Yen & Jaffe's

REPRODUCTIVE ENDOCRINOLOGY

PHYSIOLOGY, PATHOPHYSIOLOGY, AND CLINICAL MANAGEMENT

Eighth Edition

STRAUSS ▪ BARBIERI

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EIGHTH EDITION

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Preface

Reproduction is a basic biologic process permitting the existence of living organisms. Sexual reproduction has catalyzed the diversity of eukaryotic life. Sexual reproduction increases genetic diversity through two mechanisms: homologous recombination and the combination of the haploid genetic material of two distinct parents to produce diploid offspring. By greatly increasing genetic diversity, sexual reproduction results in diverse phenotypes in offspring and accelerates the evolution of all higher organisms, including humans. Recognizing the potential for growth in both the science of reproduction and its application to enhancing human health, Drs. Samuel S.C. Yen and Robert B. Jaffe completed the first edition of this book in 1977, one year before the first successful live birth using in vitro fertilization.

In the eighth edition of *Yen and Jaffe's Reproductive Endocrinology* our distinguished contributors present an authoritative distillation of the key advances in the field. Progress in endocrinology, gamete and embryo biology, and genetics has permitted the development and expansion of new human reproductive treatments, including aromatase inhibitors for ovulation induction, selective progesterone receptor modulators for the treatment of uterine leiomyomata, new GnRH receptor antagonists for the treatment of endometriosis, new selective estrogen receptor modulators for the treatment of menopausal symptoms, cryopreservation of oocytes to enhance fertility preservation, and genomic interrogation of embryos to improve reproductive outcomes. Evolving medical and societal concepts of sex and gender have prompted the editors to include a new chapter on transgender hormonal treatment.

Advances in the surgical treatment of reproductive disorders are accelerating. This rapid progress is best demonstrated by the successful development of human uterus transplantation to treat uterine factor infertility and of novel minimally invasive surgical techniques that improve reproductive performance. In this edition we have added a new video section, which is dedicated to current surgical and technological aspects of our field and edited by an internationally recognized expert in reproductive surgery, Dr. Antonio R. Gargiulo. This section uses the video format to provide a more direct access for the reader to surgical and laboratory procedures and techniques that cover fundamental topics in modern reproductive medicine, including the diagnosis and treatment of müllerian anomalies, endometriosis, uterine leiomyomata, other uterine pathologies, and ovarian tissue cryopreservation and transplantation. An embryologist's point-of-view on current techniques of assisted human reproduction is also featured.

We appreciate the collaboration of past and new authors for their scholarly and practical updates in their respective areas of specialty expertise. The contributions of the authors have always been the key to the success of *Yen and Jaffe's Reproductive Endocrinology*. The editors are deeply grateful to our authors. Our team of editors and authors hope to carry forward the tradition of excellence that Drs. Jaffe and Yen started when they completed the first edition of this text.

Video Contents

PART 1

ENDOCRINOLOGY OF REPRODUCTION

CHAPTER 1

Neuroendocrinology of Reproduction

Christopher R. McCartney
John C. Marshall

Central Control of Reproduction

Successful reproduction is essential to the survival of a species. The reproductive system represents a highly complex functional organization of diverse tissues and signaling pathways that, when properly functioning, ensures a number of key endpoints, the most important of which are the adequate production of gametes (ova and sperm); successful delivery of gametes for fertilization; and, in women, physiologic preparation for possible pregnancy. Neuroendocrine systems are the principal drivers of reproductive function in both men and women. In particular, hypothalamic gonadotropin-releasing hormone (GnRH) is the primary, if not exclusive, feedforward signal to gonadotrope cells of the anterior pituitary, stimulating the synthesis and secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Together, these two gonadotropins direct the primary functions of the reproductive axis: gametogenesis and gonadal sex steroid synthesis.

Given its critical importance to a species, the reproductive system must be robust, continuing to function properly in the face of various physiologic perturbations. In contrast, in settings of marked physiologic stress (e.g., significantly reduced energy availability), mechanisms that temporarily limit fertility—the usual outcome of which is metabolically expensive in women—are biologically advantageous for the individual and, ultimately, the species. Appropriate function (or quiescence) of the reproductive system is governed by a number of intricate relationships. For example, feedback signals from the gonads (e.g., sex steroid concentrations) communicate the status of gonadal function to the hypothalamic-pituitary axis; these signals in turn influence GnRH and gonadotropin output, rendering a coordinated and tightly regulated feedback system that maintains gonadal function within narrow limits. The reproductive system also has extensive interactions with other neuroendocrine systems, such as those involved with energy balance and adaptations to stress. The reproductive neuroendocrine system integrates these myriad feedback signals, and the GnRH-secreting neuronal network represents the final common pathway for the central control of reproduction. Thus the regulation of GnRH secretion represents a major focus of reproductive neuroendocrinology.

Much of our understanding of reproductive neuroendocrinology has emerged from the study of rodents, sheep, and nonhuman primates, which largely reflects the ethical boundaries inherent to human research. Because many neurobiologic principles are similar among all mammals, these animal studies have been (and continue to be) indispensable. Nonetheless, certain aspects of reproductive neuroendocrinology may differ markedly among species. Thus, when available, human data will be prioritized throughout this chapter, but animal studies will also be discussed when appropriate, recognizing that specific findings may or may not be generalizable to humans. The reader is referred to Chapters 3, 7, 12, 17, and 20 for additional treatment of neuroendocrine physiology and pathophysiology related to reproduction.

Neuroendocrinology: The Interface Between Neurobiology and Endocrinology

Endocrinology is the study of cell-to-cell signaling that occurs via specific chemicals (hormones) that travel through the bloodstream to influence remote targets. The term "neuroendocrinology" refers to the involvement of the central nervous system (CNS)—the hypothalamus in particular—in this process. This field of study has traditionally focused on hypothalamic neuron-derived factors that influence various target tissues, either directly, as with the hormones of the neurohypophysis, or indirectly, as with hypothalamic releasing factors that control anterior pituitary hormone secretion. Neuroendocrine systems direct a wide variety of critical biologic

Abstract

Pulsatile gonadotropin-releasing hormone (GnRH) release from the hypothalamus governs pituitary secretion of luteinizing hormone and follicle-stimulating hormone, which in turn regulate the production of gonadal sex steroids and gametes (ova and sperm). GnRH secretion is markedly influenced by a complex array of higher-level afferent inputs, such as neurons releasing kisspeptin, neurokinin B, and dynorphin. Feedback signals communicate the status of gonadal function and other facets of whole-body homeostasis to the neural systems regulating GnRH release and to pituitary gonadotropes, rendering a coordinated and tightly regulated feedback system that maintains appropriate gonadal function.

Keywords

Gonadotropin-releasing hormone,
luteinizing hormone,
follicle-stimulating hormone,
kisspeptin,
neurokinin B,
dynorphin,
KNDy neurons

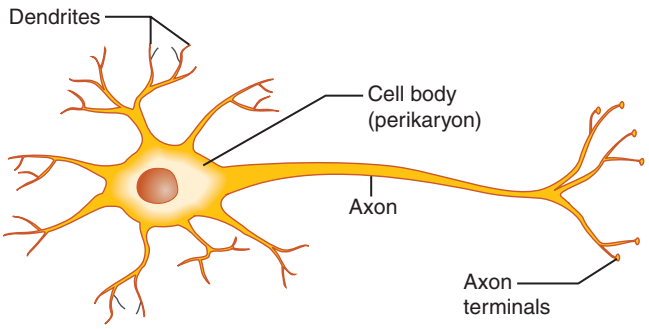

FIGURE 1.1 Morphologic components of a neuron.

processes, such as growth and development, energy and fluid homeostasis, responses to stress, and reproduction.

Neurons are highly specialized and morphologically diverse cells that transmit information via electrical impulses called action potentials. Neurons have a cell body containing the cell nucleus, mitochondria, and synthetic organelles. Neurons also have cell processes that participate in the reception and delivery of electrical impulses (Fig. 1.1). Dendrites are short processes—often extensively branched to increase surface area—that typically receive information (afferent electrical impulses). The axon is a single neuronal extension that generally transmits efferent electrical impulses away from the cell body in a process called *neuronal firing*. However, as described later, GnRH neuron fibers extending from the cell body to the median eminence (the location of GnRH release) in mice demonstrate characteristics of both axons and dendrites and, thus, have been called dendrons.[1]

In unstimulated neurons, the inner portion of the neuronal membrane is negatively charged compared with the outer membrane surface; this *resting membrane potential* is typically between −50 and −75 mV in GnRH neurons. Such electrical polarization reflects transmembrane ionic differences, which are maintained by protein channels that govern transmembrane passage of specific ions (e.g., sodium, potassium, chloride). Regulated changes of transmembrane ion differences may cause the membrane potential to become more or less negative (hyperpolarization and depolarization, respectively). Depolarization to a certain threshold results in a rapid and temporary reversal of membrane potential (an action potential), which is propagated along the neuronal membrane. Notably, the amplitude of the action potential does not vary with the strength of stimulation; instead, once a threshold is reached, a full action potential occurs—the so-called *all-or-none phenomenon*. However, the degree of neuronal stimulation can alter the frequency of action potentials generated. In this way, neurons transmit information to other neurons and effector tissue cells.

Neuronal signals are transferred across neuron-to-neuron connections (synapses) via chemical neurotransmitters. This process begins with bursts of neuronal firing, which result in the opening of voltage-gated calcium channels at the axonal terminal. The influx of calcium promotes exocytosis of neurotransmitter-containing synaptic vesicles, releasing neurotransmitters into the synaptic cleft. Neurotransmitters then bind to specific ligand-dependent ion channels in the postsynaptic membrane, which can stimulate an action potential in the postsynaptic neuron. A wide variety of factors serve as neurotransmitters, including amino acids (e.g., acetylcholine, glutamate, γ-aminobutyric acid [GABA]), biogenic amines (e.g., norepinephrine, epinephrine, dopamine, serotonin), and neuropeptides (e.g., kisspeptin, neurokinin B [NKB], dynorphin, β-endorphin, somatostatin, proopiomelanocortin [POMC], neuropeptide Y [NPY]).

Bursts of neuronal firing can also elicit release of neuronal products into the bloodstream to influence remote targets (i.e., neurosecretion of neurohormones). Hypophysiotropic neurons are specialized hypothalamic neurons that secrete peptide-releasing factors—GnRH, corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), and growth hormone–releasing hormone (GHRH)—into the hypophyseal portal circulation. These releasing factors in turn stimulate specific anterior pituitary cell populations. In contrast, hypothalamic release of dopamine into the portal circulation provides tonic inhibition of pituitary prolactin secretion. Hypothalamic neurosecretion of vasopressin and oxytocin, which are released directly into the systemic circulation, alter the function of distant targets, such as the renal tubules and uterus, respectively.

Neuroglial cells (e.g., astrocytes, ependymal cells, oligodendrocytes, and microglia) represent approximately 90% of cells in the CNS. Neuroglia do not conduct action potentials, but they perform critical supportive functions. For example, astrocytes form the supportive framework of the CNS; help isolate synaptic junctions (to prevent nonspecific spread of neuronal impulses); facilitate nutrient delivery to neurons; and contribute to the blood-brain barrier. In addition, astrocytes have been implicated in the control of GnRH secretion and the mechanisms underlying pubertal onset.[2] For example, astrocytes may impact neuronal activity via secretion of numerous growth factors, and astrocytes abundantly appose GnRH neurons; these contacts can influence synaptic input, and they may be influenced by estrogen in both rodents and nonhuman primates. Similarly, specialized ependymal cells (tanycytes) in the median eminence appear to modify access of GnRH neuron terminals to the hypophyseal portal blood.

Anatomy of the Reproductive Hypothalamic-Pituitary Axis

- *GnRH neuronal cell bodies are located in the infundibular (arcuate) nucleus and the medial preoptic area of the hypothalamus.*
- *GnRH neurons extend processes (dendrons) to the median eminence, where GnRH gains access to the hypophyseal portal system.*
- *The hypophyseal portal circulation represents the functional connection between hypothalamic GnRH neurons and the gonadotropes of the anterior pituitary.*

Portions of the hypothalamus and the anterior pituitary gland constitute the primary effector arm of the central reproductive axis. In particular, hypothalamic neural systems regulate GnRH release into the hypophyseal portal veins, with GnRH being the signal to gonadotropes (anterior pituitary) to secrete LH and FSH. In turn, these gonadotropins direct gonadal (ovarian and testicular) function.

Hypothalamus

The hypothalamus is located at the base of the brain (Fig. 1.2). Although small (approximately 10 g, less than 1% of total

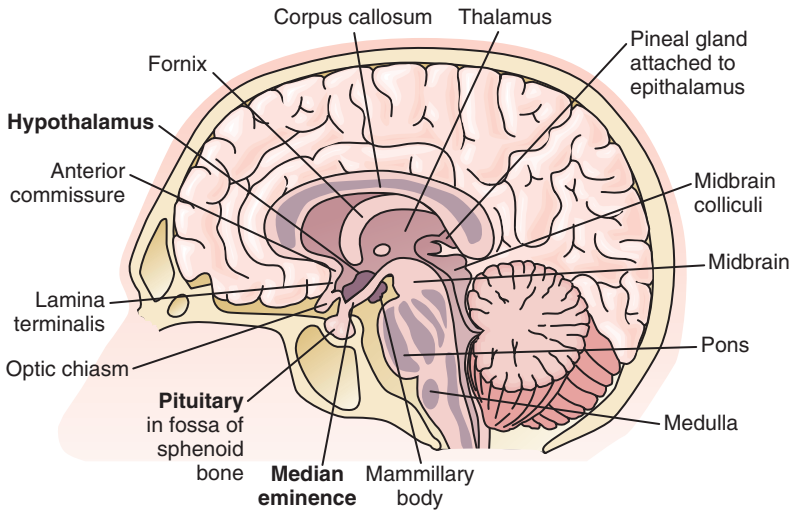

FIGURE 1.2 Cross-sectional representation of the human brain (sagittal plane), including hypothalamus, median eminence, and pituitary gland. *(Modified from Johnson MH, Everitt BJ:* Essential Reproduction*, ed 5, Blackwell, MA, 2000, Blackwell Science, Fig. 6.1.)*

brain weight), it performs critical functions for maintenance of whole-organism homeostasis, including regulation of hunger and body weight, growth, various aspects of metabolism, thirst and renal water handling, body temperature, autonomic function, sleep, circadian rhythms, and emotion. Importantly, the hypothalamus is also a primary control center for reproduction and influences sexual behavior.

As an anatomic structure, the hypothalamus does not have discrete borders, but in general it forms the floor and inferior-lateral walls of the third ventricle (Fig. 1.3). The medial portions of the hypothalamus are primarily made up of cell bodies, whereas the lateral portions are mostly composed of neuron fibers (axons), such as those connecting the medial hypothalamus to other areas of the brain. (The hypothalamus is extensively interconnected with other brain areas.) By convention, closely associated collections of neuron cell bodies are called nuclei; and the paraventricular, dorsomedial, ventromedial, and infundibular nuclei contain a majority of the neurons that secrete hypophysiotropic hormones into the portal circulation. (The human infundibular nucleus is the analogue to the arcuate nucleus in lower mammalian species.) GnRH cell bodies do not form discrete nuclei but are instead diffusely located throughout the preoptic area and the mediobasal hypothalamus (Fig. 1.4); the latter is situated caudal to the preoptic area, extending from the retrochiasmatic area (i.e., the area situated behind the optic chiasm) to the mammillary bodies, and including both the infundibular (arcuate) nucleus and the median eminence.

Median Eminence

Positioned at the base of the third ventricle, the median eminence is part of the anatomic link between the hypothalamus and anterior pituitary. The internal zone of the median eminence is located along the ventral floor of the third ventricle and is largely composed of axonal fibers from both magnocellular neurons (larger neurons that secrete vasopressin and oxytocin) and hypophysiotropic neurons as they travel from hypothalamic nuclei/areas to their final destinations—the neurohypophysis (posterior pituitary) and the external zone of the median eminence, respectively (Fig. 1.5). The external zone contains hypophysiotropic neuron terminals, which release hypophysiotropic hormones into an extensive capillary plexus—the proximal end of the hypophyseal portal system. Some nerve terminals in this zone act on other nerve terminals to influence hormone release (e.g., kisspeptin neurosecretion at GnRH neuron terminals influences GnRH release).

The ependymal layer lining the third ventricle includes a population of specialized ependymal cells called tanycytes, which have a short process extending toward the ventricular surface and a long process extending into the median eminence toward areas around portal capillaries. The latter tanycyte projections envelop or retract from GnRH nerve terminals during episodes of low and high GnRH neuronal activity, respectively. Thus tanycytes may influence GnRH secretion by physically isolating GnRH neuron terminals from portal capillaries—a regulated process.[3] Tanycytes may also represent a link between cerebrospinal fluid and the external zone of the median eminence (e.g., by transporting substances from the third ventricle to portal blood).

The median eminence is among the so-called circumventricular organs, which lie adjacent to the ventricular system and represent openings in the blood-brain barrier. Although lipid-soluble molecules can diffuse in and out of the CNS relatively easily, and cellular transport mechanisms allow selective entry of ions, the blood-brain barrier functions to protect certain regions of the brain and hypothalamus from larger charged molecules, with physical protection provided by (1) tight junctions between endothelial cells and (2) neuron-capillary separation by both astrocyte foot processes and microglia. However, the CNS requires feedback signals—including hormonal, metabolic, and toxic cues—via macromolecules of peripheral origin that would otherwise be excluded by the blood-brain barrier; accordingly, capillaries of the circumventricular organs are fenestrated and permit transcapillary exchange of larger charged molecules (e.g., proteins, peptide hormones). Thus the median eminence represents a key access point for central sensing of peripheral cues. Similarly, fenestrated vessels readily allow entry of hypothalamic releasing factors into portal blood.

