HHS Public Access

Author manuscript

Curr Opin Endocr Metab Res. Author manuscript; available in PMC 2021 June 01.

Published in final edited form as:

Curr Opin Endocr Metab Res. 2020 June ; 12: 78–84. doi:10.1016/j.coemr.2020.04.005.

Abnormal GnRH Pulsatility in Polycystic Ovary Syndrome: Recent Insights

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Abstract

Although the fundamental symptoms of polycystic ovary syndrome (PCOS) relate most directly to ovarian dysfunction, central neuroendocrine systems play a prominent role in its pathophysiology. Gonadotropin-releasing hormone (GnRH) pulse generator resistance to negative feedback contributes to rapid GnRH pulse secretion, which promotes gonadotropin abnormalities that foster ovarian hyperandrogenemia and ovulatory dysfunction. The causes of GnRH neuron dysfunction, however, have remained enigmatic. In this review, we highlight a number of recent preclinical and clinical studies pertinent to the neuroendocrine abnormalities of PCOS, including those that have provided important insights into the relevance of animal models with PCOS-like features, the potential roles of kisspeptin and γ-aminobutyric acid (GABA)-ergic neurons, and the potential role of anti-Müllerian hormone.

Introduction

Although the definitional characteristics of polycystic ovary syndrome (PCOS)—androgen excess, oligo-/anovulation, and polycystic ovarian morphology—relate most proximately to ovarian dysfunction, central neuroendocrine systems play a prominent role in the pathophysiology of PCOS. Women with PCOS exhibit exaggerated luteinizing hormone (LH) production—related to persistently high LH (and by inference gonadotropin-releasing hormone [GnRH]) pulse frequency, increased LH pulse amplitude, and exaggerated LH responses to exogenous GnRH—and relative follicle-stimulating hormone (FSH) deficiency. These abnormalities of gonadotropin secretion materially contribute to the ovarian hyperandrogenemia and ovulatory dysfunction of PCOS. In addition, ovarian hyperandrogenemia in PCOS is LH-dependent: PCOS typically manifests during or shortly after the pubertal increase in LH secretion, and long-acting GnRH agonists markedly reduce androgen production in women with PCOS. More recently, gonadotropin-related genes have

Declarations of interest: none.

been implicated in the etiology of PCOS, including those for FSH subunit beta (*FSHB*), the FSH receptor (*FSHR*), LH subunit beta (*LHB*), and the LH/choriogonadotropin receptor (*LHCGR*) [1, 2].

GnRH neurons represent the final common pathway for the central control of reproductive function. High and low frequency GnRH pulses favor LH or FSH production, respectively. Thus, persistently high GnRH pulse frequency prominently contributes to the gonadotropin abnormalities of PCOS. Yet mechanisms underlying rapid pulsatile GnRH secretion remain unclear. While high GnRH pulse frequency in PCOS partly reflects anovulation (i.e., infrequent progesterone secretion from corpora lutea), relative resistance to sex steroid negative feedback also plays an important role as estradiol and progesterone do not appropriately restrain GnRH pulse generator activity in PCOS [3, 4]. This resistance to negative feedback appears to relate, at least in part, to hyperandrogenemia *per se*, as it can be reversed by the androgen-receptor antagonist flutamide [5]. Thus, PCOS involves a vicious cycle of androgen excess contributing to poor negative feedback suppression of GnRH pulsatility, leading to gonadotropin abnormalities that promote both additional hyperandrogenemia and on-going ovulatory dysfunction.

Prenatally-androgenized animal models of PCOS: utility and potential drawbacks

For ethical and practical reasons, the GnRH pulse generator and relevant neuronal afferents are inaccessible to direct and detailed interrogation in humans. Hence, animal models have remained critically-important tools to investigate neuroendocrine dysfunction in PCOS. In several animal species, experimental hyperandrogenism produces a number of PCOS-like features. Perhaps the best phenocopy of PCOS is the prenatally-androgenized (PNA) female rhesus macaque: these animals exhibit ovarian and adrenal hyperandrogenism, ovulatory dysfunction, increased LH secretion, and central resistance to the feedback effects of sex steroids [6]. Similar findings pertain to PNA rodents and sheep [7, 8]. However, the relevance of such PNA animal models has been somewhat controversial, in part because of inter-species differences in reproductive physiology and in part because no animal model perfectly conforms to PCOS. It also remains unclear whether methods of model generation (e.g., prenatal androgenization) faithfully recapitulate the events leading to PCOS.

