neurons in the CNS. The chromosomal walking technique was used to detect a deleted gene in patients with Kallmann's syndrome (130). This gene is responsible for synthesis of proteins involved in neural cell adhesion and cell growth, giving a genetic explanation for the unique pathophysiology of the syndrome.

GnRH gene structure in families with pubertal disorders (familial central precocious puberty and idiopathic hypogonadotropic hypogonadism) was studied, and structural abnormalities were found by means of DNA sequencing of PCR products. The nucleotide sequence coding for the GnRH prohormone was found to code for serine instead of tryptophan in the −8 position (Fig. 12). This polymorphism

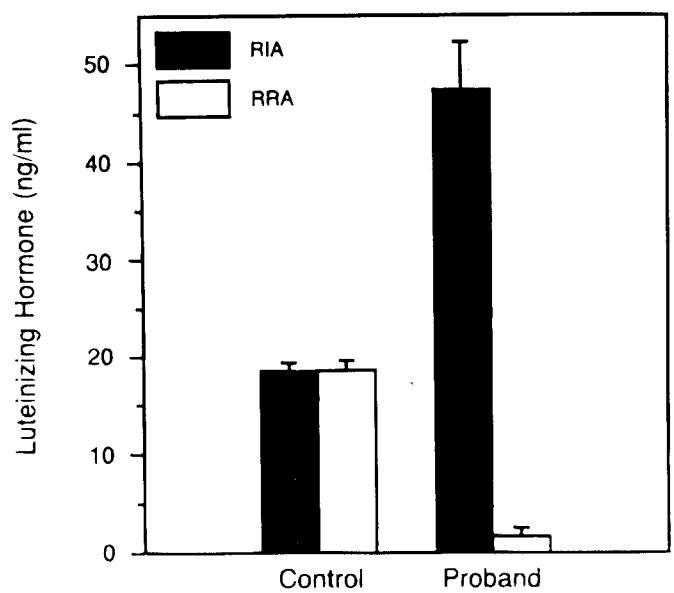

FIG. 11. Receptor-binding activity of the mutant LH expressed in Chinese hamster ovary cells. Mutant LH is undectectable in the radioreceptor assay (RRA); by radioimmunoassay (RIA) the level is elevated compared with control. Chinese hamster ovary cells were transfected with LH expression vectors containing the LH-α and LH-β genes. LH activity in media from stable cell lines expressing either the normal LH-β (control) or the mutant LH-β (proband) gene was measured by RIA and RRA. Values are means ± standard deviations. (From ref. 127.)

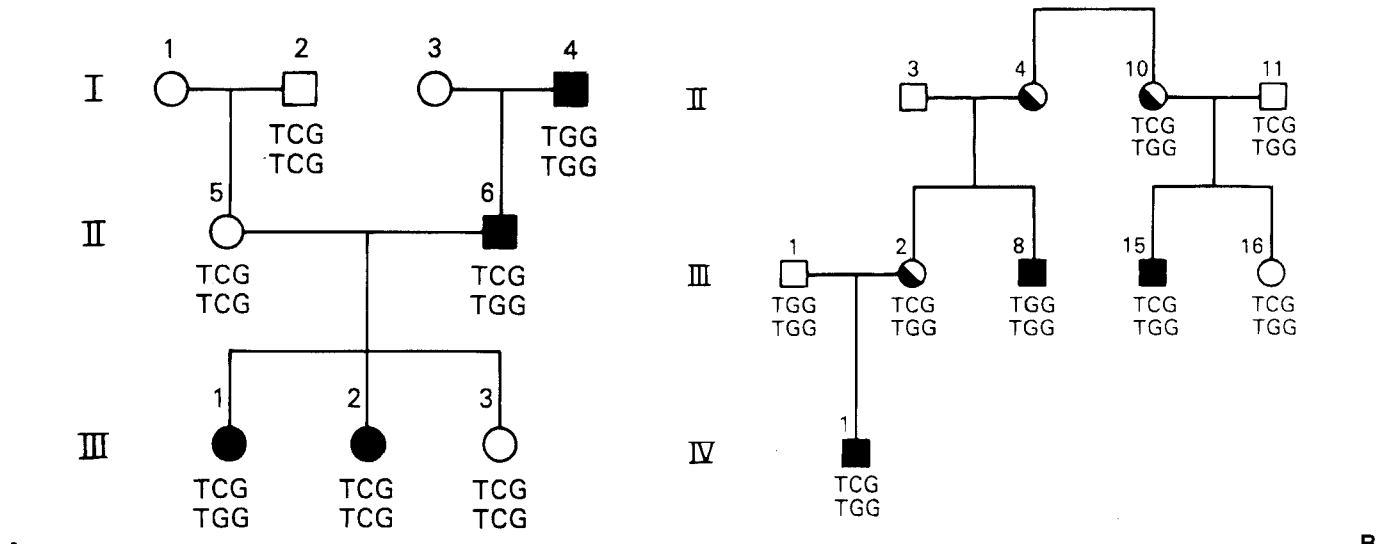

FIG. 12. Polymorphism in the nucleotide sequence in a family with familial central precocious puberty **(A)** and a family with idiopathic hypogonadotropic hypogonadism **(B).** The nucleotide sequence at the site of polymorphism is shown beneath the symbol for each family member. Family members were considered homozygous at this site if the same sequence was observed in at least five separate PCR products. (From ref. 131.)

in itself does not explain the pubertal disorders because the GnRH molecule was intact, but it indicates that GnRH gene abnormalities in the signal sequence may be factors in the etiology of pubertal disorders (131).