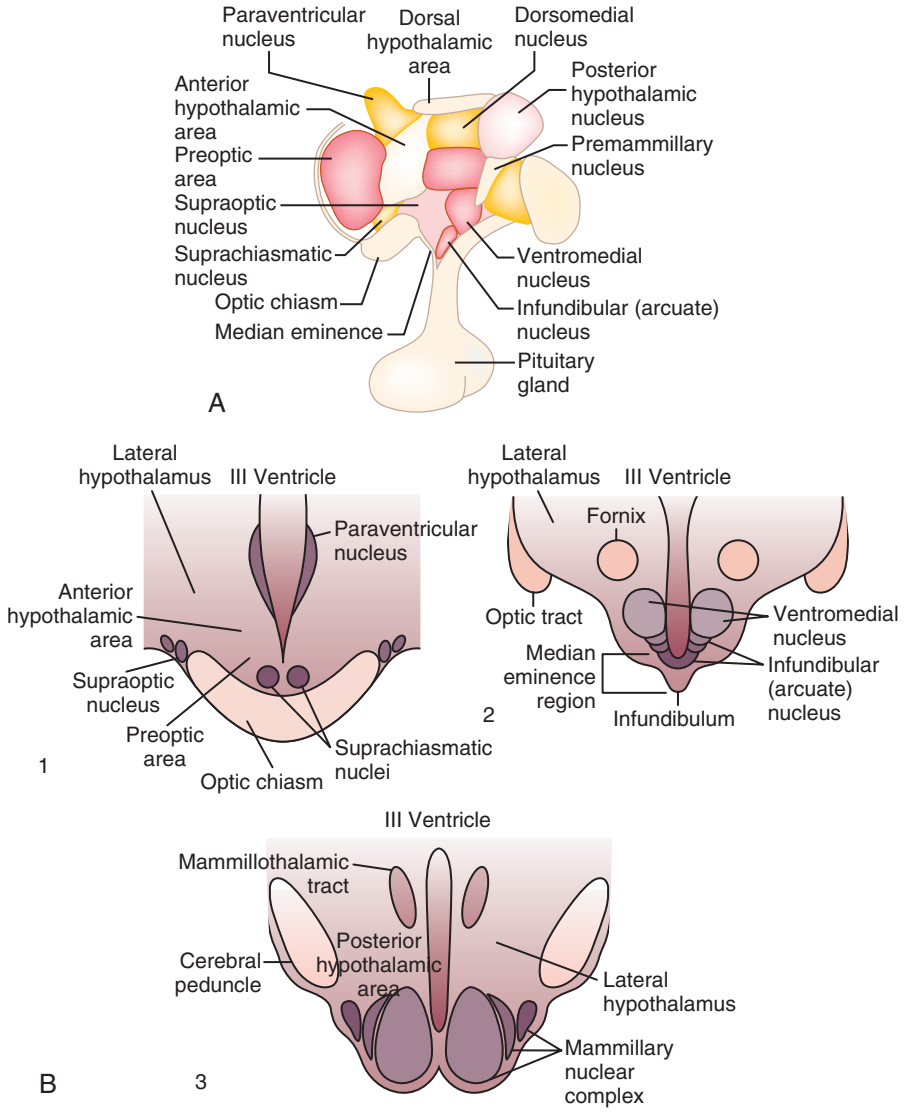

FIGURE 1.3 Nuclei and areas of the hypothalamus. (A) By custom, the nuclei and areas of the hypothalamus are often divided into three groups according to their location along the anteroposterior plane: the anterior group, tuberal group, and posterior (or mammillary) group. The anterior group is formed by the paraventricular, supraoptic, and suprachiasmatic nuclei along with the anterior hypothalamic and preoptic areas. The tuberal group—so-called because of its position above the tuber cinereum (from which the infundibulum or pituitary stalk extends)—contains the dorsomedial, ventromedial, and infundibular (arcuate) nuclei along with the median eminence. Along with the paraventricular nucleus, the nuclei of the tuberal group contain a majority of the neurons that secrete hypophysiotropic hormones (i.e., hypothalamic hormones regulating hormone synthesis and release from cells in the anterior pituitary). Finally, the posterior group includes the posterior hypothalamic nucleus and mammillary nuclei. (B) Cross-sectional representations (coronal planes) of the rostral (1), mid (2), and caudal (3) portions of the human hypothalamus. *([B] Modified from Johnson MH, Everitt BJ:* Essential Reproduction*, ed 5, Blackwell, MA, 2000, Blackwell Science, Fig. 6.3.)*

Hypophyseal Portal Circulation

No direct neuronal connections exist between the hypothalamus and the anterior pituitary. However, the hypophyseal portal circulation (hypothalamic-hypophyseal portal system, pituitary portal system) represents the functional connection between the median eminence and anterior pituitary (see Fig. 1.4). The superior hypophyseal artery—a branch of the internal carotid artery—subdivides to form an extensive capillary network in the external zone of the median eminence, with loops that reach into the inner zone. Capillary blood then drains into sinusoids that converge into the hypophyseal portal veins. Traversing the pituitary stalk, the hypophyseal portal system forms the primary blood supply of the anterior pituitary. The direction of blood flow is primarily, but not exclusively, from the hypothalamus to the anterior pituitary; some retrograde flow allows for short-loop hypothalamic feedback.

Pituitary Gland (Hypophysis)

The pituitary gland appears as an extension at the base of the hypothalamus and resides cradled within the sella turcica, a saddlelike structure of the sphenoid bone (see Fig. 1.2). The adenohypophysis (anterior pituitary) is of ectodermal origin, derived from an upward invagination of pharyngeal epithelium (Rathke pouch) during embryologic development.

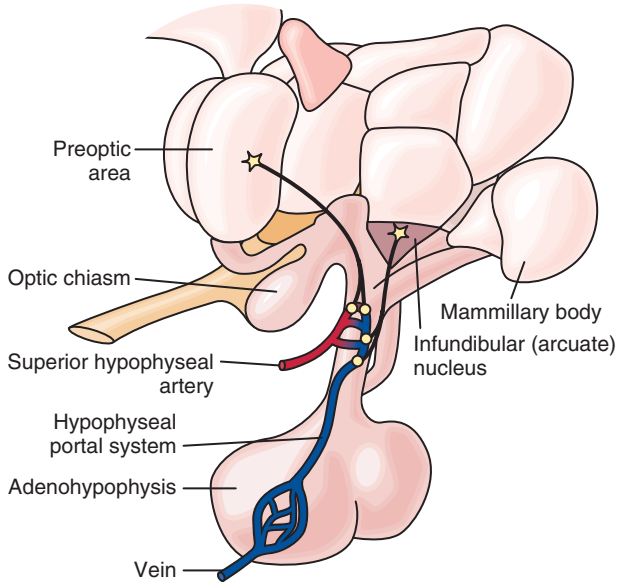

FIGURE 1.4 Anatomic relationship between hypothalamic gonadotropin-releasing hormone *(GnRH)* neurons and their target cell populations in the adenohypophysis (anterior pituitary). GnRH neuron cell bodies are located in the preoptic area and the mediobasal hypothalamus. GnRH neuron projections (dendrons) terminate at the median eminence, where GnRH is secreted into the hypophyseal portal system. *(Modified from Johnson MH, Everitt BJ:* Essential Reproduction*, ed 5. Blackwell, MA, 2000, Blackwell Science, Fig. 6.4.)*

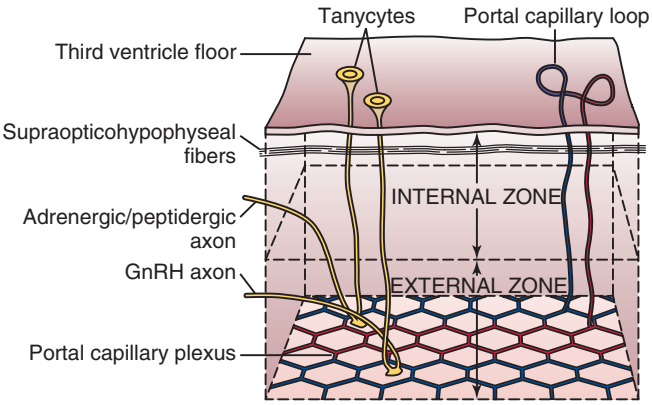

FIGURE 1.5 Diagram of the median eminence.

The adenohypophysis is composed of primarily the anterior lobe (pars distalis), which contains specialized cell populations that produce specific hormones: gonadotropes (the gonadotropins LH and FSH), mammotropes (prolactin), corticotropes (adrenocorticotropic hormone [ACTH]), thyrotropes (thyroid-stimulating hormone [TSH]), and somatotropes (growth hormone). The intermediate lobe is vestigial in adult humans but includes a small population of cells (e.g., POMC cells) in contact with the posterior lobe; the pars tuberalis is a slender layer of tissue (e.g., LH-producing cells and TSH-producing cells) surrounding the infundibulum (the funnel-shaped connection between the hypothalamus and the posterior pituitary) and pituitary stalk.

In contrast to the adenohypophysis, the neurohypophysis (posterior pituitary) is composed of neural tissue and forms as a downward extension of neuroectodermal tissue from the infundibulum during embryologic development. It is thus a direct extension of the hypothalamus. The neurohypophysis includes the infundibular stalk and the pars nervosa (posterior lobe of the pituitary). The supraoptic and paraventricular nuclei include magnocellular neurons that produce oxytocin and arginine vasopressin (AVP; also known as antidiuretic hormone [ADH]), respectively; these axons project to the posterior lobe of the pituitary, where oxytocin and AVP are secreted into a capillary network that drains into the hypophyseal veins (i.e., directly into the systemic circulation). The posterior lobe also includes specialized glial cells called pituicytes, which envelop or retract from magnocellular nerve terminals during episodes of low and high neuronal activity, respectively.

Gonadotropin-Releasing Hormone: The Final Common Pathway for the Central Control of Reproduction

- *Pulsatile GnRH secretion is the proximate stimulus for LH and FSH synthesis and secretion by pituitary gonadotropes.*
- *Although numerous internal and external factors influence gonadotropin secretion via numerous neuronal pathways, GnRH is the final common pathway for the stimulation of LH and FSH release.*

GnRH, previously called luteinizing hormone–releasing hormone (LHRH), is synthesized and released from a relatively small population of specialized hypothalamic neurons. GnRH was initially isolated from porcine hypothalami and shown to stimulate pituitary gonadotropin release.[4] Although the primary function of GnRH is to regulate pituitary gonadotropin secretion, GnRH also appears to have autocrine and paracrine functions in diverse tissues (e.g., ovary, placenta).[5]

The regulation of GnRH secretion is complex and involves overlapping pathways, which likely increases the robustness of central reproductive function. However, there are no known parallel or backup pathways for the stimulation of gonadotropin secretion. Thus natural fertility is absolutely dependent on appropriate GnRH secretion. For example, mice with mutations of the GnRH-1 gene are hypogonadal, but reproduction can be restored via GnRH-1 gene therapy[6] or transplantation of fetal GnRH neurons.[7] Similarly, a variety of human conditions associated with absent (or near-absent) GnRH secretion lead to pubertal failure, hypogonadotropic hypogonadism, and infertility, all of which can be fully reversed with exogenous GnRH therapy.[8]

GnRH secretion is influenced by numerous factors, including sex steroids, energy availability, and stress. In some mammalian species, GnRH secretion is also affected by circadian rhythms, photoperiod (e.g., seasonal breeders such as sheep), social cues, and pheromones.

Gonadotropin-Releasing Hormone Structure

GnRH (GnRH-1 in particular) is a decapeptide, with the amino acid structure (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. The amino acid structure of GnRH is identical in essentially all mammalian species; with the exception of the central Tyr-Gly-Leu-Arg segment, the amino acids of GnRH are highly conserved among vertebrate species.[9] The GnRH-1 gene *(GNRH1)* is located on human chromosome 8 (8p11.2-p21) and produces a 92–amino acid

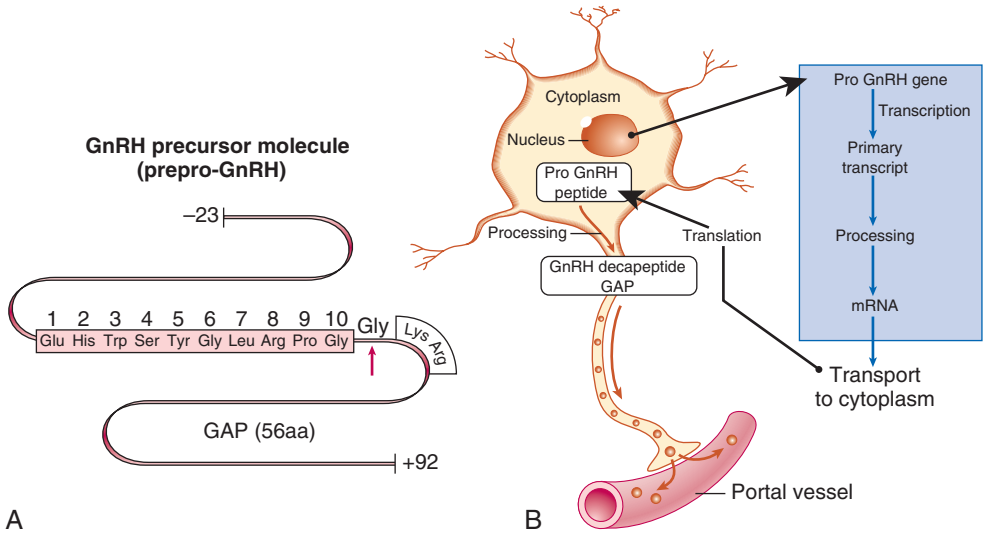

FIGURE 1.6 Schematic of gonadotropin-releasing hormone *(GnRH)* synthesis. (A) Representation of prepro-GnRH, including a 23–amino acid signal sequence, GnRH, a proteolytic processing site (Gly-Lys-Arg), and GnRH-associated peptide. The *arrow* indicates the site of proteolytic cleavage and C-amidation. (B) Schematic of neuronal GnRH synthesis and secretion.

precursor peptide called prepro-GnRH, which includes a signal sequence (23 amino acids), GnRH (10 amino acids), a proteolytic processing site (3 amino acids), and GnRH-associated peptide (56 amino acids) (Fig. 1.6). The latter peptide can stimulate gonadotropin secretion and inhibit prolactin secretion, although its precise physiologic role, if any, remains unclear. The actions of GnRH are mediated through the GnRH type I receptor.

Another form of GnRH (GnRH-2) and its receptor have been identified in a variety of animal species, including humans.[10] GnRH-2 is a decapeptide with similar structure to GnRH-1: (pyro)Glu-His-Trp-Ser-*His*-Gly-*Trp*-*Tyr*-Pro-Gly-NH2 (italicized amino acids denote differences compared with GnRH-1). However, the gene for GnRH-2 is located on human chromosome 20 (20p13). GnRH-2 is widely expressed in the CNS and extra-CNS tissues, and it may contribute to reproductive behavior regulation in some species. In lower animals, GnRH-2 can act via its own receptor, which is structurally and functionally distinct from the GnRH type I receptor. Although a homologue of the GnRH-2 receptor gene has been detected in humans, it includes a frameshift and premature stop codon. Thus the physiologic role of GnRH-2 in humans remains unclear.

Anatomy of Gonadotropin-Releasing Hormone-Secreting Neurons

GnRH neurons are a heterogeneous population of hypothalamic neurons. They are relatively few, numbering approximately 1500, and the majority of GnRH neuronal cell bodies are located in the infundibular (arcuate) nucleus—part of the mediobasal hypothalamus—and the medial preoptic area.[11] Although GnRH neurons are rather loosely affiliated anatomically, they are functionally integrated and form a complex network with numerous interconnections, in addition to connections to other neuronal populations. Of interest in this regard, work in mice suggests that the GnRH neuron fibers extending from the cell body to the median eminence are morphologically atypical: although they do not exhibit many of the molecular markers classically associated with axons or dendrites, they demonstrate morphologic and functional characteristics of both axons and dendrites, including functional synaptic inputs throughout the length of the fiber.[1] The term *dendron* has been proposed for such projections.

The GnRH neurons in the mediobasal hypothalamus—the infundibular (arcuate) nucleus in particular—appear to be requisite for gonadotropin secretion. For example, selective radiofrequency ablation of the arcuate nucleus in adult female monkeys obliterates gonadotropin secretion.[12] GnRH neurons extend projections through the tuberoinfundibular tract to the median eminence, where neuron terminals gain access to the hypophyseal portal system.

The physiologic function of other GnRH neurons, which arise from the anterior and posterior hypothalamus and project to the limbic system and posterior pituitary, respectively, remains unclear, although some of these circuits may possibly be involved with various behavioral responses.

