NEUROMODULATION IN POLYCYSTIC OVARY SYNDROME

Brinda N. Kalro, MD, Tammy L. Loucks, MPH, and Sarah L. Berga, MD

Polycystic ovary syndrome (PCOS) is classically defined as chronic anovulation in the presence of hyperandrogenism of ovarian origin. The proximate cause of anovulation in PCOS is aberrant gonadotropin secretion characterized by elevated circulating luteinizing hormone (LH) and insufficient follicle-stimulating hormone (FSH) to sustain folliculogenesis. These gonadotropin aberrations, which result in an increased LH to FSH ratio, are sustained by a rapid gonadotropin-releasing hormone (GnRH) pulse frequency that manifests in the peripheral circulation as increased LH pulse amplitude and frequency. The development of stromal and thecal hyperplasia with ensuing ovarian hyperandrogenism characteristic of this syndrome is posited to be a consequence of chronic exposure to this endogenous LH excess. Decreased FSH secretion is the most immediate cause of follicular arrest and anovulation because exogenous FSH administration permits ovulation. The relatively lower FSH concentrations can be explained by a more rapid GnRH pulse frequency or may be secondary to a differential impact of paracrine and endocrine factors on the pituitary and its response to GnRH. Although increased LH and decreased FSH secretion are believed to be an integral part of this disorder and are necessary to sustain the metabolic and

The work was supported in part by grants R01-MH50748, RR-00056, and U54-HD08610 from the Magee-Womens Research Institute and the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

From the Departments of Obstetrics, Gynecology, and Reproductive Services (BNK, SLB) and Psychiatry (SLB), Division of Reproductive Endocrinology and Infertility (BNK, SLB), Magee-Womens Hospital, University of Pittsburgh School of Medicine; and Magee-Womens Research Institute (TLL), Pittsburgh, Pennsylvania.

OBSTETRICS AND GYNECOLOGY CLINICS OF NORTH AMERICA

VOLUME 28 • NUMBER 1 • MARCH 2001
endocrine features, the hypothalamic/pituitary abnormalities are not necessarily the primary etiology. Nevertheless, because increased central drive is a fundamental feature of PCOS, any proposed explanation for PCOS/hyperandrogenic anovulation must account for the gonadotropin aberrations. Strategies to slow the GnRH pulse generator may expand therapeutic options.

As understanding of the basic mechanisms contributing to the neuromodulation of GnRH pulsatility has evolved, so too have the explanations for altered GnRH drive. The cellular and neural mechanisms that permit a more rapid GnRH pulse frequency are a subject of ongoing investigation; however, it is difficult to study the role of these cellular mechanisms directly in women with PCOS. The effect of altering a given endocrine or other parameter on GnRH pulsatility can be estimated by following LH pulse parameters in the peripheral circulation. This article reviews what is known about the neuromodulation of GnRH in PCOS.

FEATURES OF POLYCYSTIC OVARY SYNDROME

It is estimated that 6% to 10% of reproductive-aged women have PCOS. This heterogeneous disorder with its characteristic endocrine and metabolic components is a common cause of anovulatory infertility. Hyperandrogenism and increased GnRH/LH pulsatility constitute the central endocrine components responsible for anovulation, whereas insulin resistance and hyperinsulinemia characterize the key metabolic components. The symptoms associated with this disorder are clinical manifestations with varying degrees of phenotypic expression in affected individuals. Features of PCOS can develop in women who have long-standing hyperandrogenic states, such as congenital adrenal hyperplasia or exogenous chronic androgen administration.

In 1921 Achard and Thiers described the association between hyperandrogenism and abnormal carbohydrate metabolism and called this condition the diabetes of bearded women. In 1935 Stein and Leventhal described the classic triad of bilateral polycystic ovaries, anovulation, and hirsutism.

Chronic anovulation, hyperandrogenism, and an increased LH to FSH ratio are cardinal features of this disorder. Fifty percent to 70% of women with PCOS are obese. Another pathognomonic feature of PCOS is hyperplasia of the stromal and thecal compartment with a resultant increase in stromal and total ovarian volume. Atretic and immature follicles are typically peripherally arranged, resulting in a "pearl necklace" appearance. Both features account for the typical polycystic ovary morphology on ultrasonography that distinguishes this condition from multicystic ovaries, which accompany anovulation owing to a variety of other causes. Excess LH stimulation from increased GnRH drive seems to be essential in maintaining the morphologic features that are characteristic of PCOS. Nevertheless, a small proportion of women do not display the typical polycystic ovary morphology on ultrasonography.
despite documented endocrine features of PCOS,110 and some eumenorrheic women have polystic-appearing ovaries but do not manifest anovulation or other typical endocrine features of PCOS.56

Koskinen and co-workers92 demonstrated that the highest sensitivity (98%) and specificity (93%) for the diagnosis of PCOS were obtained by using the combination of LH, FSH, and androstenedione. Testosterone levels alone were not as useful, and adding testosterone levels to the triad of LH, FSH, and androstenedione did not further improve the diagnostic yield.

HISTORICAL BACKGROUND

Neural regulation of gonadotropin secretion was first demonstrated by Harris.67 Haterius and Derbyshire71 induced ovulation by stimulation of the tuber cinereum and preoptic area. In 1933 Popa and Fielding131 described the hypothalamic–hypophysial portal system, suggesting the concept that the hypothalamus was critical in regulating the function of the anterior pituitary.62 Several models, such as stalk transection69 and pituitary transplantation68,121 supported the hypothesis that hypothalamic substances regulated pituitary function. The suggestion that the adrenergic system may be involved in the regulation of hypothalamic-hypophysial release of gonadotropins was first made by Sawyer and colleagues in 1947.156 In 1955 Harris70 proposed a classic model describing the relation between the external environment and hypothalamic-pituitary-gonadal function. Although these components were known at that time, their complex interactions were poorly understood. Two peptide hormones, LH and FSH, were believed to control gonadal hormones and, in turn, were thought to be regulated by feedback from the gonads and pituitary. FSH and LH were hence termed gonadotropic hormones. Higher vertebrate studies revealed that a hypothalamic neurohormone, a decapeptide, reached the pituitary from the hypothalamus via the hypophysseal portal circulation. Several years later, Knobil demonstrated pulsatile LH secretion.39 These LH pulses were found to occur approximately once per hour, could be induced by a bolus injection of the decapeptide hormone into the circulation, and were believed to reflect the pulsatile nature of GnRH secretion from the hypothalamus into the hypophysseal portal system. A few years later, Karsch and colleagues86 confirmed the existence of the pulsatile nature of GnRH secretion in the ewe. This observation led to the notion that a signal generator existed in the central nervous system (CNS) that was responsible for the rhythmic release of the neuropeptide GnRH. This system was called "the GnRH pulse generator." Yen and co-workers197 identified three essential components: (1) the CNS–hypothalamic complex that served as a signal generator, (2) the pituitary as a signal transmitter, and (3) the cyclic ovarian steroid output as the signal modulator.197