PCR and allele-specific oligonucleotides were used to analyze 86 pituitary tumors (of which 1 was LH-FSH secreting and 33 were nonfunctioning) for the presence of mutations (132). Mutations in the α subunit of the G protein stimulating adenylate cyclase could be identified in some of these tumors, suggesting a role for these mutations in the pathogenesis of these tumors. Similarly, DNA from aggressive hypersecretory pituitary adenomas was tested and found to have allele loss on different chromosomes (133), suggesting that loss of heterozygosity at multiple alleles may serve as a marker for aggressive pituitary tumors.

CONCLUSION

It is expected that molecular biology techniques will have significant ramifications in future routine clinical reproductive endocrinology practice. For example, in the investigation of infertility, and especially male infertility, the exact diagnosis at the molecular level will be reached, enabling us to abandon empiric treatments in favor of a more specific approach for each indication.

MAJOR TAKE HOME POINTS

Altered gonadotropin secretion, presented as either a monohormonal or multihormonal defect, usually can be traced to anatomic and functional derangement in a given region of the hypothalamic–pituitary axis. Excessive secretion of gonadotropins and their subunits may occur as a result of gonadotroph cell adenoma. This tumor appears to represent a significant percentage of all pituitary adenomas. Because of the morphologic and biologic similarities between null cell (nonsecreting) adenomas and gonadotroph cell adenoma, it is possible that null cell adenoma is actually a variant of gonadotroph adenoma.

Primary (pituitary) gonadotropin deficiency is most commonly a result of pituitary infarction and is usually associated with other pituitary hormone deficiencies. Secondary (hypothalamic) gonadotropin deficiency also coincides most of the time with other hormonal derangements. An exception is isolated gonadotropin deficiency (Kallmann's syndrome). Its pathogenesis, failure of GnRH neurons to migrate into the hypothalamus, has recently been defined.

Recent molecular biology techniques enable us to expand our knowledge and understanding of the molecular and genetic basis for several endocrine disorders affecting the normal function of the gonadotrophs. Two such newly described clinical entities are male hypogonadism caused by LHβ gene mutation and an anomalous LH variant in a healthy woman lacking LH immunoreactivity but with normal LH bioactivity.

EYE TO THE FUTURE

With respect to primary gonadotroph disorders, future developments will delineate their specific molecular pathogenesis. This trend applies both to hypersecretion states (gonadotroph cell adenoma) and to deficiency disorders. Specifically, somatic cell mutations giving rise to adenomas will be elucidated. Diverse mutations rendering the gonadotropins inactive will be defined with specific reference to idiopathic infertility, where normal serum LH may reflect a biologically deficient hormone.

Medical treatment of hypogonadotropic and hypergonadotropic states will make use of recombinant LH and FSH, probably with attenuated half-lives for maximal therapeutic flexibility. Ongoing efforts will yield side effect–free and potent GnRH antagonists that, among other indications, will be used for treatment of gonadotroph cell adenomas.