Embryologic Development of the Gonadotropin-Releasing Hormone Neuronal Network

The ontogeny of GnRH neurons in vertebrate species is unique among neuronal systems of the CNS: nascent GnRH neurons are initially identified outside of the CNS in the nasal placode (sometimes called the olfactory placode). However, GnRH cells migrate during embryologic development, as directly observed in embryonic nasal explant cultures[13] and in embryonic head slices (mouse model).[14] The specific migratory pathway of GnRH neurons was first demonstrated in mice by documenting the presence of GnRH-immunoreactive cells in different areas at different stages of embryonic development (Fig. 1.7).[15-17] Specifically, GnRH expression is first observed within the nasal placode circa embryonic day 10 or 11. By embryonic day 13, GnRH-expressing cells are primarily located around the cribriform plate, and GnRH-expressing cells begin to reach the

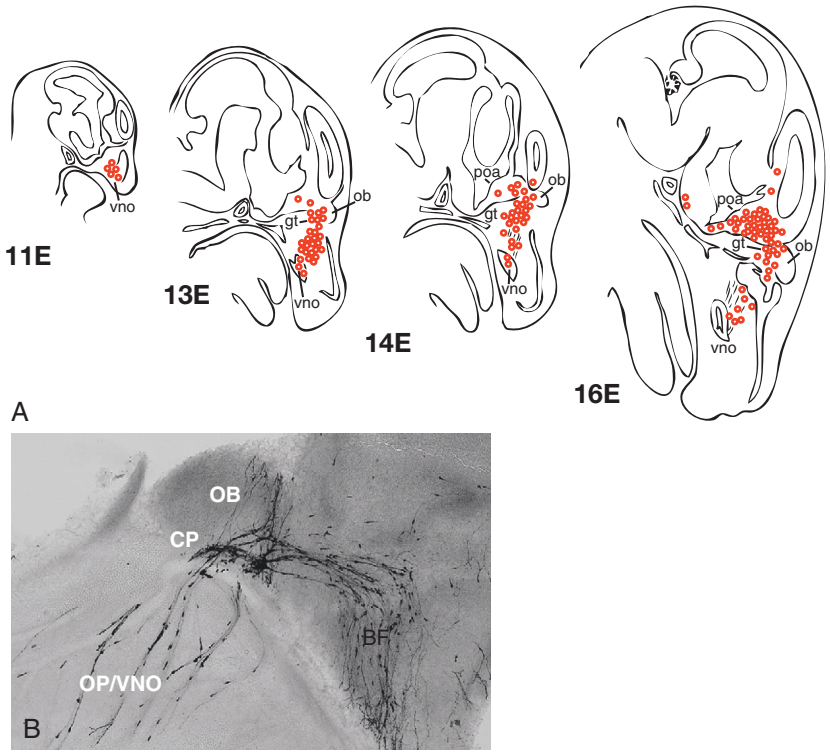

FIGURE 1.7 Gonadotropin-releasing hormone *(GnRH)* neuron migration during embryogenesis. (A) Location of GnRH-immunoreactive cells *(red circles)* as a function of embryologic age (mouse). On embryologic day 11 *(11E),* GnRH cells are located in the nasal (olfactory) placode and presumptive vomeronasal organ *(vno).* GnRH cells migrate across the cribriform plate toward the olfactory bulb *(ob).* GnRH neurons then follow the caudal branch of the vomeronasal nerve toward the forebrain and hypothalamus. By day 16 *(16E),* GnRH neurons largely reside in the preoptic area *(poa)* of the hypothalamus. (B) Sagittal brain slice (mouse, embryonic day 15) demonstrating the migratory route of GnRH-immunoreactive cells. Staining is for GnRH and peripherin (a neuronal intermediate filament). *BF,* Basal forebrain; *CP,* cribriform plate; *gt,* ganglion terminale; *OB,* olfactory bulb; *OP/VNO,* olfactory placode-vomeronasal organ. *([A] Modified from Schwanzel-Fukuda M, Pfaff DW: Origin of luteinizing hormone-releasing hormone neurons,* Nature *338:161–164, 1989; and [B] Modified from Wierman ME, Pawlowski JE, Allen MP, et al: Molecular mechanisms of gonadotropin-releasing hormone neuronal migration,* Trends Endocrinol Metab *15:96–102, 2004.)*

hypothalamus by embryonic day 14, approaching their final positions around embryonic day 16. This migratory pathway has been confirmed in both nonhuman primates[18] and humans.[19]

Successful migration of GnRH neurons is inextricably intertwined with olfactory system development, perhaps reflecting the close functional relationship between reproduction and the olfactory system (e.g., pheromones) in mammalian phylogeny. The nasal placode gives rise to nasal epithelium and olfactory sensory neurons, the latter of which extend axonal projections to the olfactory bulb. Vomeronasal neurons are a subset of olfactory neurons believed to be involved with pheromone detection; these axons originate in the vomeronasal organ and largely extend to the accessory olfactory bulb. At the level of the cribriform plate, some olfactory (vomeronasal) axons separate and form a branch that extends caudally into the forebrain. Of great importance, migrating GnRH neurons maintain adhesion to these axons; thus these olfactory neurons form a critical guidance track for GnRH neuronal migration across the nasal epithelium and through the forebrain toward the hypothalamus.[20,21]

The dependence of GnRH neuronal migration on normal olfactory system development is exemplified by Kallmann syndrome, a form of congenital hypogonadotropic hypogonadism accompanied by absent sense of smell (anosmia). In this syndrome, faulty development of the olfactory system renders an inadequate guidance infrastructure for migrating GnRH neurons, leading to failure of GnRH neurons to reach the hypothalamus. The first identified cause of Kallmann syndrome was deletion of the Kallmann syndrome 1 sequence *(KAL1)* gene, which is located on the X chromosome (Xp22.3) and encodes anosmin-1, a secreted matrix glycoprotein expressed in the presumptive olfactory bulb. Although precise mechanisms are unclear, anosmin-1 is believed to be important for the formation of olfactory elements that provide migratory guidance to GnRH neurons as they move out of the nasal placode. Evaluation of a 19-week-old human fetus with X-linked Kallmann syndrome demonstrated GnRH-immunoreactive cells within a tangle of olfactory and vomeronasal nerves at the dorsal surface of the cribriform plate, along with the absence of olfactory tracts and bulbs.[22] In a second human fetus (16 weeks) with X-linked Kallmann syndrome, GnRH was detected along terminal nerve fascicles in the nasal mucosa only.[23] This syndrome illustrates that, without the guidance framework provided by the olfactory neuronal system, GnRH neurons fail to migrate into the hypothalamus and thus cannot release GnRH into the hypophyseal portal system.

A number of additional single-gene defects have been associated with Kallmann syndrome, including mutations in the genes for prokineticin 2 *(PROK2)* and its receptor *(PROKR2),*[24] fibroblast growth factor-8 *(FGF8)* and its

receptor fibroblast growth factor receptor 1 *(FGFR1)*,[25] NMDA receptor synaptonuclear signaling and neuronal migration factor (*NSMF*; formerly called nasal embryonic LH-releasing hormone factor [NELF]),[26] and chromodomain helicase DNA binding protein 7 *(CHD7)*.[27] The importance of these genes in GnRH neuronal development is corroborated by mouse studies. For example, in fetal mice lacking either *Prok2* or *Prokr2*, GnRH neurons are trapped in a tangled web of olfactory/vomeronasal axons, with few, if any, reaching the forebrain.[28] Although these gene products are clearly important for GnRH neuron ontogeny, their precise roles remain uncertain.

Mouse studies suggest other important factors underlying GnRH neuron migration during prenatal development. For example, the chemokine (C-X-C motif) receptor 4 (CXCR4) is expressed on murine GnRH neurons and interacts with a secreted chemokine called stromal cell–derived factor-1 (SDF-1), which is present as a gradient in the nasal mesenchyme. This gradient, with highest concentrations at the cribriform plate, provides directional information as GnRH neurons migrate toward the cribriform plate; GnRH cell migration across the nasal compartment is markedly impaired in CXCR4 knockout mice.[29] As another example, extension of the caudal branch of the vomeronasal nerve toward the ventral forebrain involves chemoattraction via interactions between netrin-1—a chemokine expressed as a gradient in the forebrain—and its receptor, deleted in colorectal cancer (DCC). In mice without either netrin-1 or DCC, the caudal branch of the vomeronasal nerve extends toward the cerebral cortex rather than the ventral forebrain; and GnRH neurons follow this path, ultimately residing in the cerebral cortex.[30,31] Animal studies have suggested a number of such interactions in (1) guidance of olfactory neurons toward the forebrain and (2) the association between migrating GnRH neurons and axons of olfactory/vomeronasal nerves; but specific relevance to humans remains unclear.

After reaching the hypothalamus, GnRH neurons detach from olfactory nerve axons and may disperse further before resting. A critical next step is extension of GnRH neuronal projections (dendrons) to the median eminence, where GnRH gains access to the hypophyseal portal system.

Gonadotropin-Releasing Hormone Neuronal Firing and Gonadotropin-Releasing Hormone Secretion

GnRH neuronal activity is marked by bursts of action potentials (burst firing), the patterns and rates of which change across time; changes in GnRH neuron firing rates presumably relate to changes in GnRH secretion. Variable firing rate patterns (e.g., times of high and low firing rates) appear to be intrinsic to GnRH neurons, but they can also be altered by neurotransmitters and neuromodulators (e.g., glutamate, GABA, kisspeptin). Although sex steroids can markedly influence GnRH neuronal firing rates, GnRH neurons lack the primary receptors mediating sex steroid feedback (i.e., estrogen receptor alpha, progesterone receptor, androgen receptor); however, many studies suggest that sex steroid actions on GnRH neuronal activity are mediated primarily by afferent neurons (e.g., those secreting glutamate, GABA, kisspeptin).

GnRH neuron cell bodies are relatively scattered across the mediobasal hypothalamus and preoptic area, yet GnRH is secreted into the hypophyseal portal system in a coordinated, pulsatile fashion. Specifically, GnRH secretion is marked by episodic bursts of hormone release into the portal system, as demonstrated in rats,[32] sheep,[33] and monkeys.[34] After being released into the portal vascular compartment, GnRH is rapidly degraded via enzymatic proteolysis, and the half-life of GnRH in the blood is very short—approximately 2 to 4 minutes. Thus GnRH presentation to gonadotrope cells is intermittent.

Pulsatile GnRH secretion is an absolute requirement for long-term stimulation of gonadotropin synthesis and secretion, and there is a relatively narrow window of GnRH pulse frequency and amplitude that will optimally stimulate gonadotropin secretion. Intermittent GnRH stimulation of gonadotrope cells can increase (or maintain) GnRH receptors on gonadotropes—the self-priming or autopriming effect. Thus intermittent GnRH stimulation facilitates or maintains gonadotrope responsiveness to GnRH. However, more frequent exposure to GnRH pulses can reduce gonadotropin responses to GnRH[35]; at one extreme, continuous GnRH receptor stimulation leads to marked desensitization of gonadotropin synthesis and secretion. In a classic experiment involving rhesus monkeys with hypothalamic lesions that abolished GnRH secretion, intermittent (once an hour) exogenous GnRH administration restored pituitary gonadotropin secretion. However, changing from intermittent to continuous GnRH administration resulted in marked desensitization of gonadotropin release (Fig. 1.8).[36] Although reduced GnRH receptor expression on gonadotropes (i.e., receptor downregulation) plays a role in desensitization, additional mechanisms contribute to the uncoupling of GnRH receptor agonism and gonadotropin synthesis.[37]

The foregoing phenomenon can be exploited therapeutically with the use of long-acting GnRH receptor agonists. Such agonists are peptides with structures very similar to that of GnRH but with amino acid substitutions that enhance receptor binding affinity, increase resistance to proteolytic degradation, or both (Fig. 1.9), thus providing continuous GnRH receptor stimulation. Although initial GnRH receptor agonism temporarily (e.g., for 1 to 2 weeks) increases

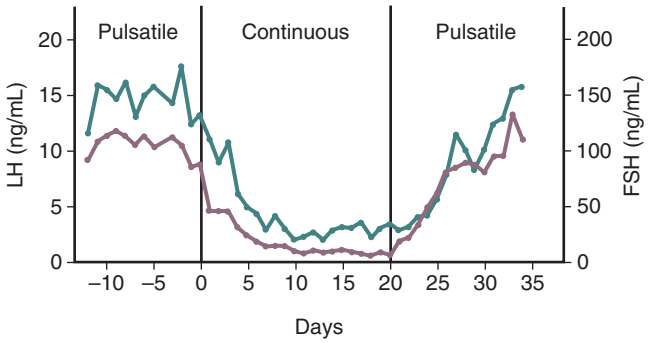

FIGURE 1.8 The influence of pulsatile versus continuous gonadotropin-releasing hormone *(GnRH)* administration to GnRH-deficient monkeys. Intermittent exogenous GnRH administration reconstitutes normal gonadotropin secretion. However, continuous GnRH infusion leads to a marked reduction (downregulation) of luteinizing hormone *(LH; green)* and follicle-stimulating hormone *(FSH; purple)* concentrations. Resumption of pulsatile GnRH administration restores LH and FSH secretion. *(Modified from Belchetz PE, Plant TM, Nakai Y, et al: Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone,* Science *202:631–633, 1978.)*

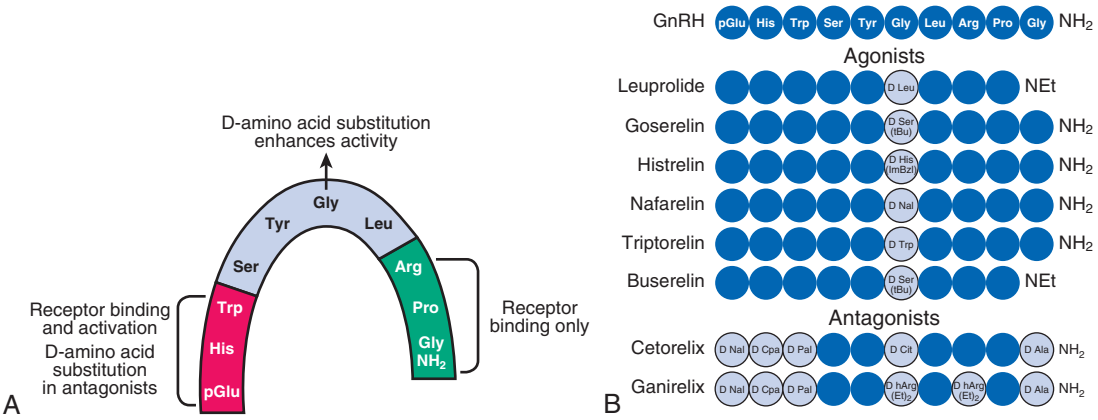

FIGURE 1.9 Structure of gonadotropin-releasing hormone *(GnRH)* and GnRH receptor agonists and antagonists. (A) Schematic of GnRH-1 in its folded conformation. Folding around the glycine in position 6 enhances GnRH receptor binding. Substitution of the glycine in position 6 with D-amino acids stabilizes the molecule in the folded conformation, which increases affinity for the GnRH receptor and reduces metabolic clearance. The amino-terminal *(red)* is involved with receptor binding and activation, and GnRH antagonists involve modifications of these residues that prevent receptor activation. The carboxyl-terminal *(green)* participates in receptor binding, but not activation. Substitution at position 10 (e.g., replacement of glycinamide by ethylamide) can increase binding affinity. (B) Amino acid structure of GnRH along with selected GnRH receptor agonists and antagonists. *Solid blue circles* represent amino acids that are unchanged compared with native GnRH. *(From Millar RP, et al: Gonadotropin-releasing hormone receptors,* Endocr Rev *25:235–275, 2004.)*

gonadotropin release (gonadotropin "flare"), continued agonism leads to desensitization of gonadotropin secretion with accompanying reductions of gonadal sex steroid concentrations to castrate levels ("medical oophorectomy," "medical castration," "pseudomenopause"), typically over several weeks. These agents are useful in the therapy of gonadotropin-dependent disorders such as central precocious puberty, endometriosis, and prostate cancer.

Peptide GnRH receptor antagonists are also available for clinical use. These antagonists reversibly bind to, but do not stimulate, the GnRH receptor (i.e., competitive antagonism). Thus these agents do not initially stimulate gonadotropin release, and they reduce gonadotropins more rapidly than GnRH agonists—usually within 24 to 72 hours.

Gonadotropin-Releasing Hormone Stimulation of Gonadotrope Cells

The specialized cells that synthesize and secrete gonadotropins (i.e., gonadotropes) are located mainly in the lateral portions of the anterior pituitary gland and constitute 7% to 10% of the adenohypophysis cell population. GnRH action at the pituitary gonadotrope begins with GnRH binding to the GnRH type I receptor on the plasma membrane.[9] The GnRH type I receptor is a member of the seven-transmembrane receptor family, a G protein–coupled receptor, and encoded on chromosome 4. GnRH receptor density varies in different physiologic conditions and exhibits a positive correlation with gonadotrope responsiveness to GnRH (e.g., with both being high in rodents during preovulatory gonadotropin surges[38]). GnRH receptor density appears to be modulated primarily by GnRH, with intermittent GnRH stimulation leading to increased GnRH receptor expression; this is a central facet of the self-priming effect of GnRH and an important mechanism by which GnRH action is modulated in different physiologic states.

A majority of gonadotropes synthesize and secrete both LH and FSH. A detailed description of intracellular mechanisms of GnRH action on the gonadotrope is provided in Chapter 2. Briefly, GnRH receptor binding activates the guanosine triphosphate (GTP)-binding protein $G_{q/11}$, leading to an increase in second messengers inositol 1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG). Further intracellular signaling involves increased intracellular calcium and activation of various protein kinase C (PKC) isoforms, mitogen-activated protein kinases (e.g., extracellular signal–regulated kinase [ERK], c-Jun NH_2-terminal kinase [JNK], and p38), calcium/calmodulin-dependent kinase II (Ca/CaMK II), and adenylate cyclase.

Each gonadotropin consists of two protein subunits, α and β. The 92–amino acid α-subunit is common to both LH and FSH—in addition to human chorionic gonadotropin (hCG) and TSH. β-Subunits for LH (LHβ) and FSH (FSHβ) are 121 and 117 amino acids in length, respectively, and account for the biologic specificity of these two hormones. GnRH stimulates gene expression of LHβ, FSHβ, and α-subunit, and the latter noncovalently dimerizes with either LHβ or FSHβ to form LH or FSH, respectively. Gonadotropins also undergo variable posttranslational modification, primarily glycosylation (addition of oligosaccharide moieties to specific amino acids), which influences bioactivity and elimination half-life.[39] The gonadotropins are then packaged into secretory granules for eventual secretion.