In 1977 Guillemin and Schally received the Nobel Prize for their remarkable feat of synthesizing and characterizing this neurohormone.
The hormone was called luteinizing hormone–releasing hormone (LHRH) or, more appropriately, GnRH because it regulates the secretion of FSH and LH.

In 1970 Yen and co-workers described the classic derangement in the hypothalamic–pituitary secretion as a relative increase in LH and relative decrease in FSH levels in PCOS. They obtained daily blood samples from women with PCOS diagnosed by ovarian wedge resection and used ovulatory women as controls. It was not clear whether the LH hypersecretion was caused by a primary defect in the hypothalamic–pituitary axis or was a consequence of inappropriate feedback from the abnormal steroidogenesis of the polycystic ovary. An intravenous infusion of 17β-estradiol to women with PCOS was effective in rapidly reducing circulating LH levels and attenuating the amplitude of the LH pulses, suggesting an intact negative feedback loop; however, FSH levels remained unchanged. When these women were given clomiphene, there was an appropriate increment in LH and FSH levels comparable with the effects of clomiphene in normally cycling women. The administration of exogenous GnRH resulted in a significantly greater increase in net LH and FSH in women with PCOS when compared with eumenorrheic women in the early and late follicular phases of the cycle, indicating increased activity of the GnRH pulse generator in the women with PCOS. In vitro studies revealed that estrogen increased the gonadotroph fraction responding to GnRH, suggesting that the increased pituitary sensitivity displayed in PCOS may be partially mediated by excess estrone from aromatization of excess androgens.

Accelerated LH pulse frequency is evident in lean and obese women with PCOS and is most likely a reflection of an increase in the GnRH pulse frequency; however, increased GnRH pulsatility may not be the only explanation for the increased LH pulse amplitude. To date, the cause of the increased GnRH pulse frequency in PCOS remains controversial. Because the frequency of LH, and presumably GnRH, slows in response to progestin-induced opioidergic tone, at least some central neuroregulatory mechanisms remain inducible.

THE GnRH PULSE GENERATOR

It is not clear that there is only one cause of PCOS. The proximate causes of the accompanying anovulation are excess LH and insufficient FSH. These abnormalities, in turn, are at least partly sustained by increased GnRH drive. Given these considerations, it becomes material to discover the cause of the alteration in GnRH input. A prerequisite is a clear understanding of the GnRH pulse generator.

The GnRH pulse generator refers to the synchronized activity of GnRH neurons that are widely distributed in the mediobasal hypothalamus. GnRH is synthesized in neuronal cell bodies that have migrated during fetal life from the olfactory placode into the hypothalamus.
GnRH is secreted in pulses from neuroendocrine terminals localized in the median eminence. GnRH neurons are inherently pulsatile and synapse primarily with one another, but the secretory activity is also controlled by classic neurotransmitter systems and by glial regulatory processes that most likely involve growth factors. Recently, neurovascular regulatory processes have been identified in the median eminence where the endothelial cells of the pituitary portal vessels wrap around GnRH secretory terminals. In the median eminence, various signaling molecules conveyed in the blood can communicate with GnRH and other neurons without penetrating the classic blood–brain barrier. In theory, GnRH pulse frequency and pulse amplitude are modifiable, but the exact neurophysiologic mechanisms mediating the modulation of GnRH pulsatility are not clear. The increased GnRH pulse frequency observed in PCOS could result from metabolic, central, or paracrine factors modifying GnRH-to-GnRH connections or neuronal, glial, or neurovascular processes. Although the resumption of ovulation in women with PCOS when insulin and androgens are reduced would suggest a reversible slowing of the GnRH pulse generator, the exact mechanisms mediating the onset of ovulatory function following these interventions have not been well described.

The extensive spatial distribution of GnRH neurons, their unique morphology, and the projection of these neurons into areas of the brain that lack a blood–brain barrier would suggest that their regulation is mediated by local factors and not solely affected by circulating or peripheral factors. Stimulation of GnRH neurons is selective. A given stimulus does not evoke uniform responses in all cells. Identification of cFos, a proto-oncogene product, served as a marker that permitted differentiation of activated neurons from neurons that were not activated. cFos is normally not expressed by GnRH neurons. Its expression can be induced within 45 minutes of electrochemical stimulation and during induced or spontaneous LH surges. This technique has proved useful in separating the subpopulation and location of neurons that are activated from neurons that remain quiescent during an LH surge. GnRH neurons in contact with catecholamine or neurotensin axons were noted to be distributed in a pattern similar to that observed for cFos expression in GnRH neurons during an LH surge.

The regulation of GnRH release by neurotransmitters has been investigated extensively, and several factors, including gamma aminobutyric acid (GABA), β-endorphin, dopamine, catecholamines, serotonin, galanin, leptin, and neuropeptide Y, have been implicated as neuromodulators of the GnRH pulse generator. Initially, it was unclear whether GnRH neurons were innervated. Several investigators have been able to demonstrate the presence of synaptic input to these neurons, although the input is sparse when compared with the input to neighboring neurons. The complex nature of GnRH cellular organization has made it difficult to define these interactions clearly. Several investigators have successfully demonstrated interactions between GnRH neurons
and other GnRH neurons, neuropeptide neurons, catecholaminergic neurons, serotonergic neurons, GABA neurons, substance P neurons, vasoactive intestinal peptide neurons, vasopressin neurons, glutaminergic neurons, proopiomelanocortin neurons, corticotrophin releasing hormone (CRH) neurons, and neurotensin neurons. Nevertheless, the contribution of any of these putative neuromodulators to the increased GnRH drive characteristic of PCOS remains obscure. Although extensive experiments have been possible in animal models, it is not clear whether similar neuroregulatory and neurosecretory mechanisms exist in humans. To date, there is no animal model of PCOS that would permit these questions to be addressed.