While a number of studies have suggested that cord blood androgen concentrations are elevated in newborn daughters of mothers with PCOS, others studies have not supported this notion [9]. Maternal androgen concentrations, however, are an imperfect surrogate for fetal androgen concentrations, and cord blood assessments in these studies were performed at the time of delivery. It remains possible that androgen exposure is high during gestational windows that are difficult to assess directly in humans. Supporting prenatal androgenization in PCOS etiology, recent studies suggest that anogenital distance, which correlates with fetal androgen exposure, is longer in adult women with PCOS [10–12], although data are mixed in neonatal daughters of mothers with PCOS [13, 14]. Additionally, a recent study suggested that neonatal sebum production—another androgen-responsive process—is increased in newborn daughters of mothers with PCOS [15].

In the aforementioned study [15], sebum production was undetectable by the next observation at four weeks of age, compatible with a maternal source of neonatal androgen excess. While one might expect the placenta to protect a fetus from maternal hyperandrogenemia, one study suggested that placental tissue from women with PCOS exhibited lower aromatase activity and higher 3β-hydroxysteroid dehydrogenase type 1 activity—changes that could render fetuses vulnerable to maternal androgen excess [9]. Fetal hyperandrogenemia could also be of fetal origin in some instances. In support of this, women with virilizing congenital adrenal hyperplasia appear to have a high prevalence of PCOS-like features (e.g., ovarian hyperandrogenemia, elevated LH) [16].

Recent interest has focused on the contribution of 11-oxygenated androgens (e.g., 11-ketotestosterone) to the hyperandrogenism of PCOS [17, 18] and other androgenic disorders [19]; to our knowledge, however, these androgens have not been assessed in pregnant women with PCOS or in fetal cord blood from newborn daughters of mothers with PCOS. Moreover, alternative (“backdoor”) pathways to dihydrotestosterone production—pathways that may be fed by placental progesterone [20]—could plausibly be relevant to the fetal androgenization hypothesis of PCOS, but this notion requires further study.

In contrast to monkey and sheep models, rodent models are relatively inexpensive to create and maintain, their reproductive lifespans are short, and their genomes can be readily manipulated. This affords the use of neuroscience tools that enable the dissection of specific neuronal circuits within the GnRH neuronal network. Accordingly, much of our recent insight into the likely neurobiological mechanisms underlying androgen-mediated neuroendocrine dysfunction in PCOS has been derived from rodent models.

Mechanisms of androgen-mediated GnRH neuron dysfunction

Earlier studies demonstrate that progesterone and dihydrotestosterone (DHT; a nonaromatizable androgen) decrease and increase, respectively, GnRH neuron firing rates in murine brain slices [21]. Similarly, PNA mice exhibit increased GnRH neuron firing frequency [22–24], and both PNA mice and sheep exhibit impaired progesterone negative feedback on LH (GnRH) pulse frequency [25, 26]. The latter impairment likely reflects reduced basal and estradiol-induced progesterone receptor expression in relevant hypothalamic regions, as described in rodents [27]. Indeed, PNA mice and sheep demonstrate reduced hypothalamic progesterone receptor expression in the arcuate nucleus [28, 29], although results are mixed in PNA rats [30, 31].

GnRH neuron responsiveness to these hormonal cues is mediated indirectly, primarily via a complex network of afferent neuronal systems. This suggests that the central pathology underpinning neuroendocrine impairments in PCOS originates within specific neuronal circuits afferent to GnRH neurons.

The potential role of kisspeptin neurons

Kisspeptin is a neuropeptide that directly and potently stimulates GnRH neuron activity and GnRH release, and arcuate nucleus kisspeptin neurons are implicated as a crucial component of the GnRH pulse generator [32]. The majority of kisspeptin-expressing neurons in the

arcuate nucleus co-express neurokinin B and dynorphin—hence the name KNDy (kisspeptin/neurokinin B/dynorphin) neurons—and studies suggest that neurokinin B augments while dynorphin reduces kisspeptin release. Importantly, KNDy neurons play a prominent role in mediating sex steroid negative feedback on GnRH secretion.

Many but not all studies suggest that PNA rodents exhibit increased *Kiss1* expression, and some indicate an increased number of kisspeptin neurons, in the arcuate nucleus [31, 33, 34]. Some of these studies also demonstrate a higher number of NKB-expressing cells in the arcuate nucleus [31] and greater hypothalamic Tac2 mRNA expression [33] in PNA rats. Hypothalamic dynorphin mRNA expression does not appear to be altered in PNA mice [34]. In studies of PNA ewes, the number of arcuate nucleus cells expressing neurokinin B and dynorphin were reduced; and no change in kisspeptin cell numbers was observed in the arcuate nucleus, although kisspeptin cell body size was increased [29, 35]. PNA sheep also exhibited reduced progesterone receptor expression in the arcuate nucleus, but the degree of cellular colocalization between kisspeptin and progesterone receptors remained high [29], suggesting that loss of progesterone receptor density in kisspeptin-secreting cells does not explain impaired progesterone negative feedback in this model. Instead, these investigators proposed that such insensitivity may partly relate to the loss of inhibitory (dynorphin) neuropeptide input into GnRH neurons.