Although GnRH is the primary stimulus for LH and FSH synthesis and release from a common cell type, concentrations of these two gonadotropins vary differentially throughout ovulatory cycles, with FSH predominance in the early follicular phase and LH predominance in the late follicular phase. This sequential pattern of FSH and LH predominance is important for normal follicular maturation, ovarian steroid production, and subsequent ovulation. At least two mechanisms govern differential gonadotropin secretion throughout ovulatory cycles. First, both estradiol and inhibins selectively inhibit FSH release from gonadotropes during the mid- and late follicular phase and the luteal phase.[40,41] Second, different patterns of pulsatile GnRH release differentially affect gonadotropin synthesis and secretion. Specifically, rapid (high frequency) GnRH pulses favor LH, whereas slower (low frequency) GnRH pulses favor FSH synthesis and secretion. For example,

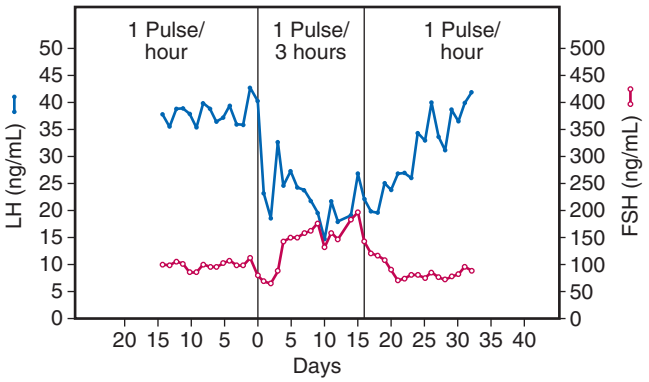

FIGURE 1.10 Luteinizing hormone *(LH)* and follicle-stimulating hormone *(FSH)* concentrations in gonadectomized (but sex steroid–replaced) monkeys after arcuate nucleus ablation—a model of isolated GnRH deficiency. Exogenous GnRH administered in a pulsatile fashion every hour reconstituted LH and FSH secretion. Changing GnRH pulse administration from a relatively high frequency (hourly) to a relatively low frequency (every 3 hours) resulted in decreased LH but increased FSH secretion. *(Modified from Wildt L, et al: Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey,* Endocrinology *109:376–385, 1981.)*

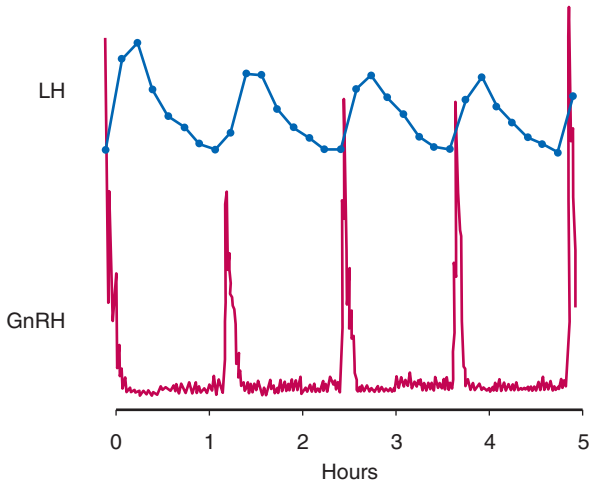

FIGURE 1.11 Close temporal relationship between pulses of luteinizing hormone *(LH)* (jugular vein) and gonadotropin-releasing hormone *(GnRH)* (pituitary portal system) in the sheep model. *(Modified from Moenter SM, et al: Dynamics of gonadotropin-releasing hormone release during a pulse,* Endocrinology *130:503–510, 1992.)*

studies in ovariectomized, GnRH-deficient monkeys reveal that a decrease in the frequency of exogenously administered GnRH pulses from one pulse per hour to one pulse every 3 hours results in a 65% increase in plasma FSH, despite a 50% decrease in LH (Fig. 1.10).[35] Similar findings have been described in sheep[42] and humans.[43,44] Detailed studies in rats demonstrate that rapid GnRH pulse stimulation favors α-subunit and LHβ mRNA expression, whereas slow GnRH pulses favor FSHβ mRNA expression.[45] The mechanisms effecting differential LH and FSH expression in response to changes in GnRH pulse frequency are complex[46] but include variations of GnRH receptor number on the gonadotrope cell surface[47] and alterations of gonadotrope activin β_B and follistatin expression (discussed later in the chapter).[48]

A pulse of GnRH release stimulates a pulse of LH release on a one-to-one basis, and LH (or α-subunit) pulse patterns, as assessed by frequent sampling of peripheral blood, accurately mirror GnRH pulse patterns in animal studies (Fig. 1.11).[33,49] Similarly, exogenous GnRH pulses elicit LH pulses in GnRH-deficient patients. Because measurable GnRH is effectively confined to the hypophyseal portal system, which is inaccessible in humans, GnRH pulse frequency is inferred from LH pulse frequency (or α-subunit pulse frequency[50,51]) in human studies. Although pulses of GnRH stimulate pulsatile release of FSH, the longer serum half-life of FSH renders FSH pulses more difficult to identify via frequent sampling of peripheral blood. In addition, although short-term LH secretion is very closely tied to continued GnRH stimulation, FSH secretion is less acutely dependent on GnRH stimulation.[52,53] For example, with GnRH antagonism, the percentage reduction in LH exceeds that of FSH.[54]

Neuronal Inputs Into Gonadotropin-Releasing Hormone Neurons

- *Normal pulsatile GnRH secretion is dependent on complex interactions among numerous afferent neuronal inputs, including those expressing kisspeptin, neurokinin B, and/or dynorphin.*
- *According to current models, kisspeptin stimulates GnRH release, whereas neurokinin B and dynorphin modulate GnRH release primarily by stimulation and suppression, respectively, of kisspeptin release.*

The governance of GnRH neurons is highly complex and involves numerous interacting neural systems using various neurotransmitters and neuromodulators. The neuronal populations upstream of the GnRH neuron play key roles in puberty and are important mediators of sex steroid feedback and the influence of nutritional cues and stress on GnRH secretion. Numerous neurotransmitters appear to be involved in the regulation of GnRH secretion, including dopamine, norepinephrine, glutamate, GABA, and nitric oxide. The control of GnRH secretion has been the subject of intense investigation, and the recent discovery of several neuronal populations upstream of the GnRH neuron (e.g., kisspeptin neurons) has markedly enhanced our understanding of reproductive neuroendocrinology.

Kisspeptin

The kisspeptin system is believed to be requisite for normal GnRH secretion, serving as a "gatekeeper" of puberty and helping to mediate the effects of sex steroids and metabolic cues on GnRH secretion. Kisspeptin was originally called metastin because of its ability to suppress metastatic spread of human melanomas and breast carcinomas. However, in recognition of its discovery at Pennsylvania State University in Hershey, Pennsylvania, it was later named *kisspeptin* after Hershey's chocolate KISSES. Herein we will use the following abbreviations[55]: *KISS1* and *Kiss1*, the human and nonhuman kisspeptin genes, respectively; *KISS1R (Kiss1R)* and KISS1R (Kiss1R), the human (nonhuman) kisspeptin receptor genes and gene products, respectively.

The *KISS1* gene product is a 154–amino acid precursor protein (kisspeptin 1-145). Variable proteolytic modification yields kisspeptins of different lengths: kisspeptin-54, -14, -13, and -10, with the numbers referring to the amino acid

length of bioactive kisspeptin fragments (Fig. 1.12). Importantly, functional native kisspeptins maintain the 10 amino acids of the carboxy-terminal (kisspeptin amino acids 112 to 121), which are important for receptor binding and function. Kisspeptin is the natural ligand of KISS1R—also known as the G protein–coupled receptor 54 (GPR54)—a seven transmembrane domain, G protein–coupled receptor.

The importance of the kisspeptin system in reproduction was initially revealed by members of two consanguineous families with *KISS1R* mutations leading to pubertal failure and normosmic hypogonadotropic hypogonadism.[56,57] Inactivating *KISS1* mutations leading to pubertal failure and normosmic hypogonadotropic hypogonadism have also been described in four sisters.[58] Murine *Kiss1* and *Kiss1R* knockout models exhibit hypogonadotropic hypogonadism with impaired sexual maturation, reduced gonadal size, failure of estrous cyclicity (females), impaired spermatogenesis (males), and infertility.[57,59,60] However, the notion that kisspeptin is an absolute requirement for puberty and reproductive function in mice is somewhat controversial.[61,62] *KISS1R* and *KISS1* mutations neither interrupt GnRH neuron migration to the hypothalamus nor impair GnRH synthesis.

Single boluses of kisspeptin markedly stimulate LH release in rodents, sheep, monkeys, and humans. This effect of kisspeptin is mediated by stimulation of GnRH neurons, as supported by the following: kisspeptin fibers appear to project to and form synaptic contacts with GnRH neurons[63,64]—connections that appear to be established in utero[65,66]; the kisspeptin receptor is expressed by a majority of GnRH neurons[67]; kisspeptin can directly depolarize GnRH neurons[68,69]; a kisspeptin antagonist inhibits murine GnRH neuron firing rates and reduces pulsatile GnRH release in female pubertal monkeys[70]; and kisspeptin stimulation of gonadotropin secretion is completely blocked by GnRH antagonists.[55,67,71] However, kisspeptin may also work indirectly because kisspeptin can increase GABAergic and glutamatergic postsynaptic currents onto GnRH neurons.[72] Kisspeptin does not stimulate LH secretion in *Kiss1R* knockout mice,[60,73] suggesting that kisspeptin acts exclusively through its cognate receptor. Moreover, mice with GnRH neuron-specific *Kiss1R* knockout exhibit hypogonadotropic hypogonadism and infertility, suggesting that kisspeptin action at GnRH neurons is critical for reproductive function.[74]

In addition to acting upon GnRH neuron cell bodies, kisspeptin neurons form synapses with GnRH neuron terminals in the external zone of the median eminence,[75] where kisspeptin can stimulate GnRH release (exocytosis).[76,77] Although kisspeptin may possibly have direct effects on gonadotropes, available data suggest that this does not play a major role in kisspeptin's ability to stimulate gonadotropin secretion. For example, pulsatile GnRH can restore normal reproductive function in patients with *KISS1R* mutations.[78]

The numbers of kisspeptin neurons are high in the infundibular (arcuate) nucleus, similar to findings in monkeys.[71,79,80] Extensive study in the rodent model discloses two primary populations of kisspeptin-expressing neurons in the hypothalamus: one in the arcuate nucleus (mediobasal hypothalamus) and the other in the anteroventral periventricular nucleus (AVPV) of the preoptic area.[81] Of interest, kisspeptin expression in the AVPV is much higher in female compared with male rodents, which appears to reflect organizational effects of sex steroids during early development[82,83]; and kisspeptin neurons in the AVPV appear to be specifically important for LH surge generation in rodents. Sexual dimorphism of kisspeptin expression has also been described in sheep[84] and humans.[80] However, in primates, including humans, the majority of the kisspeptin cell bodies reside in the infundibular (arcuate) nucleus.[79,80] Although a study of adult women revealed rare kisspeptin neurons in the preoptic area, a population homologous to the rodent AVPV has not been identified.[79]

Kisspeptin's ability to stimulate LH release in women may vary according to cycle phase or hormonal milieu. For example, although bolus kisspeptin administration consistently increases LH release in women studied in the luteal and preovulatory phases, its effects are less consistent when administered in the early to mid-follicular phase.[85-87] Compared with findings in cycling women studied during the follicular phase, acute LH responses to kisspeptin administration appear to be more pronounced in postmenopausal women[88] and in those with functional hypothalamic amenorrhea.[89]

Kisspeptin and its analogues may hold therapeutic utility in the future. Kisspeptin has been investigated as a potential treatment for several disorders marked by impaired GnRH secretion and low gonadotropins: functional hypothalamic amenorrhea,[89-91] hypogonadism associated with obesity and diabetes,[92] and hyperprolactinemia.[93] Rapid proteolytic degradation of kisspeptin may limit its therapeutic utility; although long-acting KISS1R agonists are actively being developed,[94] the precise effects of long-term KISS1R agonism on gonadotropin release remain unclear: tachyphylaxis to kisspeptin may represent a practical challenge in this regard.

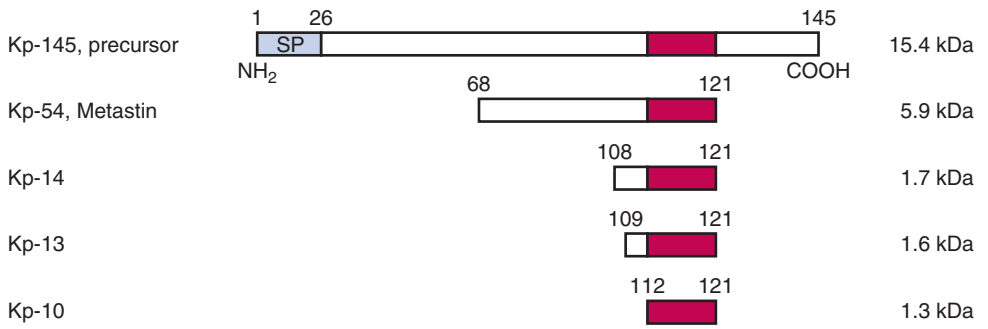

FIGURE 1.12 Schematic of the precursor kisspeptin-145 and the functional kisspeptin *(Kp)* fragments, including size and cleavage sites. Note that all functional Kp fragments maintain amino acids 112-121 *(red)*. *SP*, Signal peptide. *(Modified from Roseweir AK, Millar RP: The role of kisspeptin in the control of gonadotrophin secretion,* Hum Reprod Update *15:203–212, 2009.)*

Kisspeptin has been evaluated as a trigger for final oocyte maturation and ovulation in women at risk for ovarian hyperstimulation syndrome[95]; in this case, rapid proteolytic degradation (compared with hGC) may be central to its therapeutic utility. Finally, when complete gonadal steroid suppression is not required (e.g., endometriosis), KISS1R antagonists may permit partial inhibition of gonadotropin production.[94]

Neurokinin B

NKB—a decapeptide (Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2) encoded by the tachykinin 3 gene *(TAC3)*—is a member of the tachykinin family, which also includes substance P and neurokinin A (products of the *TAC1* gene). There are several neurokinin receptors (NK1R, NK2R, NK3R), and although NKB can produce some agonism at NK1R and NK2R, NKB binds preferentially to and acts primarily via its cognate receptor NK3R (encoded by the *TACR3* gene).[96] Studies of patients with idiopathic hypogonadotropic hypogonadism from consanguineous families revealed that homozygous loss-of-function mutations of either *TAC3* or *TACR3* can cause pubertal failure and severe hypogonadotropic hypogonadism, highlighting the importance of NKB in human reproduction.[97,98] In contrast to *Kiss1* and *Kiss1R* knockout mice, *Tacr3* knockout mice remain fertile, although they can demonstrate reproductive defects.[99,100]

The role of NKB in central reproductive function is complex and appears to vary according to species, sex, and sex steroid milieu.[101,102] The selective NK3R agonist senktide can stimulate LH secretion—albeit not as potently as kisspeptin—in rats,[103] sheep,[104] and monkeys.[105] Such stimulation of LH secretion by NKB is mediated by GnRH secretion, and GnRH receptor antagonism abolishes LH responses to senktide in the monkey.[105]

Although it remains unclear to what degree NKB may have direct actions on GnRH neurons,[106,107] a number of observations suggest that NKB primarily influences pulsatile GnRH secretion indirectly by stimulating kisspeptin release. For example, kisspeptin neurons express NK3R, and senktide increases kisspeptin neuronal activity.[103] LH responses to senktide are either absent or markedly reduced in Kiss1R knockout mice,[108] in the presence of Kiss1R antagonism,[109] or after Kiss1R desensitization.[110] Moreover, continuous kisspeptin infusion can restore pulsatile LH secretion in patients with loss-of-function mutations of *TAC3* or *TACR3*.[111] Studies in mice suggest that other members of the tachykinin family—substance P and neurokinin A in particular—may also influence GnRH secretion via actions on kisspeptin neurons.[112,113]

Regarding the therapeutic potential of NKB analogues, a phase II clinical trial in women with polycystic ovary syndrome (PCOS)—a disorder marked by persistently high GnRH pulse frequency, LH excess, and hyperandrogenemia—suggested that NK3R antagonism for 7 days reduced LH (GnRH) pulse frequency, LH area under the curve, and total testosterone concentrations, with essentially no change in FSH or estradiol concentrations.[114] NKB antagonists could also be useful in disorders requiring only partial reductions in gonadotropins and gonadal steroids (e.g., endometriosis),[94,115] although this notion has not been directly assessed in humans.

Endogenous Opioid Peptides

Endogenous opioid peptides (EOPs), which include endorphins, enkephalins, and dynorphins, participate in myriad processes such as motor activity, cognitive functions, water and food intake, and regulation of neuroendocrine function.[116] Most active EOPs share a common sequence (Tyr-Gly-Gly-Phe-[Met or Leu]) at the amino-terminal, although endorphins, enkephalins, and dynorphins are derived from different precursor proteins that undergo regulated posttranslational processing (Fig. 1.13).[117] Endorphins such as β-endorphin are products of the precursor protein POMC. POMC can be preferentially processed to produce ACTH and β-lipotropin, as occurs in corticotropes (adenohypophysis) under the control of CRH. However, in the hypothalamus, POMC processing primarily yields β-endorphin and α-melanocyte-stimulating hormone. Hypothalamic β-endorphin participates in the regulation of reproduction, temperature, and cardiovascular and respiratory functions, and it acts primarily via μ-opioid receptors. Enkephalins are derived from proenkephalin, and their primary functions appear to relate to autonomic nervous system modulation, mainly via δ-receptor activation. Dynorphins are products of the precursor prodynorphin and act chiefly at κ-opioid receptors (KORs). Importantly, although β-endorphin, enkephalins, and dynorphins act primarily via μ-, δ-, and κ-opioid receptors, respectively, each can act as agonists at more than one receptor subtype.