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REFERENCES

1. Woolf PD, Schenk EA. An FSH-producing pituitary tumor in a patient with hypogonadism. *J Clin Endocrinol Metab* 1974;38:561–568.
2. Snyder PJ, Sterling FH. Hypersecretion of LH and FSH by a pituitary adenoma. *J Clin Endocrinol Metab* 1976;42:544–550.
3. Snyder PJ. Gonadotroph cell adenomas of the pituitary. *Endocr Rev* 1985;6:552–563.
4. Bower BF. Pituitary enlargement secondary to untreated primary hypogonadism. *Ann Intern Med* 1968;69:107–109.
5. Snyder PJ, Bashey HM, Phillips JL, Gennarelli TA. Comparison of hormonal secretory behavior of gonadotroph cell adenomas *in vivo* and in cultures. *J Clin Endocrinol Metab* 1985;61:1061–1065.
6. Herman V, Fagin J, Gonsky R, Kovacs K, Melmed S. Clonal origin of pituitary adenomas. *J Clin Endocrinol Metab* 1990;71:1427–1433.
7. Biller BMK, Alexander JM, Zervas NT, Hedley-Whyte ET, Arnold A, Klibanski A. Clonal origins of adrenocorticotropin-secreting pituitary tissue in Cushing's disease. *J Clin Endocrinol Metab* 1992;75:1303–1309.
8. Davis JRE, Hoggard N. Towards the pathogenesis of human pituitary tumours. *J Endocrinol* 1993;136:3–6.
9. Nicolis G, Shimshi M, Allen C, Halmi NS, Kourides IA. Gonadotropin producing pituitary adenoma in a man with long standing primary hypogonadism. *J Clin Endocrinol Metab* 1988;66:237–241.
10. Kovacs K, Horvath E, Rewcastle NB, Ezrin C. Gonadotroph cell adenoma of the pituitary in a woman with long standing hypogonadism. *Arch Gynecol* 1980;229:57–65.
11. Heseltine D, White MC, Kendall-Taylor P, De Kretser DM, Kelly W. Testicular enlargement and elevated serum inhibin concentrations occur in patients with pituitary macroadenomas secreting follicle stimulating hormone. *Clin Endocrinol (Oxf)* 1989;31:411–423.
12. Trouillas J, Girod C, Sassolas G, Claustrat B, Lheritier M, Dubois MP, Goutelle A. Human pituitary gonadotropic adenoma: histological, immunocytolochemical, and ultrastructural and hormonal studies in eight cases. *J Pathol* 1981;135:315–336.
13. Horvath E, Kovacs K. Gonadotroph adenoma of the human pituitary: sex-related fine-structural dichotomy. *Am J Pathol* 1984;117:429–440.
14. Kontogeorgos G, Horvath E, Kovacs K. Sex linked ultrasturcture dichotomy of gonadotroph adenomas of the human pituitary: an electron microscopic analysis of 145 tumors. *Ultrastruct Pathol* 1990;14:475–482.
15. Daneshdoost LD, Gennarelli TA, Bashey HM, Savino PJ, Sergott RC, Bosley TM, Snyder PJ. Recognition of gonadotroph adenomas in women. *N Engl J Med* 1991;324:589–594.
16. Kwekkeboom DJ, de Jong FH, Lamberts SWJ. Gonadotropin release by clinically nonfunctioning and gonadotroph pituitary adenomas *in vivo* and *in vitro:* relation to sex and effects of thyrotropin-releasing hormone, gonadotropin-releasing hormone, and bromocriptine. *J Clin Endocrinol Metab* 1989;68:1128–1135.
17. Asa SL, Cheng Z, Ramyar L, Singer W, Kovacs K, Smyth H, Muller P. Human pituitary null cell adenomas and oncocytomas *in vitro:* effects of adenohypophysiotropic hormones and gonadal steroids on hormone secretion and tumor cell morphology. *J Clin Endocrinol Metab* 1992;74:1128–1134.
18. Jameson HL, Klibanski A, Black PM, Zervast NT, Lindell CM, Hsu DW, Ridgway EC, Habener JF. Glycoprotein hormone genes are expressed in clinically nonfunctioning pituitary adenomas. *J Clin Invest* 1987;80:1472–1478.
19. Lloyd RV, Jin L, Fields K, Chandler WF, Horvath E, Stefaneanu L, Kovacs K. Analysis of pituitary hormones and chromogranin A mRNA in null cell adenomas, oncocytomas, and gonadotroph adenomas by in situ hybridization. *Am J Pathol* 1991;139:553–564.
20. Chappel SC, Bashey HM, Snyder PJ. Similar isoelectric profiles of FSH from gonadotroph cell adenomas and non-adenomatous pituitaries. *Act Endocrinol (Copenh)* 1986;113:311–316.
21. Galway AB, Hsueh AJW, Daneshdoost L, Zhou MH, Pavlou SN, Snyder PJ. Gonadotroph adenomas in men produce biologically active follicle stimulating hormone. *J Clin Endocrinol Metab* 1990;71:907–912.
22. Yamada S, Horvath E, Kovacs K, Aiba T, Shimizu T, Shishiba Y, Hara M. Gonadotroph adenoma of the pituitary mimicking a prolactinoma. *Neurosurgery* 1991;28:244–249.
23. Monroe SE, Levine L, Change RJ, Keye WR Jr, Yamamoto M, Jaffe RB. Prolactin secreting pituitary adenomas. V. Increased gonadotroph responsivity in hyperprolactinemic women with pituitary adenomas. *J Clin Endocrinol Metab* 1981;52:1171–1178.
24. Vance ML, Ridgway EC, Thorner MO. Follicle stimulating hormone and alpha subunit secreting pituitary tumor treated with bromocriptine. *J Clin Endocrinol Metab* 1985;61:580–584.
25. Kwekkeboom DJ, Lamberts SW. Long term treatment with the dopamine agonist CV 205-502 of patients with a clinically non functioning, gonadotroph, or alpha subunit secreting pituitary adenoma. *Clin Endocrinol (Oxf)* 1992;36:171–176.
26. Klibanski A, Deutsch PJ, Jameson JL, Ridgway EC, Crowley WF, Hsu DW, Habener JF, Black PM. Luteinizing hormone secreting pituitary tumor: biosynthetic characterization and clinical studies. *J Clin Endocrinol Metab* 1987;64:536–542.
27. Comtois R, Bouchard J, Robert F. Hypersecretion of gonadotropins by pituitary adenoma: pituitary dynamic studies and treatment with bromocriptine in one patient. *Fertil Steril* 1989;52:569–573.
28. Abs R, Parizel PM, Beckers A. Acute effects of Parlodel-LAR and response to long-term treatment with bromocriptine in a patient with a folicle stimulating hormone–secreting pituitary adenoma. *J Endocrinol Invest* 1991;14:135–138.
29. Vos P, Croughs RJM, Thijssen JH, van't Verlaat JW, van Ginkel LA. Response of luteinizing hormone secreting pituitary adenoma to a long acting somatostatin analogue. *Acta Endocrinol (Copenh)* 1988;118:587–590.
30. Sy RAG, Bernstein R, Chynn KY, Kourides IA. Reduction in size of thyrotopin and gonadotropin secreting pituitary adenoma treated with octreotide acetate (somatostatin analog). *J Clin Endocrinol Metab* 1992;74:690–694.
31. De Bruin TWA, Kwekkeboom DJ, Verlaat JWV, Reubi JC, Krenning EP, Lamberts SWJ, Croughs RJM. Clinically nonfunctioning pituitary adenoma and octreotide response to long term high dose treatment, and studies *in vitro*. *J Clin Endocrinol Metab* 1992;75:1310–1317.
32. Katznelson L, Oppenheim DS, Coughlin JF, Kliman B, Schoenfeld DA, Klibanski A. Chronic somatostatin analog administration in patients with α-subunit secreting pituitary tumors. *J Clin Endocrinol Metab* 1992;75:1318–1325.
33. Zarate A, Fonseca ME, Mason M, Tapia R, Miranda R, Kovacs K, Schally AV. Gonadotropin secreting pituitary adenoma with concomitant hypersecretion of testosterone and elevated sperm count: treatment with LRH agonist. *Acta Endocrinol (Copenh)* 1986;113:29–34.
34. Sassolas G, Lejeune H, Trouillas J, Forest MG, Claustrat B, Lahlou N,