Yen and co-workers\(^{196, 197}\) showed that pulsatile GnRH secretion could not be abolished by sleep, by the use of general anesthetic agents in adult females, or in response to electric shock therapy in psychiatric patients. An exaggeration of pulse amplitudes in pseudocyesis, an increase in sleep-related pulse amplitude in puberty,\(^ {24, 79}\) and a decreased frequency of pulses in patients with anorexia nervosa and hypothalamic lesions\(^ {194}\) lent credence to the concept that GnRH release is regulated by CNS mechanisms and neurotransmitter systems. Table 1 reviews the evidence for and against these various putative neuromodulators that may explain the rapid GnRH/LH pulse frequency characteristic of PCOS. Two lines of evidence are considered—neuroanatomic and neurohormonal. Given the relative absence of the blood–brain barrier at the level of the median eminence, key peripheral signals that may serve as neuromodulators of GnRH are reviewed.

### Table 1. Estimated Luteinizing Hormone Pulse Frequency in Women with Polycystic Ovary Syndrome (PCOS) Hyperandrogenic Anovulation

<table>
<thead>
<tr>
<th>Study</th>
<th>Eumenorrheic Controls</th>
<th>PCOS/Hyperandrogenic Anovulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kazer, 1987</td>
<td>12.1/12 h EFP</td>
<td>12.1/12 h</td>
</tr>
<tr>
<td></td>
<td>12.0/12 h MFP</td>
<td></td>
</tr>
<tr>
<td>Waldstreicher et al, 1988</td>
<td>15.6/24 h EFP</td>
<td>24.9/24 h*</td>
</tr>
<tr>
<td></td>
<td>22.2/24 h MFP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.8/24 h LFP</td>
<td></td>
</tr>
<tr>
<td>Rossmanith, 1990</td>
<td>18.4/24 h young POF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.8/24 h PMW</td>
<td></td>
</tr>
<tr>
<td>Berga et al, 1993</td>
<td>17.1/24 h MFP</td>
<td>23.0/24 h*</td>
</tr>
<tr>
<td>Apter et al, 1994</td>
<td>15.0/24 h MFP</td>
<td>18.5/24 h*</td>
</tr>
<tr>
<td>Morales et al, 1996</td>
<td>15.9/24 h EFP lean</td>
<td>21.9/24 h lean*</td>
</tr>
<tr>
<td></td>
<td>15.9/24 h EFP obese</td>
<td>23.9/24 h obese*</td>
</tr>
<tr>
<td>Taylor, 1997</td>
<td>15.0/24 h EFP</td>
<td>18.0/24 h*</td>
</tr>
<tr>
<td>Daniels and Berga, 1997</td>
<td>5.6/12 h off OCPs</td>
<td>11.7/12 h off OCPs*</td>
</tr>
<tr>
<td></td>
<td>1.6/12 h on OCPs</td>
<td>6.3/12 h on OCPs*</td>
</tr>
</tbody>
</table>

EFP = early follicular phase; MFP = midfollicular phase; LFP = late follicular phase; POF = premature ovarian failure; PMW = postmenopausal women; OCPs = oral contraceptive pills containing 35 μg of ethinyl estradiol and 1 mg of norethindrone.

*\(P < 0.05\).
NEUROTRANSMITTERS IMPLICATED IN GnRH ABERRATIONS IN POLYCYSTIC OVARY SYNDROME

Catecholamines

Neurons releasing dopamine and noradrenaline are abundant in the hypothalamus and regulate the secretion of hypothalamic hormones.\(^\text{77, 84}\) The dopaminergic system is prominent with small neurons located in the arcuate nucleus, with axons projecting to the external layer of the median eminence.\(^\text{22, 58}\) Norepinephrine has been identified with dopamine in the basal hypothalamus. The catecholamine content has been shown to fluctuate in response to sex steroids and castration.\(^\text{58}\) The interaction between the adrenergic (catecholaminergic) neurons and peptidergic (hypophysiotropic) axons is believed to occur via axo-axonic synapses. One mechanisms by which the adrenergic system may regulate GnRH neurons would be by increasing the local blood flow, thereby increasing the permeability of the portal vascular system and permitting the entry of increased amounts of GnRH. The injection of dopamine into the third ventricle but not the general circulation led to a rapid increase in GnRH and prolactin inhibitory factor in portal blood,\(^\text{84}\) suggesting dopamine-mediated GnRH and prolactin inhibitory factor regulation. Increased central adrenergic tone has been proposed as a cause for the gonadotropin aberrations in PCOS.

Norepinephrine and Serotonin

Data from rat studies suggest that norepinephrine may have a positive influence on LH secretion; however, in humans, norepinephrine infusions have failed to elicit an effect on LH secretion.\(^\text{12}\) Urinary and plasma metabolites of norepinephrine have been reported to be elevated in women with PCOS when compared with controls.\(^\text{106, 200}\) Yoshino and co-workers\(^\text{200}\) demonstrated higher levels of plasma 3-methoxy-4-hydroxyphenylglycol (MHPG), a metabolite that closely reflects turnover of central norepinephrine, in PCOS women when compared with controls. MHPG levels correlated significantly with peak LH levels after GnRH stimulation, supporting the concept of altered catecholamine metabolism in PCOS.\(^\text{200}\) A single intramuscular injection of estradiol valerate in rodents induced polycystic ovaries and increased the sympathetic tone of neurons innervating the ovaries.\(^\text{111}\) This effect was apparent even before the appearance of cysts and correlated with the exaggerated steroid response of the ovary to β-adrenergic agonists. Desjardins and co-workers\(^\text{40}\) observed a specific reduction in β-endorphin neurons in the arcuate nucleus of the hypothalamus after estradiol valerate treatment. Because estradiol-induced polycystic ovaries share many of the characteristics of PCOS in humans, it is possible that peripheral (ovarian) sympathetic tone may be deranged, with an increased capacity to incorporate and release norepinephrine. When compared with normally cycling peers, adolescents with PCOS were noted to have lower levels of
plasma and urinary dihydroxyphenylglycol (DHPG) and higher urinary excretion of normetanephrines, suggesting alterations in noradrenaline deamination or uptake early in the course of this endocrine disorder.\textsuperscript{60}

If the increased GnRH/LH drive in PCOS is a consequence of altered sympathetic tone, it seems plausible to theorize that sympathetic blockade should rectify this phenomenon. Pulsatile release of LH was abolished by \( \alpha \)-adrenergic blocking agents (phenolamine) but not by \( \beta \)-blockers (propanolol) in ovariectomized rhesus monkeys.\textsuperscript{69} This phenomenon could not be replicated in humans; however, the doses used in humans were a tenth of those used in the primate experiments.\textsuperscript{154, 182, 195} Another explanation for the failed experiment in humans could be that pharmacologically effective concentrations of phenolamine were not achieved owing to limited permeability through the blood–brain barrier.