Numerous studies provide evidence that hypothalamic opiates partly mediate sex steroid negative feedback on GnRH release. For example, GnRH neurons express few if any progesterone receptors, but β-endorphin concentrations increase in hypophyseal blood during the luteal phase—when sex steroids suppress GnRH secretion—in monkeys.[118,119] Moreover, naloxone and naltrexone (opiate receptor antagonists acting primarily at μ- and κ-opioid receptors) increase LH pulse frequency when administered to luteal phase women[120] or progestin-treated postmenopausal women.[121] Similarly, morphine suppresses GnRH secretion from mediobasal hypothalami isolated from fetal and adult humans—an effect that is reversed by naloxone[122]; and chronic high-dose opiate administration can cause hypogonadotropic hypogonadism by suppressing GnRH and LH secretion.[116]

Several animal studies implicate dynorphin as a principal mediator of progesterone negative feedback on GnRH pulse frequency in females.[123] For example, dynorphin neurons in the arcuate nucleus colocalize with progesterone receptors in ewes,[124] and dynorphin-containing varicosities are closely associated with GnRH neuron cell bodies in the mediobasal hypothalamus.[125,126] Progesterone treatment in ewes increases dynorphin A concentrations in third ventricle cerebrospinal fluid,[127] and central infusion of dynorphin in goats reduces volleys of multiple-unit activity in the mediobasal hypothalamus and reduces LH pulses.[128] In luteal phase ewes, specific κ-opioid receptor antagonists—but not antagonists to δ- or μ-opioid receptors—reversed progesterone inhibition of LH secretion and LH pulse frequency when locally administered into the mediobasal hypothalamus.[125] However, other EOPs (e.g., β-endorphin) in other hypothalamic areas may also be involved; for example, in the aforementioned study,[125] κ- and μ-receptor antagonists locally administered into the preoptic area increased LH and LH pulse frequency.

Kisspeptin, Neurokinin B, Dynorphin Neurons

In the arcuate nucleus, kisspeptin, NKB, and dynorphin are frequently coexpressed in the same neuron. For example, kisspeptin neurons in the arcuate nucleus have been found

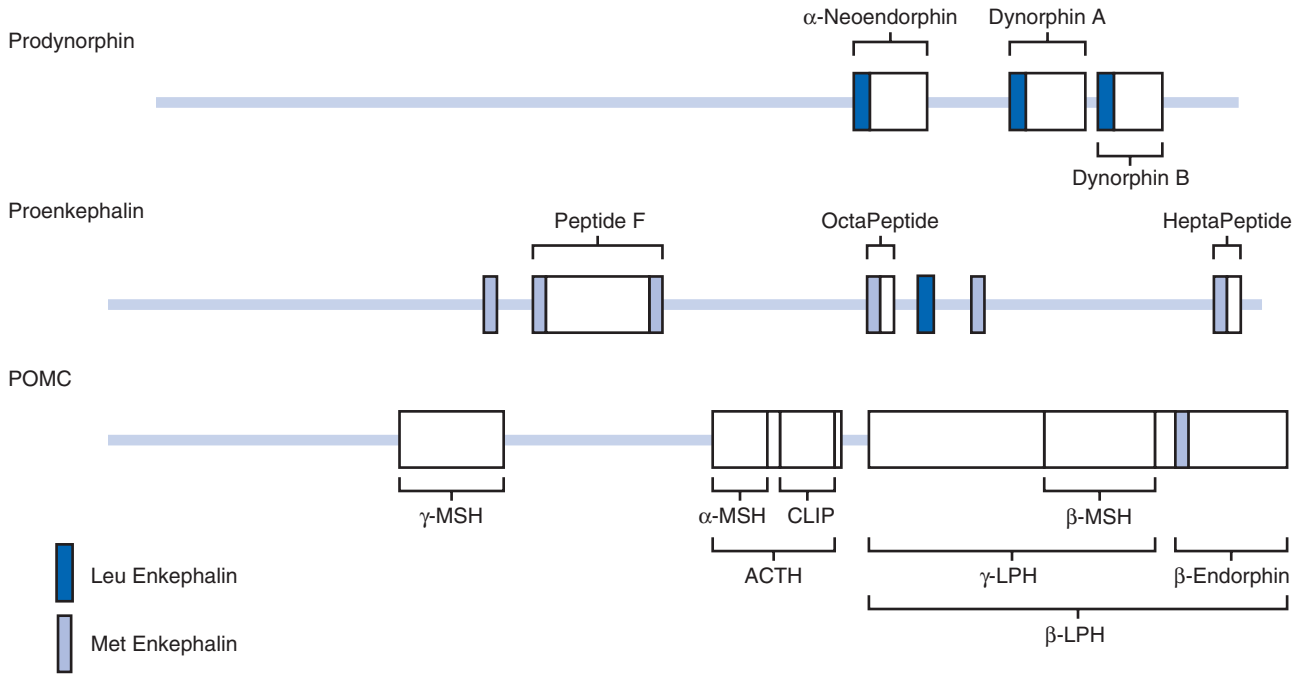

FIGURE 1.13 Schematic of endogenous opiate precursors. *ACTH*, Adrenocorticotropic hormone; *CLIP*, corticotropin-like intermediate lobe peptide; *LPH*, lipotropin; *MSH*, melanocyte-stimulating hormone; *POMC*, proopiomelanocortin. *(Modified from Akil H, et al: Endogenous opioids: overview and current issues,* Drug Alcohol Depend *51:127–140, 1998.)*

to coexpress NKB and dynorphin in rodents,[129,130] goats,[128] and sheep.[131] For convenience, and as a playful nod to kisspeptin (namesake of Hershey's chocolate KISSES), such neurons are often called KNDy neurons (*K*isspeptin, *N*eurokinin B, *Dy*norphin; pronounced *candy*).[84] KNDy neurons in the arcuate nucleus form an extensively interconnected network.[129,132,133] KNDy axons also appear to project to the internal zone of the median eminence where they are in close proximity to GnRH fibers.[106,134] As with kisspeptin neurons, KNDy neuron neuroanatomy exhibits sexual dimorphism, possibly related to perinatal sex steroid exposure.[84] As discussed further later, robust experimental data suggest that KNDy neurons are intimately involved with sex steroid feedback on GnRH secretion; a number of groups have suggested that the KNDy neuronal network represents a fundamental component of the GnRH pulse generator.[123,130,135,136]

Corresponding data are limited in humans. In one autopsy study, 77% of kisspeptin cell bodies (and 56% of kisspeptin axon fibers) in the infundibular nucleus coexpressed preproNKB, and 95% of preproNKB-expressing cell bodies coexpressed kisspeptin.[80] However, the degree of colocalization in humans appears to differ according to sex and age. For example, one autopsy study suggested that only 10% and 26% of kisspeptin-containing afferent contacts onto GnRH neurons coexpressed preproNKB in older men and women, respectively[137]; another autopsy study in young men suggested that 75% of infundibular kisspeptin-containing cell bodies also contained NKB, 33% of NKB-containing cell bodies also contained kisspeptin, and colocalization with dynorphin was uncommon.[138] Although these small studies suggested limited colocalization in humans, it is unclear to what degree postmortem degradation may have influenced these findings. Regardless, it remains well accepted that kisspeptin, NKB, and dynorphin—released from neurons that do or do not colocalize with the other peptides—substantially influence GnRH neuronal function in humans.

Gonadotropin-Inhibitory Hormone and RFamide-Related Peptides

The role of gonadotropin-inhibitory hormone (GnIH) and its mammalian orthologues, RFamide-related peptides (RFRPs), in the central control of reproduction has been recently reviewed.[139] Briefly, RFRP-immunoreactive cells have been identified in hypothalami of a number of species—including RFRP-1 and RFRP-3 in humans[140]—and RFRP-immunoreactive fibers can be found in close proximity to GnRH neurons and in the median eminence. RFRP-3 can reduce GnRH neuronal firing rates in mice[141]; RFRP-3 inhibits pituitary gonadotropin release from cultured ovine pituitary cells[142]; and intravenous RFRP-3 administration suppresses LH pulse amplitude in ovariectomized ewes.[143] Another study revealed reduced RFRP expression in the preovulatory period in ewes, suggesting a reciprocal relationship with GnRH release, and infusion of GnIH blocked the estrogen-induced LH surge.[144] GnIH and RFRPs have also been implicated in the regulation of food intake (increase), sexual motivation (decrease), and the inhibitory influence of stress on reproduction. Although a growing body of data suggests that RFRPs are important factors controlling GnRH and gonadotropin secretion in a number of mammalian species, an understanding of their role in humans awaits further investigation.

Gonadotropin-Releasing Hormone Pulse Generator

- *Discrete, intermittent bursts of coordinated GnRH neuron activity lead to pulsatile release of GnRH into the hypophyseal portal system.*

◆ *Although pulsatility is an intrinsic property of GnRH neurons, afferent inputs (e.g., neurons expressing kisspeptin, NKB, and/or dynorphin) are required for normal GnRH pulse generation and appear to represent integral components of the GnRH pulse generator.*

As described previously, intermittent GnRH receptor stimulation is an absolute requirement for physiologic maintenance of gonadotropin secretion. Although the precise basis of pulsatile GnRH release remains unclear, a number of observations strongly support the concept that neuronal systems within the mediobasal hypothalamus effect pulsatile release of GnRH into the hypophyseal portal system. In animal models, volleys of multiple unit electrical activity (i.e., detection of activity in multiple neurons near an electrode) in the area of the mediobasal hypothalamus coincide with the initiation of LH pulses (Fig. 1.14).[145,146] Similarly, electrical stimulation via electrodes placed in the mediobasal hypothalamus stimulates GnRH release into the hypophyseal portal system in monkeys.[147] Mediobasal hypothalami isolated from both fetal (20 to 23 weeks gestation) and adult humans release GnRH in discrete pulses, with a frequency approximating one pulse per 60 to 100 minutes[122]; and mediobasal hypothalami separated from the remainder of the brain can maintain pulsatile LH secretion in monkeys.[148] These data suggest that the mediobasal hypothalamus houses all requisite components for GnRH pulse generation (i.e., the GnRH pulse generator) and that pulsatile GnRH release does not require innervation from outside of the mediobasal hypothalamus. Nonetheless, mechanisms underlying episodic GnRH pulse generation, and what neuroanatomic components constitute the GnRH pulse generator, are uncertain.

Several studies suggest that pulsatility is an intrinsic property of GnRH neurons. For example, pulsatile GnRH release is exhibited by immortalized GnRH-secreting neurons[149,150] and by cultured GnRH neurons obtained from fetal rats, sheep, and monkeys.[151-153] If GnRH pulse generation reflects an intrinsic property of GnRH neurons, then coordination of GnRH release could be facilitated by cell-to-cell interconnections among GnRH neurons.[154,155]

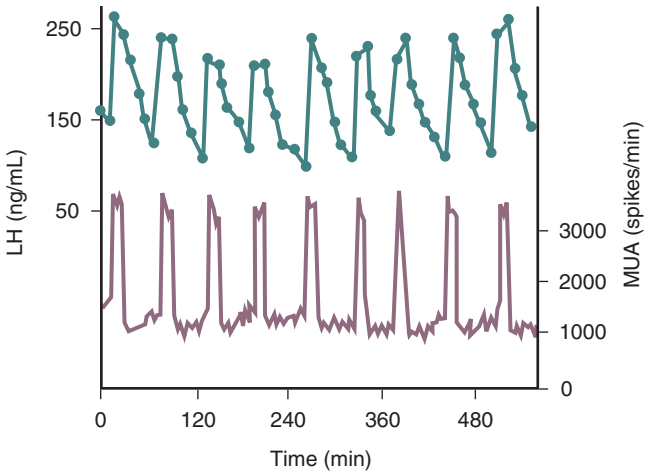

FIGURE 1.14 Temporal association between volleys of multiple unit activity *(MUA)* in the hypothalamus and luteinizing hormone *(LH)* pulses *(green)* detected in peripheral blood in an ovariectomized monkey. *(Modified from Knobil E: The electrophysiology of the GnRH pulse generator in the rhesus monkey.* J Steroid Biochem *33:669–671, 1989.)*

It is well accepted that afferent inputs (e.g., kisspeptin neurons) are important for normal GnRH secretion, and a number of investigators have suggested that kisspeptin (KNDy) neurons may represent a key component of the GnRH pulse generator—in essence orchestrating coordinated GnRH neuronal activity and, accordingly, GnRH secretion.[123,130,135,136] As described previously, LH pulses are temporally associated with volleys of multiunit activity in the arcuate nucleus, which contains both GnRH and kisspeptin (KNDy) neurons.[156] In addition, kisspeptin release at the median eminence appears to be pulsatile: although kisspeptin pulses were not clearly coincident with peripheral LH pulses in ovariectomized ewes,[157] kisspeptin pulses corresponded to GnRH pulses 75% of the time in midpubertal rhesus monkeys.[158] Moreover, a GnRH neuron cell culture study suggests that pulsatile kisspeptin administration entrains synchronous cycles of GnRH gene transcription and pulsatile GnRH secretion.[159] Work in sheep suggests that, although kisspeptin is an important mediator of GnRH pulse generation, additional elements (e.g., upstream glutamate-secreting neurons) are likely also important.[160]

Human studies also imply that kisspeptin may play a role in the GnRH pulse generator. For example, in men, continuous intravenous infusion of a relatively low dose of kisspeptin can increase LH pulse frequency[161]; and a single injection of kisspeptin may reset the GnRH pacemaker.[162] (In the latter study, the interval between the kisspeptin-induced LH pulse and the immediately preceding endogenous LH pulse was variable but on average shorter than the normal LH interpulse interval; in contrast, the interval between the kisspeptin-induced LH pulse and the subsequent endogenous LH pulse was similar to normal interpulse intervals [approximately 2 hours], suggesting that kisspeptin administration reset the hypothalamic GnRH clock.) Parallel results in women are mixed: although bolus kisspeptin administration did not appear to reset the GnRH pacemaker in women,[87] single-dose subcutaneous kisspeptin administration during the follicular phase has been reported to increase LH pulse frequency.[163]

NKB and dynorphin may also play important roles in the coordination of pulsatile GnRH release. This notion is consistent with a number of experimental observations. For example, KNDy neurons exhibit both NK3R and κ-opioid receptors[123]; murine kisspeptin neuron firing rates are increased by NK3R agonists and reduced by κ-opioid receptor agonists[112,164]—effects that appear to be modulated by gonadal steroids.[164,165] In addition, central administration of dynorphin in goats inhibits both multiple unit activity (MUA) volleys in the mediobasal hypothalamus and pulsatile LH release, whereas NKB provokes MUA volleys.[128] Studies using microimplants in the arcuate nucleus of ewes revealed consistent findings: LH pulse frequency was decreased by an NK3R antagonist, whereas LH pulse frequency was increased by either NKB or a κ-opioid receptor antagonist.[166]

Fig. 1.15 depicts a working model proposed by Goodman et al., primarily based on experiments performed in sheep.[101,123] According to this model, KNDy neurons signal to other KNDy neurons—and perhaps to other neurons within the arcuate nucleus—with NKB release stimulating kisspeptin

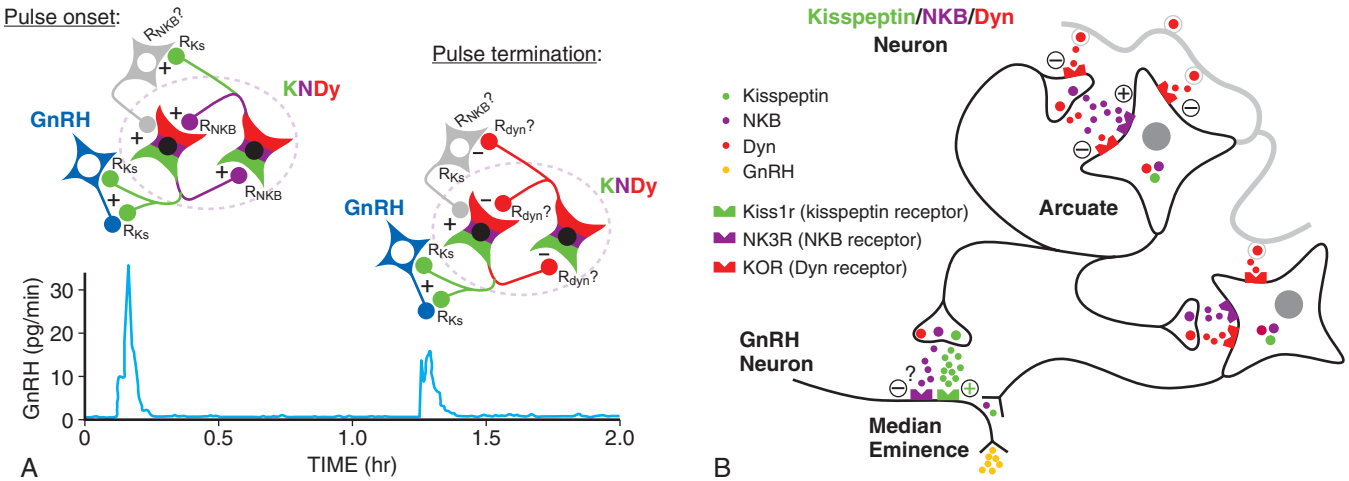

FIGURE 1.15 Working model regarding how KNDy neurons may participate in the generation of gonadotropin-releasing hormone *(GnRH)* pulses proposed by Goodman et al. (A) and Wakabayashi et al. (B). (A) By this model, neurokinin B *(NKB; magenta)* stimulates and dynorphin *(DYN; red)* suppresses kisspeptin release, with kisspeptin *(green)* stimulating GnRH neuronal firing. The onset of a GnRH pulse is triggered by an initial increase in NKB, which increases kisspeptin output. NKB also stimulates non-KNDy, kisspeptin-responsive interneurons that support or strengthen NKB stimulation of KNDy neurons. NKB stimulation of KNDy neurons also stimulates DYN release; after a short period of time, the increase in DYN suppresses kisspeptin (and NKB) release. This withdrawal of kisspeptin stimulation terminates the GnRH pulse. (B) By this model, KNDy neurons in the arcuate nucleus form a neural circuit, within which NKB *(magenta)* accelerates and Dyn *(red)* reduces KNDy neuron activation. These reciprocal effects of NKB and Dyn produce episodic activation of KNDy neurons, with KNDy neuronal activation increasing kisspeptin release at the median eminence. Kisspeptin in turn stimulates GnRH release into the hypophyseal portal system. *KOR*, κ-opioid receptor. *(Modified from Goodman RL, et al: A role for neurokinin B in pulsatile GnRH secretion in the ewe,* Neuroendocrinology *99:18–32, 2014; and Wakabayashi Y, et al: Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat.* J Neurosci *30:3124–3132, 2010.)*

secretion, which in turn initiates GnRH pulse secretion; subsequent dynorphin release then inhibits kisspeptin secretion, effecting GnRH pulse termination. Fig. 1.15 also depicts a similar working model based on experiments performed in goats.[128]

However, some data suggest that kisspeptin may not be required for GnRH pulse generation. In particular, frequent sampling studies reveal that humans with *KISSR* mutations demonstrate pulsatile LH release, albeit at low amplitude.[57,167] Similarly, a study suggested that puberty occurs and fertility is preserved in female mice with either (1) congenital absence of kisspeptin neurons or (2) congenital absence of neurons expressing Kiss1R.[61] When taken as a whole, available data imply that kisspeptin action may not be an absolute requirement for pulsatile GnRH secretion but that kisspeptin is required for normal GnRH pulse secretion and normally exerts an important influence on GnRH pulse generation.