Loras B. Gonadotropin releasing hormone agonists are unsuccessful in reducing tumoral gonadotropin secretion in two patients with gonadotropin secreting pituitary adenomas. *J Clin Endocrinol Metab* 1988; 67:180–185.
35. Klibanski A, Jameson JL, Biller BMK, Crowley WF Jr, Zervas NT, Rivier J, Vale WW, Bikkal H. Gonadotropin and α-subunit responses to chronic gonadotropin releasing hormone analog administration in patients with glycoprotein hormone secreting pituitary tumors. *J Clin Endocrinol Metab* 1989;68:81–86.
36. Daneshdoost L, Pavlou SN, Molitch ME, Gennarrelli TA, Savino PJ, Sergott RC, Bosley TM, Rivier JE, Vale WW, Snyder PJ. Inhibition of follicle stimulating hormone secretion from gonadotroph adenomas by repetitive administration of a gonadotropin releasing hormone antagonist. *J Clin Endocrinol Metab* 1990;71:92–97.
37. Rabinovici J, Rothman P, Monroe SE, Nerenberg C, Jaffe RB. Endocrine effects and pharmacokinetic characteristics of a potent new gonadotropin releasing hormone antagonist (ganirelix) with minimal histamine releasing properties: studies in postmenopausal women. *J Clin Endocrinol Metab* 1992;75:1220–1225.
38. Ebersold MJ, Quast LM, Laws ER, Scheithauer B, Randall RV. Long term results in transsphenoidal removal of nonfunctioning pituitary adenomas. *J Neurosurg* 1986;64:713–719.
39. Sheehan HL, Murdoch R. Postpartum necrosis of the anterior pituitary: pathological and clinical aspects. *J Obstet Gynecol Br Emp* 1938; 45:456–488.
40. Pastore RA, Andersson JW, Herman RH. Anterior and posterior hypopituitarism associated with sickle cell trait. *Ann Intern Med* 1969;71: 593–598.
41. Sheehan HL, Stanfield JP. The pathogenesis of postpartum necrosis of the anterior lobe of the pituitary gland. *Acta Endocrinol* 1961;37: 479–510.
42. Imura H. Hypopituitarism. In: Imura H, ed. *The pituitary gland.* New York: Raven; 1985:501–525.
43. Aono T, Minagawa J, Kinugasa T, Tanizawa O, Kurachi K. Response of pituitary LH and FSH to synthetic LH releasing hormone in normal subjects and patients with Sheehan's syndrome. *Am J Obstet Gynecol* 1973;117:1046–1052.
44. Veldhuis JH, Hammond JM. Endocrine function after spontaneous infarction of the human pituitary: report, review, and reappraisal. *Endocr Rev* 1980;1:100–107.
45. Grimes HG, Brooks MH. Pregnancy in Sheehan's syndrome: report of a case and review. *Obstet Gynecol Rev* 1980;35:481–488.
46. Schalch DS, Burday SZ. Antepartum pituitary insufficiency in diabetes mellitus. *Ann Intern Med* 1971;74:357–360.
47. Dorfman SG, Dillaplain RP, Gambrell RD. Antepartum pituitary infarction. *Obstet Gynecol* 1979;53:21s–24s.
48. Wakai S, Fukushima T, Teramoto A, Sano K. Pituitary apoplexy: its incidence and clinical significance. *J Neurosurg* 1981;55:187–193.
49. Reid RL, Quigley ME, Yen SSC. Pituitary apoplexy associated with diabetes insipidus: case report and review of the literature. *Arch Neurol* 1985;42:712–719.
50. Arafah BM, Taylor HC, Salazar R, Saadi H, Selman WR. Apoplexy of a pituitary adenoma after dynamic testing with gonadotropin releasing hormone. *Am J Med* 1989;87:103–105.
51. Post MJ, Davis NJ, Glauser JS, Safran A. Pituitary apoplexy: diagnosis by CT. *Radiology* 1980;134:665–670.
52. Neelon FA, Goree JA, Lebovitz HE. The primary empty sella: clinical and radiographic characteristics and endocrine function. *Medicine* 1973;52:73–92.
53. Weisberg LA, Zimmerman EA, Franz GA. Diagnosis and evaluation of patients with an enlarged sella turcica. *Am J Med* 1976;61:590–596.
54. Pestell RG, Best JD, Alford FP. Lymphocytic hypophysitis: the clinical spectrum of the disorder and evidence for an autoimmune pathogenesis. *Clin Endocrinol* 1990;33:457–466.
55. Feigenbaum SL, Martin MC, Wilson CB, Jaffe RB. Lymphocytic adenohypophysitis: a pituitary mass lesion occurring in association with pregnancy: proposal for medical treatment. *Am J Obstet Gynecol* 1991;164:1549–1555.
56. Woolf PD, Jacobs LS, Donofrio R, Burdy SZ, Schalch DS. Secondary hypopituitarism: evidence of continuing regulation of hormone release. *J Clin Endocrinol Metab* 1974;38:71–76.
57. Schlaff WD. Dynamic testing in reproductive endocrinology. *Fertil Steril* 1986;45:589–606.