Some researchers have postulated that psychologic stress may initiate inappropriate gonadotropin secretion, chronic anovulation, and hyperandrogenemia in women with PCOS. Psychologic stress seemed to be more prevalent among women with PCOS than in controls and in women with hypothalamic-pituitary dysfunction from other causes. This stress was reflected by greater levels of urinary MHPG sulfate but not MPHG glucuronide in PCOS women. MPHG primarily reflects brain norepinephrine turnover.\textsuperscript{106} There was a positive correlation between urinary MPHG and serum LH in women with PCOS and hypothalamic-pituitary dysfunction. Platelet serotonin levels were elevated in PCOS, which is believed to reflect brain serotonin levels. Whether this finding truly reflects an abnormality in the central serotonergic pathway is unclear. Infusion of 5-hydroxytryptophan caused an elevation in prolactin levels but had no influence on LH secretion.\textsuperscript{116} Serotonin is believed to have an inhibitory effect on GnRH secretion in animals, but human data are sparse. Despite evidence implicating an altered sympathetic tone in women in PCOS, it is not apparent whether this observation is purely coincidental or truly induces increased GnRH pulsatility.

**Dopamine**

Dopamine is considered to be a major factor governing the secretion of immunoreactive LH. It exerts an inhibitory effect on LH secretion in normally cycling women.\textsuperscript{94, 80, 115} In PCOS, the increased frequency of LH pulses has been attributed to a central impairment of the dopaminergic or opioidergic inhibitory activity on LH secretion. This increase in LH can be blocked with metoclopramide, a dopamine receptor blocker, and resumption of menses and ovulation can be achieved with the dopamine agonist bromocriptine. It has been suggested that decreased inhibitory influence of tuberoinfundibular dopamine results in increased GnRH neuronal activity, which accounts for the alterations in LH secretion.\textsuperscript{81} Because of the invasive nature of studies required to assess central catecholamine concentrations and activity in humans, one must rely on indirect methods with their inherent limitations. Several investigators have pursued indirect assessments and have shown conflicting yet inter-
esting results. Although observations from pharmacologic interventions suggest a possible mechanism, they do not truly explain physiologic events.

Several observations support the concept that the dopaminergic system may be responsible for the GnRH aberrations seen in PCOS. Exogenous dopamine has been shown to lower circulating immunoreactive LH in PCOS. Moreover, bromocriptine, a dopamine agonist, restored ovulatory cycles and decreased LH pulses in some normoprolactinemic women with PCOS. Metoclopramide, a dopamine receptor antagonist, did not elicit a rise in LH levels in women with PCOS but did so in normally cycling women. Ovulation induction in these women with gonadotropin injections reversed the dopaminergic/opioidergic dysfunction, with a normal increase in preovulatory and midluteal LH response to metoclopramide and naloxone. In contrast, Lobo and co-workers did not demonstrate an increase in bioactive immunoreactive LH secretion in response to either dopamine alone or a dopamine-carbidopa combination and concluded that dopamine did not have a vital role in increasing GnRH release from the pituitary. L-dopa alone and L-dopa-carbidopa treatment attenuated the LH response to GnRH, suggesting that there may be a relative deficiency of dopamine at the level of the pituitary gland.

Urinary homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) are urinary dopamine metabolites, the former largely unconjugated and the latter also existing in the conjugated form. MHPG is the major central dopamine metabolite. More than 50% of urinary MHPG reflects CNS-derived norepinephrine metabolism. HVA, a major urinary metabolite of dopamine in humans, represents central and peripheral dopamine turnover but accounts for 30% to 60% of central dopamine turnover. Women with PCOS had lower levels of urinary HVA and DOPAC at baseline, suggesting decreased dopamine turnover; however, total urinary MHPG and the MHPG to HVA ratio were significantly higher in the PCOS group than in controls, suggesting increased norepinephrine turnover, and correlated with serum LH levels. In contrast, Segos and co-workers showed that normoprolactinemic women with PCOS had increased excretion of urinary HVA when compared with normally cycling controls, with a negative correlation between urinary HVA and serum LH levels in the latter group but not in the PCOS group. This observation supports the notion that dopamine synthesis and use in PCOS are increased rather than deficient. Disulfiram, an inhibitor of dopamine hydroxylase activity, significantly altered urinary HVA and MHPG ratios as anticipated in women with PCOS but had no effect on LH secretion, refuting the hypothesis that dopamine hydroxylase activity is increased in PCOS.

Szilagyi and co-workers examined the effects of surgical ovarian manipulation (wedge resection and laser vaporization) on central dopaminergic/opioidergic activity. Despite the resumption of menstrual cyclicity and ovulation, the gonadotropin secretory pattern remained unaltered in response to naloxone and metoclopramide before and after
surgery. The administration of cabergoline, a long-acting D2 dopaminer-
gic agent, blunted plasma prolactin levels but did not affect LH pulsati-
lity in PCOS women and controls despite improvement of menstrual
cyclicity and normalization of androgens in the former group. This
observation would indicate that hyperandrogenism in PCOS is mediated
via peripheral effects of prolactin on the ovary and liver (such as de-
creased sex hormone-binding globulin [SHBG]) rather than central ef-
effects.124 Data from a study by Ferriani and co-workers95 refuted the
theory that central dopamine deficiency triggers a release in prolactin
and LH hypersecretion in women with PCOS, questioning the benefit of
bromocriptine therapy in these women.