Gonadotropin-Releasing Hormone Secretion During Development and in Adulthood

- *Gonadotropin secretion is robust during fetal development and early infancy but quiescent during childhood; puberty represents the reemergence and amplification of gonadotropin secretion, which stimulates gametogenesis, gonadal sex steroid secretion, and the physical manifestations of puberty.*
- *GnRH pulse frequency changes across the normal menstrual cycle, being highest in the late follicular phase and lowest in the luteal phase; these day-to-day changes primarily reflect the imposition or removal of progesterone negative feedback, and they contribute to the normal cyclic patterns of LH and FSH secretion.*
- *Men demonstrate consistent day-to-day GnRH pulse patterns, with GnRH pulse frequency approximating one pulse every 2 hours.*

Physiologic Development of Reproductive Neuroendocrine Function

Patterns of GnRH secretion change markedly across human development. Reproductive neuroendocrine events throughout early maturation, including both before and during the establishment of reproductive competence, are discussed in detail in Chapter 17. Briefly, GnRH and gonadotropin secretion is robust in utero, peaking in midgestation. In males, gonadotropin secretion markedly stimulates testicular androgen secretion, which is important for normal genital differentiation. The gestational increase in sex steroid (e.g., estradiol) production from the fetoplacental unit provides negative feedback to limit fetal GnRH and gonadotropin secretion. Birth is followed by a marked but transient (3 to 9 month) increase in GnRH and gonadotropin secretion (the "minipuberty of infancy"), perhaps related to the withdrawal of fetoplacental sex steroids. A marked sex difference of gonadotropin release is evident at this time, with LH concentrations being higher in males and FSH levels higher in females. The possibility that kisspeptin is important for the minipuberty of infancy is suggested by a patient with a compound heterozygote mutation of *KISSR*, who had micropenis, undescended testes, and undetectable serum gonadotropins at 2 months of age—a time usually marked by robust gonadotropin secretion.[168]

By late infancy or early childhood (earlier in boys than in girls), GnRH and gonadotropin secretion markedly

decreases, leading to a hypogonadotropic phase of childhood marked by low sex steroid concentrations—the *juvenile pause*. Studies of gonadotropin secretion in children reveal low LH and FSH concentrations, a high FSH-to-LH ratio, and low LH pulse amplitude and frequency.[169] Mechanisms accounting for low GnRH secretion during this time appear to include inhibition of the GnRH pulse generator *(neurobiologic brake)* by higher-order neuronal systems (e.g., involving GABA- and NPY-secreting neurons) and a developmental removal of stimulation (e.g., involving neurons secreting glutamate and norepinephrine).

Near the close of the first decade, a marked nocturnal amplification of pulsatile LH secretion indicates the neuroendocrine initiation of puberty. A majority of studies suggest that early pubertal subjects demonstrate sleep-entrained increases in LH (GnRH) pulse frequency and amplitude.[170] Gonadotropin concentrations rise across puberty,[171,172] stimulating gametogenesis, gonadal sex steroid secretion, and the development of secondary sexual characteristics. Mechanisms underlying puberty are poorly understood, but they likely reflect developmental remodeling of inhibitory and stimulatory neural circuits in the hypothalamus. For example, puberty has been associated with reductions of GABAergic inhibitory neurotransmission and an increase in excitatory neurotransmitters such as glutamate. Kisspeptin and NKB also appear to play important roles in human puberty because inactivating mutations of *KISS1*, *KISS1R*, *TAC3*, or *TACR3* result in pubertal failure. Conversely, central precocious puberty has been associated with gain-of-function *KISS1R* mutations[173] and *KISS1* mutations that may impair kisspeptin degradation.[174] In addition, loss-of-function mutations in the maternally imprinted gene *MKRN3* (encoding makorin ring finger protein 3) have been discovered as a common cause of central precocious puberty,[175-177] suggesting that *MKRN3* contributes to the neurobiologic brake. In addition to these transsynaptic mechanisms, neuroglial cells may contribute to the pubertal reactivation of GnRH secretion (e.g., by secretion of growth factors).[2]

Patterns of Pulsatile Gonadotropin-Releasing Hormone Secretion in Adults

Human studies using frequent blood sampling and pulse detection analysis have documented significant changes of LH (and by inference GnRH) pulse frequency throughout ovulatory cycles. Briefly, average LH (GnRH) pulse frequency is around one pulse every 90 minutes in the early follicular phase, and this gradually increases to approximately one pulse per hour by the late follicular phase. Although monkey studies suggest that GnRH pulse frequency slows during the mid-cycle surge,[178] human studies suggest no change in either LH or α-subunit pulse frequency at mid-cycle.[179,180] LH pulse frequency decreases markedly during the luteal phase, approximating one pulse every 3 to 8 hours. These day-to-day changes of GnRH pulse frequency appear to be important for normal hormonal changes across ovulatory cycles.[181,182]

In adult humans and nonhuman primates, GnRH pulses occur approximately once per hour in the (near) absence of sex steroid negative feedback (e.g., after surgical or natural menopause).[183-185] Similarly, the isolated human mediobasal hypothalamus secretes GnRH pulses every 60 to 100 minutes,[122] and LH pulses do not appear to exceed a once hourly frequency during any phase of the cycle.[184,186] These findings have contributed to the concept that a pulse frequency of approximately one per hour (a *circhoral* frequency) may be an inherent characteristic of the adult GnRH pulse generator and that day-to-day changes of GnRH pulse frequency in women reflect the imposition or removal of sex steroid (primarily progesterone) negative feedback.

LH pulse amplitude also changes across the menstrual cycle. LH pulse amplitude decreases slightly across the follicular phase, but it is greatly amplified at mid-cycle (i.e., during the LH surge). During the luteal phase, LH pulse amplitude is variable, but in general it is approximately twofold higher than that of the follicular phase. It is important to note that the amplitude of LH pulses can be modulated centrally via changes of GnRH released per pulse, at the pituitary gonadotrope via changes of gonadotrope responsiveness to GnRH, or both. Also of interest, LH pulse amplitude varies inversely with the preceding LH interpulse interval.[187]

In women, dynamic changes of gonadotropin secretion are required to achieve follicular development, ovulation, and preparation for possible pregnancy. In contrast, young men demonstrate consistent daily patterns of GnRH and gonadotropin secretion, with GnRH pulse frequency approximating one pulse every 2 hours. This achieves continuous spermatogenesis, and healthy men are prepared for fertilization at all times. In addition, day-to-day testosterone secretion remains relatively constant, although testosterone concentrations exhibit diurnal changes with peaks in the morning.

Feedback Regulation of Gonadotropin-Releasing Hormone and Gonadotropin Secretion

- *Throughout most of the cycle in women, estradiol restrains GnRH pulse amplitude and gonadotropin secretion, whereas progesterone restrains GnRH pulse frequency, but high estradiol concentrations at mid-cycle exert positive feedback on pituitary gonadotropes, provoking marked gonadotropin release—the gonadotropin surge.*
- *In men, GnRH secretion is restrained by testosterone and dihydrotestosterone (DHT) in addition to estradiol, a product of testosterone aromatization, but estradiol is the primary mediator of testosterone negative feedback at pituitary gonadotropes.*
- *Afferent neuronal pathways (e.g., kisspeptin and dynorphin neurons) are key mediators of sex steroid negative feedback on GnRH secretion.*

After puberty, gonadal hormones continually relay information about the state of gonadal function to the hypothalamic-pituitary axis. Hypothalamic areas involved with the regulation of GnRH secretion (and pituitary gonadotropes) express receptors for estrogen, progesterone, and androgen; and sex steroid feedback plays a predominant role in the physiologic modification of GnRH and gonadotropin secretion. These steroid feedback signals can thus alter gonadotropin feedforward signals to the gonads by influencing GnRH secretion, modulating pituitary (gonadotrope) responses to GnRH, or both. Under normal circumstances, these regulatory feedback loops maintain appropriate gonadal function. The negative feedback actions of pharmacological doses of sex steroids (e.g., combined oral contraceptives) suppress gonadotropins

and can be used for temporary contraception in women. Similar strategies are being developed for men.[188]

Negative Feedback Regulation of Gonadotropin-Releasing Hormone and Gonadotropin Secretion in Women

In women, estradiol concentrations correspond to follicular development during the follicular phase and corpus luteum function in the luteal phase. When concentrations are relatively low (i.e., excluding preovulatory concentrations), estrogens restrain gonadotropin secretion. This effect is most dramatically illustrated by markedly increased gonadotropin secretion in states of estrogen deficiency (i.e., the open loop condition) such as menopause[189] and aromatase deficiency.[190] The negative feedback effects of estradiol appear to be mediated primarily at the hypothalamus.[191] GnRH release (by direct measurement) is increased in ovariectomized sheep and monkeys, and this is reversed with estrogen replacement.[192,193] In human studies, GnRH release can be estimated using GnRH antagonists, with the premise that the degree of LH suppression after incomplete GnRH antagonism is inversely related to endogenous GnRH secretion. In postmenopausal women, the percent reduction in LH concentrations after incomplete GnRH receptor blockade is increased by estradiol replacement.[194]

Overall, studies indicate that estradiol reduces GnRH pulse amplitude but not GnRH pulse frequency.[193,195] Although one study in ovariectomized monkeys suggested that estradiol reduces the frequency of both hypothalamic multiple unit electrical activity and LH pulses,[196] available studies in postmenopausal women imply that estrogen replacement primarily reduces LH pulse amplitude rather than LH pulse frequency.[185,197] In addition, LH pulse frequency is maximal at one pulse per hour during the late follicular phase in women, when estradiol concentrations are relatively high.

Estrogens may also decrease pituitary LH responses to GnRH, although available data are mixed. For example, although estradiol acutely reduces LH release in GnRH-deficient monkeys and sheep receiving fixed-dose exogenous pulsatile GnRH,[198,199] relatively low (i.e., not preovulatory) doses of estradiol do not markedly influence LH release in GnRH-deficient women receiving fixed-dose exogenous pulsatile GnRH.[40] Interestingly, initial reductions of LH release with higher-dose estradiol may be followed by increased LH release[200]; this biphasic pattern presumably reflects initial negative feedback and later positive feedback.

Progesterone is the primary negative feedback regulator of day-to-day GnRH pulse frequency in women. LH pulse frequency slows in tandem with increases of progesterone (from the corpus luteum) in the early luteal phase,[186] and LH pulse frequency is inversely correlated with progesterone (but not estradiol) concentrations during the luteal-follicular transition.[201] Moreover, administration of progesterone to women during the follicular phase, when progesterone concentrations are usually low, slows LH pulse frequency.[202] Similarly, progesterone plus low-dose estradiol, but not estradiol alone, slows LH pulse frequency in postmenopausal women.[185,197] Importantly, the ability of progesterone to slow GnRH pulses appears to require the permissive presence of estradiol,[203,204] which likely reflects the ability of estrogen to increase hypothalamic progesterone receptor expression.[205,206] In contrast, progesterone inhibition of GnRH pulse frequency appears to be antagonized by androgens. For example, androgens increase GnRH neuronal firing rates in the mouse model[207]; and in hyperandrogenic women with PCOS, high GnRH pulse frequencies are relatively resistant to negative feedback suppression by progesterone and estradiol[208]—a defect that can be reversed by androgen receptor blockade.[209] These findings may be a consequence of androgen-mediated reductions of hypothalamic progesterone receptor expression.[210]

Positive Feedback and the Mid-Cycle Gonadotropin Surge

Most examples of endocrine feedback regulation involve negative feedback loops. However, the ovulatory menstrual cycle is unique in that it also depends on the positive feedback effects of sex steroids on the hypothalamic-pituitary axis. Specifically, high estradiol concentrations from the dominant ovarian follicle can produce a marked increase in gonadotropin release—a mid-cycle (or preovulatory) gonadotropin surge. In effect, estradiol from the preovulatory follicle signals to the hypothalamic-pituitary axis that follicular development is adequate for ovulation. Estradiol positive feedback appears to be related to both achieved estradiol concentrations and the duration of estradiol elevation, as demonstrated in both monkeys[211] and women.[212] Although the mid-cycle surge is characterized by a marked discharge of both gonadotropins, the increase in LH release exceeds that of FSH, with blood concentrations increasing approximately tenfold versus fourfold, respectively; thus it is often called the LH surge.

The mid-cycle gonadotropin surge uniformly involves positive feedback at the pituitary, markedly increasing gonadotrope responsiveness to GnRH stimulation.[213,214] However, whether the gonadotropin surge involves positive feedback at the hypothalamus is likely species dependent. GnRH secretion is augmented during the LH surge in rats[215] and sheep[216]; such GnRH surges appear to be physiologically important in these species. Similarly, GnRH release appears to increase in response to estradiol positive feedback in female monkeys.[217,218] However, LH surges can be induced by high estradiol concentrations in GnRH-deficient monkeys receiving constant dose (exogenous) GnRH delivered as once hourly pulses,[219] implying that a GnRH surge is not essential for LH surge generation in these animals.

One study using incomplete GnRH receptor antagonism to estimate GnRH secretion in women suggested that GnRH secretion may actually be reduced at mid-cycle compared with the late follicular and early luteal phases.[220] Similarly, pulsatile administration of constant-dose exogenous GnRH produces LH surges in GnRH-deficient women[214]; indeed, LH surges can occur in such women even when pulsatile GnRH doses are reduced at mid-cycle.[221] In addition, although pituitary metabolic activity (by positron emission tomography) increases in women at mid-cycle, hypothalamic metabolic activity does not.[222] Thus, although continued GnRH stimulation plays a critically important permissive role in LH surge generation in women (e.g., the surge can be prevented with GnRH receptor antagonists[223,224]), available data do not suggest that the gonadotropin surge is accompanied by increased GnRH release in women.

Progesterone increases pituitary gonadotropin responses to GnRH[225,226]; and when given in the luteal phase, the

progesterone receptor antagonist mifepristone decreases mean LH and LH amplitude[227,228] and reduces LH responses to exogenous GnRH.[228] Nonetheless, in ovariectomized but estradiol-replaced women, progesterone by itself (i.e., without high-dose estradiol) is unable to induce gonadotropin surges; indeed, progesterone can block LH surge generation when administered before high-dose estradiol.[229] However, progesterone augments gonadotropin secretion in the setting of preovulatory estradiol concentrations.[212,230] Although estradiol alone can provoke an LH surge, the late follicular rise in progesterone, which begins approximately 12 hours before the LH surge,[212,231] may be important for the full expression of the mid-cycle gonadotropin surge. For example, progesterone may increase the duration of the surge,[212] and the progesterone-receptor antagonist mifepristone can delay the surge.[232] Some studies suggest that progesterone may be important for the increase in FSH at mid-cycle,[229,230] whereas others suggest that estradiol alone is sufficient to produce a normal FSH surge.[212,233]

Of interest, although humans demonstrate sexual dimorphism of hypothalamic neuronal populations (e.g., kisspeptin), the circuitry required for LH surgelike activity appears to be present in male primates. For example, in male monkeys orchiectomized after puberty, estradiol administration can induce LH surges,[234] and ovarian transplants can induce LH surges and other neuroendocrine changes that maintain cyclic function of the transplanted ovary.[235] Estradiol and progesterone positive feedback can be experimentally induced in adult men,[236,237] but this is not a normal occurrence in male physiology.

Negative Feedback Regulation of Gonadotropin-Releasing Hormone and Gonadotropin Secretion in Men

In contrast to cyclic changes in women, normal postpubertal males demonstrate a relatively constant average LH pulse frequency of approximately one pulse every 120 minutes, related to relatively stable day-to-day sex steroid concentrations and corresponding negative feedback effects (tonic inhibition). In male monkeys, bilateral orchiectomy increases mean LH, LH pulse frequency, and LH pulse amplitude—effects that are prevented by physiologic testosterone replacement.[238] Similarly, testosterone-deficient men (e.g., related to either primary testicular failure or inhibition of steroidogenesis with ketoconazole) exhibit elevated mean LH, LH pulse frequency, and LH pulse amplitude—changes that are at least partially reversed by testosterone replacement.[239-241]

The importance of the androgen receptor in mediating testosterone negative feedback of LH secretion is suggested by elevated LH concentrations in the setting of androgen insensitivity[242] and androgen receptor blockade.[243,244] Moreover, mean LH is reduced in men by administration of DHT, a potent androgen that cannot be aromatized to estradiol.[245-247] However, a portion of synthesized testosterone is aromatized to estradiol, either in testicular Leydig cells or in nongonadal tissues, and estrogens can exert negative feedback actions at the hypothalamic-pituitary axis. For example, estradiol administration reduces LH secretion in normal and agonadal men.[243,245,248,249] Taken together, these findings suggest that both androgens and estrogens exert negative feedback actions at the hypothalamic-pituitary axis.