58. DiZerega G, Kletzky OA, Mishell DR Jr. Diagnosis of Sheehan's syndrome using a sequential pituitary stimulation test. *Am J Obstet Gynecol* 1978;132:348–353.
59. Sheldon WR, deBold CR, Evans WS, DeCherney GS, Jackson RV, Island DP, Thorner MO, Orth DN. Rapid sequential intravenous administration of four hypothalamic-releasing hormones as a combined anterior pituitary function test in normal subjects. *J Clin Endocrinol Metab* 1985;60:623–630.
60. Cohen R, Bouquier D, Biot-Laporte S, et al. Pituitary stimulation by combined administration of four hypothalamic-releasing hormones in normal men and patients. *J Clin Endocrinol Metab* 1986;62:892–898.
61. Kallmann FJ, Schoenfeld WA, Barrera SE. The genetic aspects of primary eunuchoidism. *Am J Ment Defic* 1944;48:203–236.
62. Chaussain JL, Toublanc JE, Feingold J, Naud C, Vassal J, Job JC. Mode of inheritance in familial cases of primary gonadotropic deficiency. *Horm Res* 1988;29:202–206.
63. Schwanzel-Fukuda M, Bick D, Pfaff DW. Luteinizing hormone releasing hormone (LHRH) expressing cells do not migrate normally in an inherited hypogonadal syndrome. *Mol Brain Res* 1989;6:311–326.
64. Goldenberg RL, Powell RD, Rosen SW, Marshall JR, Ross GT. Ovarian morphology in women with anosmia and hypogonadotropic hypogonadism. *Am J Obstet Gynecol* 1976;126:91–94.
65. Spitz IM, Diamant Y, Rosen E, Bell J, Ben David M, Polishuk W, Rabinowitz D. Isolated gonadotropin deficiency: a heterogenous syndrome. *N Engl J Med* 1974;290:10–15.
66. Yeh J, Rebar RW, Liu JH, Yen SS. Pituitary function in isolated gonadotrophin deficiency. *Clin Endocrinol (Oxf)* 1989;31:375–387.
67. Pawlowitzki IH, Diekstall P, Miny P, Balleisen L. Abnormal platelet function in Kallmann syndrome. *Lancet* 1986;2:166.
68. Lieblich JM, Rogol AD, White BJ, Rosen SW. Syndrome of anosmia with hypogonadotropic hypogonadism (Kallmann syndrome). Clinical and laboratory studies in 23 cases. *Am J Med* 1982;73:506–519.
69. Schwankhaus JH, Currie J, Jaffe MJ, Ross SR, Sherins RJ. Neurologic findings in men with isolated hypogonadotropic hypogonadism. *Neurology* 1989;39:223–226.
70. Kertzman C, Robinson DL, Sherins RJ, Schwankhaus JD, McClurkin JW. Abnormalities in visual spatial attention in men with mirror movements associated with isolated hypogonadotropic hypogonadism. *Neurology* 1990;40:1057–1063.
71. Rabin D, Spitz I, Bercovici B, Bell J, Laufer A, Beveniste R, Polishuk W. Isolated deficiency of follicle stimulating hormones: clinical and laboratory features. *N Engl J Med* 1972;287:1313–1317.
72. Snyder PJ, Rudenstein RS, Gardner DF, Rothman JG. Repetitive infusion of gonadotropin releasing hormone distinguishes hypothalamic from pituitary hypogonadism. *J Clin Endocrinol Metab* 1979;48: 864–868.
73. Barkan AL, Reame NE, Kelch RP, Marshall JC. Idiopathic hypogonadotropic hypogonadism in men: dependence of the hormone responses to gonadotropin releasing hormone (GnRH) on the magnitude of endogenous GnRH secretory defect. *J Clin Endocrinol Metab* 1985; 61:1118–1125.
74. Winters SJ, Troen P. α-Subunit secretion in men with idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1988;66: 338–342.
75. Winters SJ, Troen P. Pituitary glycoprotein hormone α-subunit secretion after thyrotropin releasing hormone stimulation in normal men and men with idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1990;70:544–547.
76. Whitcomb RW, O'dea LL, Finkelstein JS, Heavern DM, Crowley WF. Utility of free α-subunit as an alternative neuroendocrine marker of gonadotropin releasing hormone (GnRH) stimulation of gonadotrophs in the human: evidence from normal and GnRH deficient men. *J Clin Endocrinol Metab* 1990;70:1654–1661.
77. Sheckter CB, McLachlan RI, Tenover JS, Matsumoto AM, Burger HG, deKretser DM, Bremner WJ. Stimulation of serum inhibin concentrations by hypogonadotropin releasing hormone in men with idiopathic hypogonadotropic hypogonadism. *J Clin Eneocrinol Metab* 1988;67:1221–1224.
78. Vourtsis K, Illig R. The use of a low dose LHRH test for the distinction of delayed adolescence and isolated gonadotropin deficiency. *Helv Pediatr Acta* 1982;37:437–447.
79. Spitz IM, Hirsch HJ, Trestian S. The prolactin response to thyrotropin releasing hormone differentiates isolated gonadotropin deficiency from delayed puberty. *N Engl J Med* 1983;308:575–579.