Dopamine neurons containing estrogen and progesterone receptors
have been identified in the arcuate nucleus with axo-axonal synapses to
the GnRH neurons.145,155 One of the causative sites implicated in altered
dopaminergic function is signal transduction. Of the five dopamine
receptor subtypes identified, the D2 receptor has been cloned and local-
ized to chromosome 11.26,162 Its expression has been localized to the
GnRH-secreting areas of the brain, with high levels of expression in the
limbic system and olfactory tubercles.163 Although D2 receptor allelles
have been implicated in functional disturbances in reproductive function
and thought to mediate tonic inhibition of prolactin secretion,198 Legro
and co-workers99 did not find any significant association between D2
receptor haplotypes and ovulatory status in a group of infertile Hispanic
women. To date, the evidence for a role of dopaminergic and catechol-
amnergic neurotransmission in PCOS is indirect, contradictory, and in-
conclusive.

**Insulin-like Growth Factor 1**

Insulin-like growth factors (IGFs) are polypeptides that are found
in the CNS and the periphery. In addition to regulating growth, differen-
tiation, and survival, they influence reproductive function. IGFs and
IGF-binding proteins (IGFBP) are synthesized locally in the CNS, in
the hypothalamus and pituitary, and also derived from the systemic
circulation. IGF receptors have been located in all brain regions, includ-
ing the median eminence, hippocampus, and cortex.36,74,130 In tanycytes,
which are hypothalamic glial cells, IGF expression is cyclical, reflecting
the changes in sex steroid concentrations of the estrous cycle.36,51,130

In many species, IGF-1 has been implicated in the initiation and
acceleration of puberty. In prepubertal female rats, IGF-1, IGF-2, and
insulin increased GnRH release in a dose-dependent manner. It has been
postulated that IGF may influence hypothalamic and pituitary processes
to facilitate puberty-related changes in gonadotropin secretion and may
act as a metabolic indicator signaling the physiologic readiness for sexual
maturation.95 In rat anterior pituitary cell cultures, IGF-1 significantly
increased LH, FSH, and GnRH-stimulated gonadotropin secretion.85,167,168

The central mechanism by which IGFs regulate reproductive func-
tion remains unclear, as does their role in the pathogenesis of PCOS. The IGF family of growth factors seems to arrest follicular growth, with an accumulation of antral follicles that is mediated by direct ovarian effects. IGFs also increase the thecal response to LH, resulting in hyperandrogenemia. In women with PCOS, an increased ratio of IGF-1 and steroids to their binding proteins correlated significantly with increased concentrations of circulating LH. IGFBPs are synthesized by ovarian follicular cells. IGFBP-1 levels tend to be lower in women with PCOS; the reason for this difference is not known. IGF-1 levels decrease in response to GnRH agonist treatment, suggesting that altered IGF-1 in PCOS is most likely a consequence of aberrant GnRH secretion rather than the cause for this abnormality.

The observation that IGF-1 to inhibits GABA release and the ability of IGF-1 and IGF-2 to interact with the insulin receptor suggests that the insulin receptor may mediate some of the actions of IGF on endogenous opioids. The IGF system may also influence norepinephrine expression in the CNS and periphery. IGF-1 has been shown to increase the activity of enzymes involved in catecholamine biosynthesis. Pathways that connect the IGF system with norepinephrine and gonadotropin secretion have been described, but information is scarce. Most investigations into the role of the various IGFs and IGFBPs posit paracrine or peripheral endocrine actions.

**Opioids**

Endogenous opioid peptides and GABA have been thought to inhibit LH secretion in adult rats. The ability of the opioid receptor antagonist naloxone to induce a rise in LH levels supports this notion. GABA may tonically inhibit LH secretion, as evidenced by an increase in LH secondary to a GABA-A receptor-blocking drug, bicuculline. Naloxone, an opioid antagonist, stimulates glutamate and nitric oxide in neurons that modulate GnRH release. A glutamate receptor antagonist blocked this effect of naloxone; therefore, opioids may act on the glutamate neurons upstream of nitric oxide to inhibit LH release.

β-endorphins are thought to inhibit hypothalamic GnRH release, and reduced activity of this hypothalamic neurotransmitter has been implicated in GnRH hypersecretion in PCOS. Acute administration of a methionine-enkephalin analogue or β-endorphin resulted in a decrease in plasma levels of LH in men and women. Naloxone increased LH secretion under basal conditions, suggesting that endogenous peptides exert a tonic inhibitory effect on LH secretion in both sexes. Interestingly, naloxone failed to elicit a LH rise in hypogonadal and castrated men and postmenopausal women. Naloxone also seems to be ineffective in increasing LH in patients with primary GnRH deficiency. Adequate estradiol levels are necessary for hypothalamic expression of opioid peptides, naloxone-induced rises in LH, and central activities of opiate agonists and antagonists.
A differential LH response to naloxone has been observed, with maximal response observed in the luteal phase and minimal response in the follicular phase. Central opioidergic tone has been shown to be greatest during the phase of the cycle when estrogen levels are high, that is, at midcycle and during the luteal phase, and lowest in the early follicular phase. Progesterone, a potent ovarian steroid, has been shown to promote hypothalamic opioid activity that inhibits the GnRH pulse generator in monkeys in vivo. Women with PCOS lack the luteal phase but have chronic hyperstrogenism. Because naloxone has been shown to increase LH secretion and restore pulsatile LH secretion in women with hypothalamic hypogonadism and amenorrhea secondary to hyperprolactinemia, it has been proposed that women with PCOS have decreased opioid inhibition of LH secretion and pulsatility. Peripheral levels of β-endorphins have been reported to be increased in PCOS but whether this observation accurately reflects central opioidergic activity is not known.

Petraglia and co-workers observed a rise in LH in response to naloxone in obese women with PCOS but not in women PCOS with a normal body weight. They concluded that a change in the E₁/E₂ ratio or increased ovarian androgen secretion impaired the inhibitory action of opioids on gonadotropin secretion and was modified by obesity. Extended treatment with naltrexone, a long-acting opioid antagonist, effectively reduced the LH response to a GnRH stimulus in women with PCOS.