Many studies suggest that sex steroid negative feedback on the GnRH pulse generator is partly mediated by the androgen receptor. For example, DHT can reduce LH pulse frequency in men,[247] and some[243,244] but not all[250] studies suggest that androgen receptor blockade increases LH pulse frequency in men. Similarly, the marked increase in LH pulse frequency with high-dose ketoconazole (which inhibits both testicular/adrenal steroidogenesis and aromatase activity) is completely reversed by testosterone replacement despite low estradiol concentrations, whereas LH pulse frequency is only partly normalized with estradiol add-back alone (with persistently low testosterone concentrations).[241] On the other hand, aromatase inhibitors[251] and antiestrogens[248,252-254] increase LH pulse frequency in normal men, and estrogen treatment in men with aromatase deficiency reduces LH pulse frequency.[255] Overall, these data suggest that both androgens and estrogens mediate negative feedback of hypothalamic GnRH secretion.

In contrast to dual (androgen, estrogen) steroid feedback on GnRH pulse secretion, a number of studies suggest that estradiol is a primary mediator of negative feedback at the pituitary in men.[241,245,249,256] For example, androgen receptor blockade does not alter LH responses to exogenous GnRH in some studies,[244,250] and aromatase inhibition appears to prevent testosterone's ability to reduce GnRH-stimulated LH and FSH secretion.[240] Perhaps the most compelling studies were performed in GnRH-deficient men receiving pulsatile exogenous GnRH in constant doses. Under this GnRH clamp paradigm, testosterone alone and estradiol alone reduced LH and FSH concentrations, but DHT alone did not.[256] In another GnRH clamp study, LH amplitude and mean LH increased after high-dose ketoconazole, and LH parameters were normalized with estradiol, but not testosterone, add-back.[241] Overall, these study results imply that estradiol is the primary mediator of testosterone negative feedback at the pituitary gonadotrope.

Kisspeptin and KNDy Neurons as Mediators of Sex Steroid Feedback on Gonadotropin-Releasing Hormone Secretion

Evidence in rodents, sheep, and monkeys suggests that sex steroid feedback on GnRH secretion is mediated by kisspeptin (KNDy) neurons, at least in part. Although GnRH neurons express few sex steroid receptors, kisspeptin and KNDy neurons show a high degree of colocalization with estrogen receptors,[81] progesterone receptors,[124] and androgen receptors.[257] In the female monkey, kisspeptin expression is markedly reduced by estrogen or estrogen plus progesterone.[79] In addition, sex steroid deficiency is associated with increased kisspeptin expression in the infundibular (arcuate) nucleus—in parallel with circulating gonadotropins—and this is reversed by estradiol replacement in females[79,81] and either testosterone or estradiol replacement in males.[257] Compared with premenopausal women, the numbers of infundibular prodynorphin-expressing neurons are decreased in postmenopausal women.[258] A autopsy study in men suggests that the numbers of infundibular kisspeptin-containing cell bodies, fibers, and contacts onto GnRH neurons increase with age, hypothesized to reflect reduced steroid negative feedback.[259] In addition to influencing kisspeptin expression, estrogen modulates GnRH neuron responsiveness to kisspeptin in mice.[69] A model regarding the influence of KNDy neurons in negative feedback is shown in Fig. 1.16.

Kisspeptin also appears to play a key role in the mid-cycle LH surge in the female rodent. *Kiss1* and *Kiss1R* null mice do not exhibit LH surges,[260] and the LH surge can be prevented by a kisspeptin antagonist[261] or a monoclonal antibody to kisspeptin.[262] Of particular interest, available data in rodents suggest that kisspeptin neurons in the AVPV are stimulated by estradiol, whereas those in the arcuate nucleus are inhibited by estradiol.[81] In addition, estradiol administration directly into the medial preoptic area (location of the AVPV) induces LH surges, whereas estradiol administration directly into the mediobasal hypothalamus—which also raises estradiol levels in the pituitary—does not.[263] These and other findings in rodents have led to a model in which kisspeptin neurons in the arcuate nucleus regulate tonic GnRH release by mediating estrogen negative feedback, whereas kisspeptin neurons in the AVPV mediate the positive feedback effects of estrogen (Fig. 1.17).

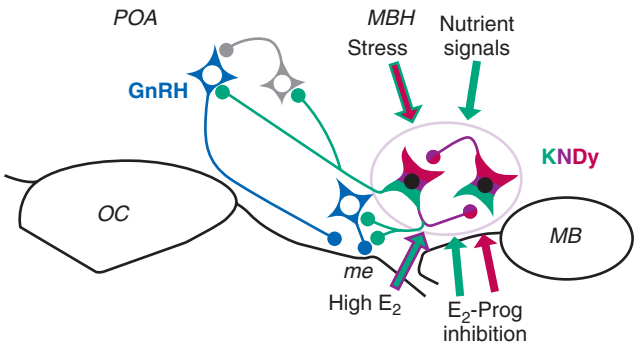

FIGURE 1.16 Model of KNDy signaling to gonadotropin-releasing hormone *(GnRH)* neurons, largely based on data obtained in sheep. KNDy peptides are kisspeptin *(green)*, which stimulates GnRH neurons, neurokinin B *(NKB; magenta)*, and dynorphin *(DYN; red)*. The major influences on GnRH secretion are shown, with putative effects on KNDy peptides denoted by the color of the arrow. For example, estradiol *(E_2)* inhibition may involve reductions of kisspeptin *(green arrow)*, whereas progesterone *(Prog)* inhibition likely involves an increase in DYN. *Arrows* with two colors signify that more than one KNDy peptide may mediate a given effect (e.g., in the ewe, stimulation of GnRH secretion by high E_2 may involve an increase in both kisspeptin and NKB). The possibility that kisspeptin stimulation of GnRH neurons is mediated by interneurons is shown by the gray cell. *MB*, Mammillary bodies; *MBH*, mediobasal hypothalamus; *ME*, median eminence; *OC*, optic chiasm; *POA*, preoptic area. *(Modified from Lehman MN, Coolen LM, Goodman RL: Mini review: kisspeptin/neurokinin B/dynorphin [KNDy] cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion,* Endocrinology *151:3479–3489, 2010.)*

It remains unclear whether a population of kisspeptin neurons homologous to those in the AVPV of rodents plays a similar role in primates. Although LH surge generation requires an intact preoptic area in rodents,[264] monkeys retain the ability to produce LH surges after isolation of the mediobasal hypothalamus from the remainder of the brain.[148] In addition, LH surges persisted after destruction of the preoptic area, which included the AVPV and suprachiasmatic nuclei, in one monkey study[265] but not in another.[266] Although human and monkey studies suggest the presence of kisspeptin neurons in the preoptic area,[79,80,267] it is unclear whether these are analogous to those of the AVPV in rodents. Of interest, kisspeptin expression increases in a caudal portion of the arcuate nucleus during the preovulatory period in both monkeys and sheep,[267-269] and some have suggested that kisspeptin neurons in this region could possibly represent a special population important for surge generation. Regardless, it is not certain that a unique population of kisspeptin neurons need be invoked in women, as increased GnRH secretion at mid-cycle (i.e., a GnRH surge) does not clearly occur in women (described earlier in the chapter). Thus how these intriguing observations in animal models relate to human neurophysiology during mid-cycle remains unclear.

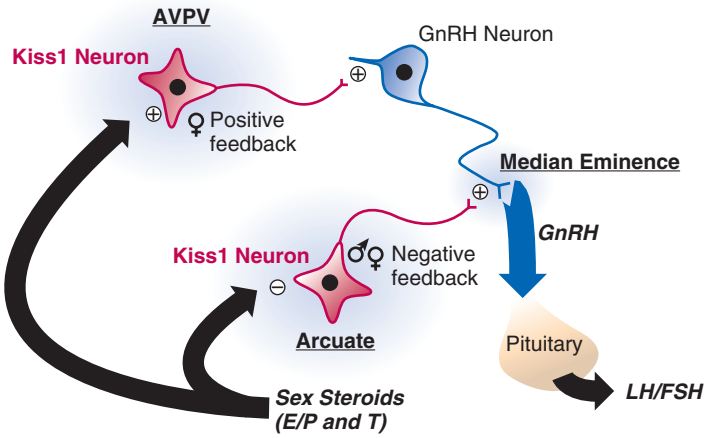

FIGURE 1.17 Model of kisspeptin-mediated feedback regulation of gonadotropin-releasing hormone *(GnRH)* and gonadotropin secretion in rodents. By this model, kisspeptin *(Kiss1)* neurons in the arcuate nucleus of males and females project to and stimulate GnRH neurons. This population of kisspeptin neurons is inhibited by sex-appropriate gonadal steroids (i.e., estradiol *[E]*, progesterone *[P]*, and testosterone *[T]*). Thus tonic GnRH secretion is primarily regulated by relatively low concentrations of estradiol via kisspeptin neurons in the arcuate nucleus. Females have another population of kisspeptin neurons in the anteroventral periventricular nucleus *(AVPV)* that also projects to and stimulates GnRH secretion. However, estradiol stimulates kisspeptin neurons in the AVPV—in contrast to estradiol inhibition of kisspeptin neurons in the arcuate nucleus. Thus, although high estradiol concentrations inhibit arcuate kisspeptin neurons in females, they stimulate AVPV kisspeptin neurons, resulting in a GnRH surge. *FSH*, Follicle-stimulating hormone; *LH*, luteinizing hormone. *(From Oakley AE, Clifton DK, Steiner RA: Kisspeptin signaling in the brain,* Endocr Rev *30:713–743, 2009.)*

Selective Regulation of Pituitary Follicle-Stimulating Hormone Secretion

Inhibins, activins, and follistatin preferentially influence FSH secretion and contribute to divergent release of LH and FSH throughout the menstrual cycle.[41] During the mid- to late follicular phase and the luteal phase, both estradiol and inhibins selectively inhibit FSH release from gonadotropes. Inhibins are heterodimer peptide members of the transforming growth factor (TGF)-β superfamily with two isoforms, inhibin A and inhibin B, which contain identical α-subunits but different β-subunits (βA for inhibin A, βB for inhibin B). Most inhibin is derived from the ovaries: inhibin B is secreted by ovarian granulosa cells, mainly during the early follicular phase in response to FSH stimulation; and inhibin A is primarily produced by the corpus luteum during the luteal phase in response to LH stimulation. The chief function of both inhibins is to inhibit FSH release from pituitary gonadotropes. In men, inhibin B is produced from Sertoli cells and is a key negative feedback regulator of pituitary FSH release, although estradiol also inhibits pituitary FSH release.[270]

Activin is a dimer peptide with three isoforms: activin A (βAβA), activin B (βBβB), and activin AB (βAβB). The activin β-subunits and the inhibin β-subunits are identical. Activin produced in pituitary gonadotropes stimulates production of FSH in a paracrine fashion. Follistatin is a monomer peptide synthesized by the anterior pituitary (including folliculostellate cells); it inhibits pituitary FSH secretion by binding activin, thus rendering it inactive. Of interest, gonadotrope follistatin production varies in parallel with GnRH pulse frequency—one of the mechanisms contributing to the differential effects of GnRH pulse frequency on LH and FSH release.[48,271] In contrast to inhibins, which act primarily via endocrine signaling, activin and follistatin produced in the pituitary influence FSH secretion via autocrine-paracrine signaling.

Reproductive Neuroendocrine Adaptations in Settings of Reduced Energy Availability, Stress, and Lactation

- *Reproductive function is impaired in the setting of decreased energy availability and/or stress; this primarily reflects central inhibition of GnRH pulse frequency and reduced gonadotropin secretion.*
- *Lactation is associated with suppressed pulsatile GnRH secretion and low gonadotropin concentrations; this relates to the high energy demands of lactation (reduced energy availability), hyperprolactinemia, and other (e.g., neural) mechanisms.*

Interface Between Reproductive Neuroendocrine Function and Energy Availability

Organisms require metabolic energy to support a number of processes, including maintenance of cellular function, muscle contraction (e.g., cardiac function, locomotion), thermogenesis, and growth. Low energy availability may result from short- or long-term reductions in calorie intake (e.g., famine, anorexia nervosa), insufficient calorie intake for metabolic demands (e.g., in the setting of significant exercise loads), or reduced ability to use energy sources (e.g., as may occur in severe diabetes). In such situations, energy use has opportunity costs: energy used for one process is no longer available for another. Thus energy-requiring processes are prioritized to favor those that are life sustaining.

Reproduction in women, pregnancy and lactation in particular, is metabolically demanding. For example, pregnancy requires an estimated additional 80,000 kilocalories.[272] Because reproduction is not imperative for individual survival, it is metabolically gated: reductions in energy availability can suppress reproductive function (nutritional infertility). This is biologically advantageous for the individual and, ultimately, the species. As such, it can be seen as an appropriate adaptive response. This process is believed to be at the center of functional hypothalamic amenorrhea, a reversible condition of suppressed hypothalamic-pituitary function occurring in the absence of anatomic abnormalities and often accompanied by reduced body weight, disordered eating (e.g., restrictive eating patterns), excessive exercise, and/or psychological stress.

The functional relationships between metabolic status and reproductive function are mediated by neural systems located in the hypothalamus, and functional hypothalamic amenorrhea is characterized by impaired GnRH and gonadotropin secretion. Although a majority of women with functional hypothalamic amenorrhea demonstrate low LH pulse frequency,[273,274] such patients may demonstrate variable LH pulse patterns as a group—including absent pulses, low frequency and amplitude, low frequency only, low amplitude only, and (apparently) normal frequency and amplitude; and patterns can change across time in the same woman.[8,273,274] In these patients, GnRH and gonadotropin secretion is inadequate for normal follicular development, estrogen production, and mid-cycle gonadotropin surges, but cyclic ovulation and fertility can be restored with pulsatile administration of exogenous GnRH.[8]

Some investigators have proposed that reduced reproductive function in functional hypothalamic amenorrhea primarily reflects insufficient body fat stores—the *critical fatness hypothesis*.[275] However, a substantial body of data suggests that reduced energy availability is the primary cause of reduced reproductive function in these settings. For example, body fat does not reliably distinguish amenorrheic from eumenorrheic athletes.[276] In addition, calorie restriction can result in amenorrhea before weight loss; in those with a history of eating disorders, amenorrhea can persist after weight restoration.[277] Likewise, findings consistent with functional hypothalamic amenorrhea can be observed shortly after bariatric surgery for severe obesity—in the setting of negative energy balance but while still obese (e.g., body mass index approximately 35 kg/m^2).[278] Moreover, altered LH pulsatility is observed very quickly (within 5 days) of controlled reductions of energy availability.[279] Experiments in women and monkeys suggest that reproductive function may not be impaired until energy availability is reduced by more than 30% (Fig. 1.18).[279,280] Overall, these findings suggest that altered reproductive function in this setting reflects altered energy availability rather than body composition per se. It is important to note that energy balance (and thus body weight) can be maintained in the face of calorie restriction by reducing metabolic rate[281] and by suspending "noncritical" but energy-requiring functions such as reproduction.

Although some have posited a specific influence of exercise, calorie supplementation to maintain adequate energy availability appears to prevent alterations of LH secretion despite significant daily exercise loads.[282] Similarly, whereas

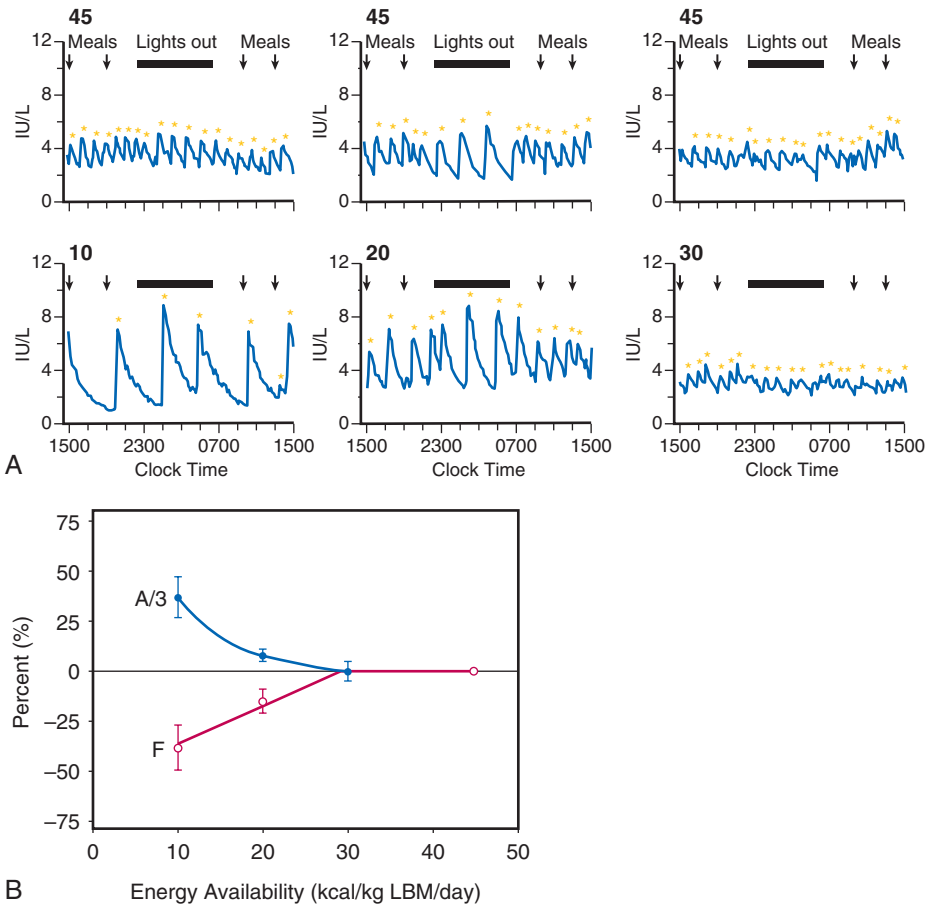

FIGURE 1.18 Influence of energy availability on luteinizing hormone *(LH)* pulsatility in healthy women. (A) Representative 24-hour LH time series in three women under different conditions of energy availability. These studies were designed to assess the effects of exercise and reduced energy availability in habitually sedentary women with regular menstrual cycles. In this study, exercise energy expenditure was substantial for all participants at approximately 840 kcal/day, and energy availability was altered via variable calorie intake. When energy availability is defined as dietary energy intake minus exercise energy expenditure—an estimate of the energy available for non-exercise-related functions—and normalized to fat-free mass (thus expressed as kcal/kg lean body mass *[LBM]* per day), 45 kcal/kg LBM per day approximates balanced energy availability, and LH profiles under this condition are shown on top. Conditions of restricted energy availability (i.e., 10, 20, and 30 kcal/kg LBM per day) are shown along the bottom. Significant LH pulses are denoted by *asterisks*; *arrows* denote the timing of meals; and *black bars* denote lights out periods. (B) Association of energy availability and LH pulse characteristics. Energy availability is shown on the *x*-axis; on the *y*-axis, LH pulse amplitude *(solid circles)* and LH pulse frequency *(open circles)* are expressed as changes relative to values observed at 45 kcal/kg LBM per day. (Note that changes of LH pulse amplitude are divided by three.) Although energy availability reductions to 30 kcal/kg LBM per day did not alter LH pulse characteristics, reductions below 30 kcal/kg LBM per day were associated with progressive reductions of LH pulse frequency and corresponding increases of LH pulse amplitude. *(Modified from Loucks AB, Thuma JR: Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women,* J Clin Endocrinol Metab *88:297–311, 2003.)*

amenorrhea can be induced in monkeys by gradually increasing daily exercise in the setting of constant food intake,[283] providing supplemental calories reverses amenorrhea despite continued exercise.[284]