80. Dickerman Z, Cohen A, Laron Z. Growth in patients with isolated gonadotropin deficiency. *Arch Dis Child* 1992;67:513–516.
81. James CSJ, Pride SM, Yuen BH. Failure of ovulation induction with pulsatile gonadotropin releasing hormone and human menopausal gonadotropins in isolated gonadotropin deficiency. *Obstet Gynecol* 1990;76:921–924.
82. Ley SB, Leonard JM. Male hypogonadotropic hypogonadism: factors influencing response to human chorionic gonadotropin and human menopausal gonadotropin, including prior exposure to androgens. *J Clin Endocrinol Metab* 1985;61:746–752.
83. Burris AS, Rodbard HW, Winters SJ, Sherins RJ. Gonadotropin therapy in men with isolated hypogonadotropic hypogonadism: response to human chorionic gonadotropin is predicted by initial testicular size. *J Clin Endocrinol Metab* 1988;66:1144–1151.
84. Hoffman AR, Crowley WF Jr. Induction of puberty in men by long term pulsatile administration of low dose gonadotropin releasing hormone. *N Engl J Med* 1982;307:1237–1241.
85. Liu L, Banks SM, Barnes KM, Sherins RJ. Two year comparison of testicular response to pulsatile gonadotropin releasing hormone and exogenous gonadotropins from the inception of therapy in men with isolated hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1988;67:1140–1145.
86. Fok ACK, Wong, MC, Cheah JS. Syndrome of cerebellar ataxia and hypogonadotrophic hypogonadism: evidence for pituitary gonadotropin deficiency. *J Neurol Neurosurg Psychol* 1989;52:407–409.
87. Abs R, Van-Vleymen E, Parizel PM, Van-Acker K, Martin M, Martin JJ. Congenital cerebellar hypoplasia and hypogonadotropic hypogonadism. *J Neurol Sci* 1990;98:259–265.
88. Bovet P, Reymond MJ, Rey F, Gomez F. Lack of gonadotropic response to pulsatile gonadotropin releasing hormone in isolated hypogonadotropic hypogonadism associated to congenital adrenal hypoplasia. *J Endocrinol Invest* 1988;11:201–204.
89. Warren MP, Siris ES, Petrovich C. The influence of severe illness on gonadotropin secretion in the postmenopausal female. *J Clin Endocrinol Metab* 1977;45:99–104.
90. Fries H, Nillius SJ, Pettersson F. Epidemiology of secondary amenorrhea: II. A retrospective evaluation of etiology with special regard to psychogenic factors and weight loss. *Am J Obstet Gynecol* 1974;118:473–479.
91. Quigley ME, Sheehan KL, Casper RF, Yen SSC. Evidence for an increased dopaminergic and opioid activity in patients with hypothalamic hypogonadotropic amenorrhea. *J Clin Endocrinol Metab* 1980;50:949–954.
92. Yen SSC, Rebar RW, Quesenberry W. Pituitary function in pseudocyesis. *J Clin Endocrinol Metab* 1976;43:132–136.
93. Devane GW, Vera MI, Buhi WC, Kalra PS. Opioid peptides in pseudocyesis. *Obstet Gynecol* 1985;65:183–188.
94. Mecklenburg RS, Loriaux DL, Thompson RH, Andersen AE, Lipsett MB. Hypothalamic dysfunction in patients with anorexia. *Medicine* 1974;53:147–159.
95. Schweiger U, Pirke KM, Laessle RG, Fichter MM. Gonadotropin secretion in bulimia nervosa. *J Clin Endocrinol Metab* 1992;74:1122–1127.
96. Veldhuis JD, Evans WS, Demers LM, Thorner MO, Wakat D, Rogol AD. Altered neuroendocrine regulation of gonadotropin secretion in women distance runners. *J Clin Endocrinol Metab* 1985;61:557–563.
97. Kapen S, Sternthal E, Braverman L. Case report: A "pubertal" 24-hour luteinizing hormone (LH) secretory pattern following weight loss in the absence of anorexia nervosa. *Psychosom Med* 1981;43:177–182.
98. Smith LH Jr. Overview of hemochromatosis. *West J Med* 1990;153:296–308.
99. Veldhuis JD, Urban RJ, Lizarralde G, Johnson ML, Iranmanesh A. Attenuation of luteinizing hormone secretory burst amplitude as a proximate basis for the hypoandrogenism of healthy aging in men. *J Clin Endocrinol Metab* 1992;75:707–713.
100. Kaufman JM, Giri M, Deslypere JM, Thomas G, Vermeulen A. Influence of age on the responsiveness of the gonadotrophs to luteinizing hormone releasing hormone in males. *J Clin Endocrinol Metab* 1991;72:1255–1260.
101. Kwekkeboom DJ, De Jong FH, Van Hemert AM, Vandenbroucke JP, Valkenburg HA, Lamberts SWJ. Serum gonadotropins and α-subunit decline in aging normal postmenopausal women. *J Clin Endocrinol Metab* 1990;70:944–950.