Although the LH levels of cycling women in the early follicular phase decline in response to an intravenous bolus of β₇-human-endorphin, women who have PCOS with inappropriate LH levels fail to respond to intravenous β₇-human-endorphin or naloxone, posing the question that these women have an aberrant opioidergic control of GnRH secretion. Treatment with medroxyprogesterone acetate (MPA) for 10 days in PCOS women was effective in slowing LH pulsatility. Naloxone reversed the MPA-induced slowing, thereby establishing that the neuromodulation of GnRH by central opioidergic tone is inducible. The fact that the induction of this neuroendocrine regulation can be readily achieved by progestogen administration indicates that the apparent opioidergic impairment in PCOS women represents a functional state caused by ovarian acyclicity rather than an inherent hypothalamic defect.

The GnRH pulse generator of women with PCOS differs to some extent from that of eumenorrheic women when exposed to sex steroids. In the past, it was popular to attribute the increased GnRH drive in PCOS to diminished opioidergic inhibition. Using slightly different experimental paradigms, Daniels and Berga and Pastor and colleagues found that the GnRH pulse generator of women with PCOS was more resistant to suppression by combined estrogen/progestin exposure than that of eumenorrheic women. This observation suggests a primary hypothalamic defect in PCOS but not a primary defect in opioidergic tone.
Leptin, Neuropeptide Y, and Galanin

The observation that obesity is a key feature of PCOS led to the logical notion that neuropeptides and hormones that influence and modify eating behavior, satiety, and body fat distribution may in some way manipulate the GnRH pulse generator. The hormone implicated was leptin and the neurotransmitters, galanin and neuropeptide Y. The action of neuropeptides on the regulation of food intake and body weight is modulated by leptin.\textsuperscript{153, 157, 172} Delineating the role of each of these substances is not appropriate because of the complexity of their interactions and actions within the CNS, which are susceptible to further alteration in the presence of obesity; therefore, leptin, galanin, and neuropeptide Y are discussed collectively.

Leptin, a 16 kd protein hormone, is synthesized and secreted by adipocytes.\textsuperscript{204} It is a product of the \textit{ob} gene that signals the amount of energy stores to the brain and has been implicated in the regulation of food intake and energy balance. Leptin levels are elevated in obese animal models and obese humans. Leptin is believed to be the molecular signal for the regulation of energy balance and possibly the reproductive axis by serving as an endocrine signal that relays the extent of body fat stores and the nutritional status to the reproductive axis. In rats, leptin seems to be essential for reproduction. The identification of leptin receptor mRNA in the brain suggests that leptin may act centrally to regulate hypothalamic-pituitary function;\textsuperscript{30, 98, 177} however, because leptin also circulates peripherally, one cannot exclude other mechanisms.

The observation that leptin levels were significantly elevated in approximately 30% of lean and obese women with PCOS when compared with controls suggests that leptin may have a role in the pathogenesis of PCOS, although the mechanism remains unknown.\textsuperscript{25} In contrast, Mantzoros and co-workers\textsuperscript{114} showed that the relationship between leptin levels and body mass index was similar in obese women with and without PCOS and that there was no correlation between leptin and circulating gonadal steroid levels. Improving hyperinsulinemia and insulin resistance with troglitazone in obese women with PCOS without altering the body mass index failed to elicit a demonstrable difference in leptin levels when the women were compared with matched controls without PCOS. Laughlin\textsuperscript{97} and Roru\textsuperscript{151} and their colleagues similarly showed that leptin levels did not differ in women with PCOS and normally cycling women.

It has been proposed that leptin may act as an afferent satiety signal on the hypothalamic centers, in part, by modulating the expression and action of the orexigenic hypothalamic neuropeptide Y\textsuperscript{174} on food regulation and body weight.\textsuperscript{153, 157, 172} Nevertheless, in humans, unlike in the \textit{ob}/\textit{ob} mouse, obesity is not necessarily a result of a leptin mutation.\textsuperscript{31, 113} In addition to its anti-obesity effects, leptin influences many neuroendocrine functions,\textsuperscript{3} including the activity of the hypothalamic-pituitary-gonadal axis. During puberty, leptin pulsatility is thought to sensitize the hypothalamus by interacting directly with GnRH neurons.
or indirectly via neuropeptide Y neurons of the arcuate nucleus. Nonetheless, Erickson and co-workers showed that neuropeptide Y–deficient mice had normal food intake, weight, growth, and fertility. This observation suggests that leptin may act on GnRH or pituitary gonadotroph through its own receptor-promoting nitric oxide release or via other pathways independent of, or in addition to, neuropeptide Y-ergic neurons.

Synchronicity between leptin and LH pulses in the mid-to-late follicular phase in healthy women suggests that leptin may regulate minute-to-minute oscillations in plasma levels of LH. Women with PCOS also exhibit synchronicity between leptin and LH pulses, but this relationship is weaker with a greater phase shift than seen in normally cycling women, most likely because of greater LH levels in PCOS women. These observations confirm the existence of a link between leptin and gonadotropin secretion but do not clarify which one regulates the other. Palmert and co-workers observed that when girls with precocious puberty were given GnRH analogues, leptin pulsatility persisted despite suppression of the gonadal axis. Sir-Petermann’s group showed that GnRH administration stimulated LH release without an increase in leptin release. Leptin concentrations increased in blood before, and concomitantly with, LH peaks but seldom after. It is possible that leptin-mediated effects on the reproductive axis are central, peripheral, or both because leptin receptors have also been identified on the ovary. Insulin resistance at the level of the adipocyte may explain the increase in leptin levels, but the role of leptin in causing or contributing to hyperinsulinemia and hypersecretion of LH in PCOS is inconclusive.

Neuropeptide Y is a potent orexigenic stimulant and is synthesized and located in the arcuate nucleus and median eminence. It is the most abundant neuropeptide in the brain and has various functions, including regulation of circadian rhythm, the response to anxiety and stress, peripheral vascular resistance and cardiac contractility, and the body’s response to starvation. Prolonged central administration has been shown to induce obesity. Neuropeptide Y administration in rats led to hyperinsulinemia, hypercortisolemia, and an increase in lipoprotein lipase activity and insulin resistance in muscles.