The neurobiologic mechanisms underlying functional hypothalamic amenorrhea, and mechanisms underlying the influence of nutritional status on the reproductive system in general, remain poorly understood. Chronic energy deprivation is associated with myriad neuroendocrine adaptations and hormonal changes, including reductions of leptin, insulin, insulin-like growth hormone-1 (IGF-1), and thyroid hormone concentrations; increases of growth hormone and ghrelin levels; and activation of the hypothalamic-pituitary-adrenal (HPA) axis.[285] A number of these alterations can influence GnRH and gonadotropin secretion and may mediate the influence of low energy availability on reproductive function. Much interest has centered on the permissive role of leptin, a hormone derived from adipose tissue that functions to signal metabolic status to central systems, influencing feeding, energy expenditure, and reproduction. Humans and mice lacking leptin or the leptin receptor (LepR) (e.g., ob/ob and db/db mice, respectively) have pubertal failure and infertility; in the setting of leptin deficiency, these manifestations can be reversed with leptin administration.[286,287] Low leptin levels have also been observed in women with functional hypothalamic amenorrhea,[288] and a specific role for leptin was suggested by a small study in which recombinant human leptin administration improved LH pulse secretion and estradiol concentrations in women with functional hypothalamic amenorrhea.[289] Although serum leptin concentrations generally correlate with fat mass, leptin levels can change rapidly and are suppressed with maneuvers known to suppress LH secretion, such as marked short-term energy restriction.[290]

A number of neuropeptides have been implicated in the influence of energy availability on reproductive function, including kisspeptin, NPY, galanin-like peptide (GALP),

β-endorphin, CRH, ghrelin, and polypeptide YY. Although metabolic signals could potentially act directly on GnRH neurons, many studies suggest that afferent neural circuits are involved. For example, studies in mice suggest that metabolic signals can be relayed to the GnRH pulse generator from different areas in the brain, such as the ventral premammillary nucleus (e.g., leptin effects)[291] and the area postrema (e.g., in the absence of usable glucose).[292] A growing body of evidence suggests that the influence of energy availability on reproductive neuroendocrine function is at least partly mediated by kisspeptin neurons.[293,294] For example, leptin-deficient (ob/ob) mice demonstrate reduced hypothalamic *Kiss1* expression, which is partially reversed with leptin administration.[295] Some studies suggest that increased opioid tone may contribute to slow GnRH pulses in functional hypothalamic amenorrhea.[274,296-298]

Even though successful reproduction is not metabolically costly for males, male reproductive function can also be affected by metabolic stress. For example, healthy young men participating in US Army Ranger training, which involves multiple stressors including intermittent extreme calorie restriction and weight loss (10 to 12 kg on average), can experience reductions in LH and suppression of testosterone concentrations to near castrate levels.[299] Increased calorie intake allowed prompt recovery of testosterone in this study, even without altering other associated stressors (e.g., exercise, sleep deprivation). Also of interest, exogenous leptin administration prevented the fall of LH release and testosterone concentrations associated with short-term fasting in men.[300] In addition, anorexia nervosa in adolescent boys and men can be associated with marked hypogonadotropic hypogonadism.[301] Energy-sensitive reproductive function in males would also be expected to delay reproduction during times of reduced energy availability; these regulatory pathways may have developed in males because of advantages imparted to other members of the species (e.g., mates and offspring).

Impact of Stress on Reproductive Neuroendocrine Function

Functional hypothalamic amenorrhea in the setting of reduced energy availability represents a particular form of stress-related reproductive suppression. The term *stressor* refers to a real or potential threat to homeostasis, such as injury, illness, temperature extremes, reduced energy availability, predator proximity, and situations that provoke psychological distress. The nature of the stress response depends on the precise nature of the stressor but typically involves both neural and neuroendocrine responses. A group of neurons in the hypothalamic paraventricular nucleus project to the median eminence, where they secrete CRH into the hypophyseal portal system. CRH (and, to some degree, cosecreted AVP) stimulates corticotrope cells in the anterior pituitary to release ACTH, which in turn stimulates adrenal glucocorticoid (cortisol) production. A subset of paraventricular neurons is also involved with the regulation of the autonomic sympathetic nervous system, which includes neural pathways linked to the brain stem, spinal cord, and adrenal medulla (e.g., the sympathoadrenal axis). Other components of the stress response include central arousal systems and the locus ceruleus, a nucleus in the brainstem involved with emotional and cognitive responses to stress. Thus stressors trigger integrated neural, endocrine, and behavioral responses that promote short-term maintenance of homeostasis and survival. For example, activation of the sympathoadrenal axis leads to increased epinephrine secretion—an important component of the fight-or-flight response—whereas activation of the HPA axis with increased cortisol secretion enhances energy mobilization.

Chronic stress and marked acute stress can inhibit reproductive function—an appropriate adaptive response when homeostasis is threatened. For example, critical illness is associated with a reversible hypogonadotropic hypogonadism.[302,303] Mechanisms underlying the suppression of reproductive function during stress are highly complex; although suppression of GnRH secretion is a major component, direct pituitary and gonadal effects may also occur. Notably, the specific effects of stress on various aspects of reproductive function appear to depend on a number of factors including species, sex, hormonal milieu (e.g., gonad intact vs. castrate), and the specific type of stress experienced.

A number of mediators have been implicated in stress-related inhibition of GnRH secretion, including CRH (which activates the HPA axis but also appears to have central effects), CRH-like peptides called urocortins, AVP, ACTH, EOPs (e.g., β-endorphin), and cortisol, in addition to noradrenergic, GABAergic, and serotoninergic neural pathways. For example, intracerebroventricular injection of CRH reduces multiple unit electrical activity in the mediobasal hypothalamus in monkeys[304]; and CRH antagonists can prevent some forms of stress-related LH suppression.[305] Naloxone can block CRH-related LH suppression in monkeys,[306] suggesting the involvement of EOPs in this process.

Notably, some data suggest that stress plays a role in functional hypothalamic amenorrhea. For example, amenorrheic athletes and women with anorexia nervosa demonstrate evidence of HPA axis activation (e.g., elevated cortisol concentrations).[285,307] In addition, functional hypothalamic amenorrhea in women may be associated with evidence of higher psychological stress, including perfectionism, a history of unfavorable childhood experiences, and difficulty coping with stressors[308,309]; reproductive function may be improved in some with cognitive behavioral therapy[310] or hypnotherapy.[311] Studies in female monkeys provide corroborating evidence: in one study, very few monkeys demonstrated altered reproductive function when exposed to either (1) psychosocial stress (relocation to new housing setting with unfamiliar monkeys) or (2) mild dietary restriction plus daily exercise, but the combination was associated with altered cycle length or anovulation in a majority.[312]

For unclear reasons, the degree to which stressors (e.g., reduced energy availability) interrupt reproductive function is variable among individual women (i.e., hypothalamus robustus vs. hypothalamus fragilis; stress sensitive vs. stress resilient). As suggested previously, it is likely that a number of factors (e.g., reduced energy availability, stress) can interact to impact GnRH secretion. In addition, a study suggested that mutations in genes associated with hypogonadotropic hypogonadism, including *KAL1*, *FGFR1*, *PROKR2*, and the GnRH receptor *(GNRHR)* genes, are more likely to be identified in women with functional hypothalamic amenorrhea compared with normally cycling women.[313] Thus it seems likely that underlying genetic (and epigenetic) architecture

plays an important role in reproductive susceptibility to reduced energy availability and stress.

Lactation and Reproductive Neuroendocrine Function

High prolactin concentrations during pregnancy and suckling in the postpartum period stimulate milk production, which, for much of human history, was effectively the only source of nutrition for infants. Suckling also leads to posterior pituitary release of oxytocin, which stimulates contraction of myoepithelial cells within mammary gland acini, causing milk ejection. Lactation is also associated with amenorrhea and subfertility. The likelihood of pregnancy during the first 6 months postpartum is low (less than 2%) in fully breastfeeding, amenorrheic women,[314] and some lactating women may remain amenorrheic for years. Because a short interval between births can place infant well-being at risk, lactational amenorrhea has ostensibly been an important adaptation enhancing infant survival in many cultures both past and present.[315]

During pregnancy, high placental sex steroid (estradiol, progesterone) and prolactin concentrations markedly suppress GnRH and gonadotropin secretion and prevent follicular development. In the absence of lactation, cyclic hypothalamic-pituitary-ovarian activity typically resumes in the 8 weeks after parturition. However, in the setting of lactation, pulsatile GnRH remains suppressed (e.g., low frequency pulses), with consequent impairment of LH secretion and estradiol production.[316] The reduction in GnRH secretion during lactation is suggested by a marked reduction in multiple unit electrical activity in the mediobasal hypothalamus in nursing monkeys[317] and by the ability of pulsatile exogenous GnRH to restore ovarian function in amenorrheic lactating women.[318]

Mechanisms underlying lactational amenorrhea are incompletely understood. Lactation is associated with a very high metabolic cost: daily production of 750 to 1000 mL of human milk requires approximately 500 to 600 kilocalories a day,[319] some of which is obtained from fat stores and increased food intake. Nonetheless, the high energy requirements of lactation—which are approximately twice that of pregnancy—may induce some or all of the aforementioned mechanisms that inhibit pulsatile GnRH secretion in the setting of reduced energy availability. Animal (chiefly rodent) studies reveal that lactation is associated with activation of orexigenic neural systems (e.g., NPY) and inhibition of anorexigenic neural systems within the hypothalamus—changes that may partly relate to alterations of peripheral metabolic cues (e.g., leptin, insulin).[320] Such alterations increase food intake and may suppress GnRH neuronal activity, either by direct effects on the GnRH neuronal network or by modification of key afferent systems such as kisspeptin. As an example of the former, NPY neuronal activation during lactation may directly inhibit GnRH neuronal activity.[321] As an example of the latter, expression of both kisspeptin and NKB in the arcuate nucleus is reduced in lactating rodents.[134,322]

Hyperprolactinemia suppresses GnRH pulsatility, at least in part via increased hypothalamic opioids,[323] and suppression of GnRH secretion during periods of lactation may partly reflect high prolactin concentrations. Data in rodent models suggest that prolactin suppression of GnRH secretion is at least partly mediated by kisspeptin: kisspeptin neurons appear to express prolactin receptors[324]; hyperprolactinemia has been associated with reduced hypothalamic *Kiss1* expression[93,325]; and kisspeptin administration reversed hyperprolactinemia-mediated hypogonadotropic anovulation.[93] However, prolactin levels gradually decrease to normal despite continued breastfeeding; thus the activity of the hypothalamic-pituitary-ovarian axis may not correlate well with circulating prolactin concentrations.

Importantly, the intensity (e.g., frequency, duration) of suckling appears to be an important determinant of contraceptive effectiveness in women, and the suckling stimulus may inhibit the hypothalamic-pituitary-ovarian axis through additional (e.g., neural) mechanisms.

Miscellaneous Physiologic Influences on Gonadotropin-Releasing Hormone Secretion

Circadian Changes

Diurnal rhythms (i.e., those that cycle once a day) are frequently observed in endocrinology, and this includes the reproductive system. Diurnal rhythms are called circadian rhythms if they are internally (endogenously) driven rhythms, although such rhythms are usually entrained (synchronized) to environmental cues (e.g., light-dark cycle). Circadian rhythms are dictated by a "master clock" located in the suprachiasmatic nucleus of the hypothalamus and are derived from complex intracellular interactions involving the so-called *clock genes*, which participate in feedback interactions that generate recurring cyclic activity.[326] Such clocks in the suprachiasmatic nucleus can be synchronized by light-dark signals received from the retina and transferred to the suprachiasmatic nucleus via the optic nerve (retinohypothalamic tract).

Depending on such factors as species and sex, both basal gonadotropin secretion and LH surges may exhibit diurnal rhythms. As a prominent example, LH surges in female rats are specifically confined to the late afternoon, which is shortly before rats become active (i.e., when copulation is most likely).[327] It is believed that this reflects a daily stimulus generated by the suprachiasmatic nucleus but relayed to the GnRH neuronal network only in the presence of preovulatory estradiol concentrations. Thus ovulation in rats is optimally timed to coincide with sexual opportunity and receptivity.

In contrast to rodents, LH surges do not appear to be constrained to a specific time of day in monkeys; for example, LH surges can be advanced by 12 to 18 hours with supraphysiologic estradiol administration.[211] Some studies in women suggest that LH surges tend to be initiated in the morning.[328,329] For example, in one study of 19 ovulatory women, LH surges were initiated in the early morning hours (approximately 4:00 to 8:00 a.m.)[328]; in one study of 155 spontaneous cycles, the estimated time of LH surge initiation was between midnight and 8:00 a.m. in 85% of cycles.[329] (These studies do not confirm a true circadian signal, and putative confinement of ovulation to the morning hours could reflect environmental cues.) In contrast to these findings, a detailed study of mid-cycle gonadotropin surges in women suggested that surge initiation is not constrained to a certain time of day.[231] In addition, the potential relevance of a specific daily timing of ovulation in women is uncertain since ovulation typically occurs some 36 hours after the LH surge; the likelihood of conception when sexual intercourse occurs on

the day before ovulation (approximately 40%) is similar to conception rates when intercourse occurs on the day of ovulation.[330]

Although humans demonstrate clear diurnal changes of gonadotropins, studies in women who were carefully assessed during the early follicular phase[331] or after menopause[332] suggest that LH and FSH secretory parameters (including LH pulse frequency and amplitude) do not exhibit circadian changes after controlling for sleep status, body position, light exposure, activity level, and nutritional cues.

Sleep

Although precise mechanisms are unclear, sleep can have a major influence on pulsatile LH (and by inference GnRH) secretion. For example, the nocturnal amplification of LH pulsatility during puberty is specifically related to sleep: it generally begins within an hour of sleep onset, and it follows sleep reversal.[333,334] Although the data relating LH pulses to sleep stage are incomplete, early studies suggested that sleep-related pulses during puberty occur primarily during non–rapid eye movement (REM) sleep.[333,334] Further refining this concept, more recent studies suggest a strong relationship between slow wave sleep and LH pulse initiation during puberty.[335,336]

Sleep also influences LH pulse secretion in adult women, primarily in the form of sleep-related slowing of LH pulse frequency, which is most prominent during the early follicular phase,[184,186,337,338] but also occurs in the late follicular phase.[279,339] Nocturnal slowing of LH pulse frequency in women during the early follicular phase is specifically related to sleep because it follows sleep reversal.[340] During this time, LH pulses are uncommon during REM and slow wave sleep and more common following brief awakenings.[340] Such slowing may be mediated by hypothalamic opioids because naloxone appeared to prevent the sleep-associated decrease in LH pulse frequency.[341]

Interestingly, sleep appears to interact with other determinants of pulsatile GnRH secretion. For example, a study in early pubertal girls suggested that progesterone rapidly and profoundly suppresses LH pulse frequency during waking hours but not during nighttime (sleeping) hours.[342] Similarly, studies performed during the late follicular phase in normal women suggested that dietary calorie restriction preferentially reduces daytime LH pulse frequency.[282,339] These findings suggest differential control of GnRH pulse frequency depending on sleep status in human females.

Taken together, the aforementioned data imply that sleep influences LH pulse frequency and that the effect of sleep can be modulated by such factors as developmental stage and sex steroid milieu. The physiologic relevance of sleep-associated changes of GnRH secretion remains unclear, but such changes have been postulated to contribute to normal gonadotropin production across puberty[170] and to the prominence of FSH secretion during the early follicular phase in postpubertal women.[340]

Pheromones

Pheromones are chemicals transmitted through the air that can influence reproductive function and sexual behavior in other individuals within many mammalian species. For example, the presence of a novel and sexually mature male mouse can synchronize estrous cycles among female mice; this so-called *Whitten effect* is presumably mediated by pheromones.[343] Similarly, pheromones produced by male sheep and goats can induce out-of-season ovulation in females—the *male effect*.[344]

The role of pheromones in humans remains unclear. Menstrual synchrony (sometimes called the McClintock effect) is a putative phenomenon in which menstrual cycles of women living in close proximity become synchronized[345]; this has been cited as physiologic evidence of pheromone functionality in humans. Axillary (armpit) compounds obtained from women during the late follicular phase have been reported to advance ovulatory timing in recipient women.[346] However, supportive research has been criticized on methodologic grounds, and the existence of this phenomenon remains controversial.[347] In addition, although the vomeronasal organ—believed to be responsible for pheromone detection in animals—develops in utero in humans, it subsequently regresses and is largely believed to be nonfunctional in adults.[348]

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