102. Morell AG, Gregoriadis G, Scheinberg IH, Hickman J, Ashwell G. The role of sialic acid in determining the survival of glycoproteins in the circulation. *J Biol Chem* 1971;246:1461–1467.
103. Miller C, Ulloa-Aquirre A, Hyland L, Chappel S. Pituitary follicle stimulating hormone heterogeneity: assessment of biologic activities of each follicle stimulating hormone form. *Fertil Steril* 1983;40:242–247.
104. Godine JE, Chin WW, Habener JF. Alpha subunit of rat pituitary glycoprotein hormones. Primary structure of precursor determined from the nucleotide sequence of cloned cDNA. *J Biol Chem* 1982;257:8368–8371.
105. Talmadge K, Boorstein WR, Fiddes JC. The human genome contains seven genes for the beta subunit of chorionic gonadotropin but only one gene for the beta subunit of luteinizing hormone. *DNA* 1983;2:281–289.
106. Jameson JL, Lindell CM, Habener JF. Evolution of different transcriptional start sites in the human luteinizing hormone and chorionic gonadotropin beta subunit genes. *DNA* 1986;5:227–234.
107. Gharib SD, Wierman ME, Shupnik MA, Chin WW. Molecular biology of the pituitary gonadotropins. *Endocr Rev* 1990;11:177–199.
108. Gordon DF, Wood WM, Ridgway EC. Organization and nucleotide sequence of the mouse alpha subunit gene of the pituitary glycoprotein hormones. *DNA* 1988;7:679–690.
109. Tsutsumi M, Zhou W, Millar RP, Mellon PL, Roberts JL, Flanagan CA, Dong K, Gillo B, Sealfon SC. Cloning and functional expression of a mouse gonadotropin releasing hormone receptor. *Mol Endocrinol* 1992;6:1163–1169.
110. Anderson L, Milligan G, Eidne KA. Characterization of the gonadotropin releasing hormone receptor in αT3-1 pituitary gonadotroph cells. *J Endocrinol* 1993;136:51–58.
111. Schoderbek WE, Kim KE, Ridgway EC, Mellon PL, Maurer RA. Analysis of DNA sequences required for pituitary specific expression of the glycoprotein hormone α-subunit gene. *Mol Endocrinol* 1992;6:893–903.
112. Gharib SD, Wierman ME, Badger TM, Chin WW. Sex steroid hormone regulation of follicle stimulating hormone subunit messenger ribonucleic acid (mRNA) levels in the rat. *J Clin Invest* 1987;80:294–299.
113. Shupnik MA, Gharib SD, Chin WW. Divergent effects of estradiol on gonadotropin gene transcription in pituitary fragments. *Mol Endocrinol* 1989;3:474–480.
114. Wray S, Zoeller RT, Gainer H. Differential effects of estrogen on luteinizing hormone releasing hormone gene expression in slice explant cultures prepared from specific rat forebrain region. *Mol Endocrinol* 1989;3:1197–1206.
115. Clayton RN, Catt KJ. Gonadotropin releasing hormone receptors: characterization, physiological regulation and relationship to reproductive function. *Endocr Rev* 1981;2:186–209.
116. Wise PM, Camp-Grossman P, Barraclough CA. Effects of estradiol and progesterone on plasma gonadotropins, prolactin, and LHRH in specific brain areas of ovariectomized rats. *Biol Reprod* 1981;24:820–830.
117. Stanley HF, Lyons V, Obonsawin MC, Bennie J, Carroll S, Roberts JL, Fink G. Regulation of pituitary α-subunit β-LH, and PRL mRNA by GnRH and estradiol in hypogonadal and normal mice. *Mol Endocrinol* 1988;2:1302–1310.
118. Veldhuis JD, Evans WS, Rogol AD, Kolp L, Thorner MO, Stumpf P. Pituitary self-priming actions of gonadotropin releasing hormone: kinetics of estradiol's potentiating effects on gonadotropin releasing hormone facilitated luteinizing hormone and follicle stimulatory hormone release in healthy postmenopausal women. *J Clin Invest* 1986;77:1849–1856.
119. Soules MR, Steiner RA, Clifton DK, Cohen NL, Aksel S, Bremner WJ. Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. *J Clin Endocrinol Metab* 1984;58:378–383.
120. Couzinet B, Brailly S, Bouchaard P, Schaison G. Progesterone stimulates luteinizing hormone secretion by acting directly on the pituitary. *J Clin Endocrinol Metab* 1992;74:374–378.
121. Dalkin AC, Paul SJ, Haisenleder DJ, Ortolano GA, Yasin M, Marshall JC. Gonadal steroids effect similar regulation of gonadotropin subunit mRNA expression in both male and female rats. *J Endocrinol* 1992;132:39–45.
122. Johnson MS, Mitchell R, Thomson FJ. The priming effect of luteinizing hormone releasing hormone (LHRH) but not LHRH-induced go-