In the medial preoptic area, neuropeptide Y neurons are in contact with GnRH neurons. Neuropeptide Y neurons regulate the reproductive axis at the hypothalamic and hypophyseal levels. Neuropeptide Y stimulates GnRH release in vivo directly via Y-1 receptor–mediated action on the GnRH neuron. In the rhesus monkey, neuropeptide Y pulses coincide with GnRH pulses. Potentiation of the GnRH-stimulated LH increase by neuropeptide Y seems to be steroid dependent. Circulating gonadal steroid concentrations increase hypothalamic GnRH and pituitary LH responsiveness to neuropeptide Y. Baranowska and co-workers demonstrated that obese and nonobese PCOS women had elevated neuropeptide Y levels and that the increase was independent of body mass index. All of these observations point to a possible contributory role of neuropeptide Y in the maintenance of aberrant
GnRH secretion. Whether NPY exerts a primary effect or is a synergistic factor in this cascade of interactions between neuropeptides and neurohormones needs further clarification.

Galanin, a 29 amino acid peptide synthesized in the hypothalamus and the anterior pituitary, has an important role in regulating food intake and GnRH secretion.\(^{100, 149}\) Galanin expression is stimulated by sex steroids in GnRH neuron\(^{72}\) and has been shown to regulate ovulatory surges of LH and prolactin.\(^{109}\) It is cosecreted with GnRH and can modulate the secretion of GnRH.\(^{149}\) Galanin stimulates GnRH and LH secretion. The galanin and neuropeptide Y pathways may synergistically stimulate GnRH release.\(^{192}\) At the hypothalamic level, neuropeptide Y and galanin produce hyperphagia and reduce energy expenditure.\(^{100}\) Baranowska and co-workers\(^{9}\) showed that obese women had increased levels of plasma neuropeptide Y, galanin, and leptin.

Because reproduction requires energy, a tight link between metabolic signals and reproductive competence is to be expected; however, it is not necessarily clear that energy excess would be expected to suppress reproductive function. Furthermore, because energy status is critical to survival, one would expect a certain amount of redundancy. Investigation in this area is hampered by the discordance between levels of a given hormone and its impact on target tissues. Despite a panoply of studies linking metabolic signals and reproductive function, it is not clear that any one factor, or any combination of factors, explains the alterations in GnRH/LH drive in PCOS.

**Insulin**

Since 1980 when Burghen and co-workers\(^{27}\) demonstrated a positive linear correlation between hyperandrogenism and hyperinsulinemia in obese women with PCOS when compared with obese controls, it has been repeatedly shown that insulin resistance occurs in lean and obese women with PCOS and that neither obesity nor androgen excess, per se, fully accounts for decreased insulin action.\(^{46}\)

It is possible that hyperinsulinemia or insulin resistance impact on the cellular physiology of GnRH neurons to alter their pulsatile output. Insulin resistance may alter the endogenous pulse attributes of individual GnRH neurons, modify the number of GnRH-to-GnRH synapses, alter glial interposition between GnRH-to-GnRH appositions, influence the secretion of glial trophic factors, or change neuromodulation by traditional neurotransmitter systems,\(^{54}\) including neuropeptide Y. Insulin may act as a neuronal growth factor and promote neuron outgrowth.\(^{184}\) A combination of mechanisms is possible. Insulin receptors have wide distribution in the brain and have been identified in the rat hypothalamus, with demonstrable insulin uptake by the periventricular hypothalamus\(^{14}\) and olfactory and limbic areas.\(^{73}\) One study demonstrated an increase in cerebrospinal fluid insulin levels following an intravenous infusion of insulin, confirming that insulin crosses the blood–brain bar-
Insulin receptors also have been identified in pituitary tissue. Pituitary cell cultures incubated with increasing concentrations of insulin exhibited a dose-dependent increase in the GnRH-stimulated release of LH and FSH. Although Dunaif and GraP showed no increase of gonadotropin release in women with PCOS in response to raising insulin levels, the lack of change may reflect the fact that the GnRH drive is maximal in PCOS and cannot be further increased. Guzick and co-workers demonstrated that weight loss in obese PCOS women decreased insulin levels, but LH pulse frequency remained unaltered. Given these considerations, it is plausible that either hyperinsulinemia or insulin resistance may modulate the GnRH pulse generator, but concrete data in support of this notion are lacking.

Androgens

Another factor that may explain the aberrations in GnRH secretion is sustained androgen excess. A study by Plant suggested that fetal ovarian androgen exposure might imprint the nascent GnRH pulse generator toward a more rapid pulse frequency. Following postnatal gonadectomy, male rhesus monkeys exhibited a more rapid LH pulse frequency than females. Nevertheless, Dumesic and co-workers showed that although female monkeys exposed in utero to exogenous testosterone oversecreted LH, they remained ovulatory. In the past, it was assumed that the fetal ovary was inactive, but two studies found follicular development and ovarian secretory activity beginning around week 16 to 20 of fetal life. Apter and co-workers observed that peripubertal girls with hyperandrogenism and increased ovarian volume displayed increased LH pulsatility and enhanced expression of ovarian 17-hydroxyprogesterone levels, supporting the concept that altered GnRH function antedates the pubertal phase of life. One hypothesis advanced to explain PCOS is a fetal diathesis toward ovarian androgen oversecretion in utero that imprints the fetal GnRH pulse generator.

Evidence against the notion that androgens irreversibly fix the GnRH pulse generator at a more rapid frequency was provided in a study by De Leo and colleagues. Following 3 months of treatment with the antiandrogen flutamide, eight young women with PCOS so treated began to ovulate, with normalization of the LH to FSH ratio. One interpretation is that although androgens sustain anovulation in PCOS, the effect is reversible and not fully caused by imprinting. In an elegant study by Balen and Jacobs, women with PCOS underwent unilateral ovarian diathermy. Following surgery, 50% began to ovulate, but ovulation occurred in both ovaries, not just in the ovary that was treated. The women who ovulated had a greater decline in androgen levels following intervention. Laparoscopic ovarian diathermy is an updated version of ovarian wedge resection and undoubtedly exerts its effects by destroying the thecal compartment, thereby reducing androgen levels. It has not been determined whether a surgically induced reduction in androgen
secretion provokes ovulatory function by altering GnRH/LH pulse frequency or by temporarily permitting FSH levels to rise. If the LH pulse frequency does not change following ovarian diathermy, the woman may be at risk for the return of anovulation if a sufficient residual of responsive theca cells remains. Because exposure to comparable ovarian sex steroid levels does not suppress the GnRH pulse generator in women with PCOS to the same extent as it does in eumenorrheic women, it remains an open question whether the GnRH pulse generator of women with PCOS is intrinsically and irreversibly faster or merely a reflection of sustained androgen excess.