Characteristics of the kisspeptin/KNDy neuronal network in women with PCOS are unknown. In a recent study, the GG genotype of the rs4889 polymorphism in the *KISS1* gene was shown to be more common in women with PCOS [36]. Women with PCOS are reported to exhibited elevated circulating kisspeptin levels in a majority, but not all, reports [37]. Importantly, the extent to which peripherally-circulating kisspeptin concentrations parallel hypothalamic kisspeptin action on GnRH neurons is unclear, although reports of temporal concordance between kisspeptin pulses and LH pulses [38, 39] may be supportive in this regard.

Together, these data provide support for the hypothesis that kisspeptin neuron overactivity may be involved in elevated GnRH pulsatility in PCOS, and support the notion that agents targeting the KNDy neuronal network may have promise in the treatment of PCOS. Indeed, a recent clinical study suggested that a neurokinin-3 receptor antagonist can reduce both LH pulse frequency (by 3.55 LH pulses over 8 hours) and circulating LH concentrations (50% reduction in LH area under the curve), while preserving FSH secretion, in adult women with PCOS [40].

The potential role of γ-aminobutyric acid (GABA)-ergic neurons

Because of the high intracellular chloride level in GnRH neurons, $GABA_A$ receptor stimulation depolarizes GnRH neurons and can have a net excitatory effect on GnRH neurons. Progesterone and DHT decrease and increase, respectively, GABAergic stimulation of GnRH neurons [41], implicating GABA neurons in the negative feedback effects of progesterone and the pathological effects of androgen excess on GnRH neuron activity [8]. PNA mice demonstrate both increased GABAergic innervation (anatomical) and excitatory GABAergic drive (functional) onto GnRH neurons [23, 28, 42, 43]. Moreover, while increased GABA input onto GnRH neurons is evident prior to puberty and the development

of a PCOS-like phenotype in PNA mice [23, 43], the abnormally high GABAergic input to GnRH neurons can be reversed with post-pubertal administration of the androgen-receptor antagonist flutamide [42, 43]. Interestingly, these GABAergic neurons are primarily derived from the arcuate nucleus, and this GABAergic neuron population shows significantly less colocalization with progesterone receptors [28]. Additionally, a recent study demonstrated in transgenic mice that long-term selective activation of arcuate nucleus GABAergic neuron terminals in the rostral preoptic area (known to densely contact GnRH neurons) renders a number of PCOS-like changes, including abnormal estrous cyclicity, increased serum testosterone concentrations, and a trend toward increased LH pulse frequency [44]. PNA ewes also demonstrate increased GABAergic synapses/inputs onto GnRH neurons in the mediobasal hypothalamus and onto arcuate nucleus KNDy neurons, suggesting that GABAergic neurons can affect GnRH neuron activity both directly and indirectly [45]. Taken together, these studies are consistent with the notion that PNA leads to organizational and functional changes within the GABAergic neuronal networks governing GnRH secretion, promoting GnRH neuron overactivity, LH excess, and other PCOS-like features.

With regard to supportive clinical research, a recent study suggested that cerebrospinal fluid GABA concentrations were elevated in women with PCOS [46]; and the use of valproate—a medication that increases GABAergic tone—is associated with a higher risk for PCOS when used for disorders such as epilepsy [47] and bipolar disorder [48]. Although an older study suggested that valproate administration for one month did not increase LH pulse frequency in normal women [49], the role of GABAergic mechanisms in PCOS pathophysiology clearly deserves additional study.

The potential role of anti-Müllerian hormone

Anti-Müllerian hormone (AMH) is a product of granulosa cells in pre-antral and small antral ovarian follicles. Serum AMH concentrations are elevated in women with PCOS, and a recent series of experiments provided compelling evidence that AMH can directly stimulate GnRH neuron activity and secretion in mice [50]. Of interest, a recent study of daughters of women with PCOS, who are at high risk for developing PCOS [51], found that these postmenarcheal adolescents exhibit high circulating LH and AMH concentrations, with a positive correlation between the two [52]— compatible with a putative role of AMH in the neuroendocrine defects of PCOS. Another series of mouse experiments suggested that maternal AMH excess produces a PCOS-like syndrome in female progeny, including increases in GABAergic appositions onto GnRH neurons, GnRH neuron firing rate, LH pulse frequency, and mean LH concentrations [53]. While circulating AMH had access to the maternal median eminence in mice, it did not appear to traverse the placental barrier; and the aforementioned manifestations in offspring were reversed by maternal cotreatment with a GnRH antagonist [53]. Accordingly, the fetal effects of maternal AMH excess may reflect GnRH-mediated effects on maternal LH secretion and subsequent ovarian androgen production. Of interest in this regard, two recent studies suggested that circulating maternal AMH levels are increased during pregnancy in women with PCOS [53, 54], and another study suggested that cord blood AMH concentrations are elevated in neonates born to women with PCOS [55].