nadotropin release, can be prevented by certain protein kinase C inhibitors. *Mol Cell Endocrinol* 1992;85:183–193.
23. Attardi B, Keeping HS, Winters SJ, Kotsuji F, Maurer RA, Troen P. Rapid and profound suppression of messenger ribonucleic acid encoding follicle stimulating hormone beta by inhibin from primate Sertoli cells. *Mol Endocrinol* 1989;3:280–287.
24. Wood SC, Glencross RG, Bleach ECL, Lovell R, Beard AJ, Knight PG. The ability of steroid free bovine follicular fluid to suppress FSH secretion and delay ovulation persists in heifers actively immunized against inhibin. *J Endocrinol* 1993;136:137–148.
25. Adashi EY, Hsueh AJW, Yen SSC. Insulin enhancement of luteinizing hormone and follicle stimulating hormone release by cultured pituitary cells. *Endocrinology* 1981;108:1441–1449.
26. Schwartz J, Cherny R. Intercellular communication within the anterior pituitary influencing the secretion of hypophyseal hormones. *Endocr Rev* 1992;13:453–470.
27. Weiss J, Axelrod L, Whitcomb RW, Harris PE, Crowley WF, Jameson JL. Hypogonadism caused by a single amino acid substitution in the β subunit of luteinizing hormone. *N Engl J Med* 1992;326:179–183.
128. Pierce JG, Parsons TF. Glycoprotein hormones: structure and function. *Annu Rev Biochem* 1981;50:465–495.
129. Pettersson K, Ding YQ, Huhtaniemi I. An immunologically anomalous luteinizing hormone variant in a healthy woman. *J Clin Endocrinol Metab* 1992;74:164–171.
130. Franco B, Guioli S, Pragliola A, Incerti B. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesions and axonal path finding molecules. *Nature (London)* 1991;353:529–536.
131. Nakayama Y, Wondisford FE, Lash RW, Bale AE, Weintraub BD, Cutler GB Jr, Radovick S. Analysis of gonadotropin releasing hormone gene structure in families with familial central precocious puberty and idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1990;70:1233–1238.
132. Pistorello M, Boggild MD, Boscaro M, Scanarini M, Perrett CW, Clayton RN. *Gsp* oncogene mutations in a group of 86 pituitary tumours. *J Endocrinol* 1992;135(Suppl):P42.
133. Boggild MD, Pistorello M, Perrett CW, Clayton RN. Multiple allelic deletions—a marker of aggressive pituitary adenomas? *J Endocrinol* 1992;135(Suppl):P41.