Other models that have addressed the question of whether androgen excess causes anovulation include studies of female-to-male transsexuals. Futterweit and Deligdisch showed that exogenous testosterone given to female-to-male transsexuals before castration induced PCOS-like morphologic changes in the ovaries. In contrast, Spinder and co-workers documented no difference in mean LH, pulse amplitude, or frequency in response to exogenous long-term testosterone exposure in agonadal female-to-male transsexuals. The observation that women with virilizing congenital adrenal hyperplasia hypersecrete LH supports the concept that androgens alter the GnRH pulse generator. Levin and co-workers showed that women with adult-onset congenital adrenal hyperplasia had a LH pulse frequency intermediate between that of eumenorrheic women and women with PCOS. Although the available evidence strongly suggests that excess androgen exposure contributes to anovulation in women with PCOS, the data do not reveal whether androgens exert their effect by causing GnRH hypersecretion.

Paracrine Factors

Several studies have demonstrated that GnRH neurosecretion seems to directly influence LH synthesis and pulsatility. Although GnRH stimulates the release of LH and FSH, the effects of GnRH on the synthesis and release patterns of both gonadotropins are differential. One explanation for this differential control could be inhibin. A glycoprotein secreted by the ovary, inhibin has been shown to suppress specifically FSH synthesis and secretion. GnRH pulse frequency also alters LH and FSH gene expression and secretion. Slower frequencies seem to favor FSH secretion and faster ones LH. Two other factors, activin and its binding protein follistatin, may also contribute to the FSH regulatory mechanism. Activin, a group of dimeric peptides, stimulates FSHβ gene expression and selectively promotes FSH secretion. Follistatin, a protein, seems to block this action by binding activin and bioneutralizing it. Activin and follistatin are present in the pituitary, where they are believed to form a local regulatory loop. This mechanism may have implications on the physiologic and pathophysiologic control of gonadotropin secretion.

In rats, pituitary follistatin mRNA exhibits a preovulatory peak
during the gonadotropin surge and is stimulated by GnRH in vitro. This observation indicates a possible interaction between hypothalamic GnRH and the presumed activin/follistatin intrapituitary loop in the specific regulation of FSH. Lockwood and co-workers\textsuperscript{108} observed that inhibin B levels were significantly higher in PCOS women when compared with controls. In addition, in women with PCOS-associated ovulatory dysfunction, no distinct periodicity in inhibin B levels (noted in normally cycling women) was observed. The restoration of ovulation following laparoscopic ovarian drilling in PCOS women reduced inhibin B levels and initiated pulsatility.\textsuperscript{108}

Evidence to support the concept that the inhibin/activin/follistatin loop has a role in regulating hypothalamic GnRH secretion is compelling. Although inhibin and activins are, in effect, peripheral factors, their effects seem to be centrally mediated in a paracrine manner. The complex interactions between various components of the endocrine system make it difficult to define the role of individual components and the extent to which they may influence the GnRH secretory pattern. Current evidence suggests that a primary defect in the inhibin/activin/follistatin system causes PCOS.

**SUMMARY**

Although central and peripheral factors have been implicated in the neuromodulation of GnRH in PCOS, there are no definitive or conclusive data to establish a primary causal role for any one factor. Because increased GnRH pulse frequency is at least a contributor to the secretion of excess LH and insufficient FSH that are the proximate cause of chronic anovulation in PCOS, strategies to slow the GnRH pulse generator are likely to promote ovulation in women with PCOS. Several pharmacologic agents, such as dopamine agonists and antagonists, have been tried, but the lack of consistent effects in women with PCOS limits their clinical utility. Current treatment strategies include the use of the combined oral contraceptive pills, antiandrogens or androgen receptor blockers, and insulin sensitizers. Oral contraceptive preparations are effective in suppressing ovarian hyperandrogenemia, regulating menstrual cycles, and reducing the risk of endometrial hyperplasia. Androgen blockade and antiandrogens provide symptomatic relief from androgen-induced acne and hirsutism and have been reported to restore ovulation in women with PCOS. Whether this effect is mediated peripherally or centrally remains to be clarified. The most recent class of pharmacologic agents to gain popularity are the "insulin modifiers." With increasing evidence that insulin resistance constitutes a key metabolic element, it seems logical that improving insulin sensitivity and glucose disposal might wholly, or partially, reverse certain features of PCOS, including anovulation. To date, insulin modifiers have proved most promising in improving the clinical features and promoting fertility, but whether this effect is centrally mediated is yet to be elucidated.
ACKNOWLEDGMENTS

The authors thank the nurses of the Magee Satellite Clinical Research Center and the General Clinical Research Center (University of Pittsburgh School of Medicine) for expert assistance in completing the research.

References


hyperprolactinemia and weight-loss related amenorrhea. Clin Endocrinol (Oxf) 17:379, 1982


68. Harris GW: Oestrous rhythm, pseudopregnancy and pituitary stalk in rat. J Physiol (Lond) 111:347, 1950


70. Harris GW: Neural Control of the Pituitary gland. London, Edward Arnold, 1955


100. Leibowitz ST: Possible contribution of brain peptides to the development of eating disorders and obesity. Int J Obes Relat Metab Disord 18(suppl 2):85, 1994
104. Levin JH, Carmina E, Lobo RA: Is the inappropriate gonadotropin secretion of patients with polycystic ovary syndrome similar to that of patients with adult-onset congenital adrenal hyperplasia? Fertil Steril 56:635-640, 1991


155. Sar M: Estradiol is concentrated in tyrosine hydroxylase containing neurons of the hypothalamus. Science 223:938, 1984


Address reprint requests to
Sarah L. Berga, MD
Departments of Obstetrics, Gynecology, and Reproductive Services
Division of Reproductive Endocrinology and Infertility
University of Pittsburgh School of Medicine
Magee-Womens Hospital
300 Halket Street
Pittsburgh, PA 15213–3180
e-mail: sberga@mail.magee.edu
学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具