Selected insights from other models of PCOS

While PCOS-like neuroendocrine dysfunction is well described in PNA mice, there is no clear evidence to date for similar neuroendocrine impairments in mice exposed to postnatal androgenization [56] despite the linkage between peripubertal androgen excess and PCOS development. Nonetheless, a recent study in mice strongly implicates the CNS in the development of PCOS-like features following postnatal DHT treatment [57]. In particular, neuron-specific AR knockout prevented DHT-induced ovulatory dysfunction, mitigated the untoward effects of DHT on ovarian morphology and large antral follicle morphology, and abrogated the untoward effects of DHT on adiposity.

Long term treatment of mice and rats with the aromatase inhibitor letrozole also generates PCOS-like features [56]. In one such study, letrozole-treated mice demonstrated elevated serum LH and reduced serum FSH concentrations, lower progesterone receptor mRNA expression in the mediobasal hypothalamus, and a trend toward higher kisspeptin receptor mRNA in the preoptic area [58]. Similarly, letrozole-treated rats exhibited greater numbers of kisspeptin-immunoreactive cells in the arcuate nucleus [59, 60]. While such neuroendocrine findings may partly reflect the effects of reduced estrogen production following aromatase blockade per se, some neuroendocrine findings appear to reflect letrozole-induced hyperandrogenemia [61].

In female rhesus monkeys, experimentally producing mild (approximately 3.7-fold elevated) hyperandrogenemia via exogenous testosterone administration beginning at one year of age (prepubertal) resulted in increased early follicular phase LH pulse frequency at 5 years of age, suggesting that peripubertal hyperandrogenemia alters GnRH pulse generator function [62]. However, this testosterone treatment-related difference in LH pulse frequency was lost within 6 months of western-style diet initiation [63].

Abbott and colleagues recently described a group of reproductive-aged female rhesus monkeys with naturally higher testosterone levels. Compared to those with lower testosterone levels, these monkeys demonstrated a number of PCOS-like features including subfertility, elevated AMH, higher serum LH concentrations, and an increased serum LH-to-FSH ratio [64]. Such monkeys may represent a natural non-human primate analogue of human PCOS, and we believe that additional study of such models will be highly informative.

Peripubertal hyperandrogenemia is believed to be a risk factor for PCOS, and hyperandrogenemic adolescents demonstrate abnormal LH secretion. A recent study suggested an association between hyperandrogenemia and the absence of a sleep-related decrease in LH pulse frequency in later-stage pubertal girls [65]. Reasons for this observation are uncertain, but it could partly reflect abnormal relationships between sleep stages and LH (GnRH) pulse initiation: while follicular phase LH pulse initiation is normally discouraged by REM sleep in adult women, LH pulse initiation is not appropriately discouraged by REM sleep, and may possibly be encouraged by slow wave sleep, in adults with PCOS [66].

Final thoughts

Taken together, the clinical and preclinical studies described herein support the notion that hyperandrogenemia contributes to abnormal GnRH secretion, in part by inducing GnRH pulse generator resistance to sex steroid (progesterone) negative feedback, and that these factors play an important role in the pathophysiology of PCOS. While it remains unknown the degree to which such neuroendocrine abnormalities reflect abnormal developmental programming in utero, available data suggest that these defects are maintained by ongoing androgen excess [5, 24, 42, 43]. This suggests the utility of androgen-receptor blockade in PCOS, although the more holistic efficacy of such treatments remains unclear: for example, while some studies suggest that antiandrogens markedly improve ovulation rates in adult PCOS, other studies have not confirmed this finding [67]. Nonetheless, it is plausible that androgen-receptor blockade may be more effective during critical developmental windows, and maneuvers that reduce androgen signaling clearly deserve additional study. Pharmacological agents that target KNDy and/or GABAergic neuron function may also represent promising treatment options. Moreover, PCOS appears to involve perturbations in a number of other neuroendocrine systems, including those involved with energy homeostasis, weight maintenance, and adrenal function, and these remain important areas for further research. Collaboration among basic, preclinical, and clinical researchers will continue to be critically important as we attempt to unravel the complex pathophysiology of —and to identify potential therapeutic targets for—PCOS.

Acknowledgements

This work was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development/National Institutes of Health (NIH) through cooperative agreement P50 HD28934 as part of the National Centers for Translational Research in Infertility (CRM); NIH R01 HD102060 (CRM); the Health Research Council of New Zealand Grant 18-671 (REC); and the Marsden Fund Grant 17-064 (REC).

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