Minireview: Timely Ovulation: Circadian Regulation of the Female Hypothalamo-Pituitary-Gonadal Axis

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The preovulatory surge in the secretion of LH is timed by a neuroendocrine integrative mechanism that involves ovarian estradiol levels and the endogenous circadian system. Studies in female rats and hamsters have established that the clock in the hypothalamic suprachiasmatic nucleus has a preeminent role in setting the LH surge, and anatomical, physiological, and pharmacological data are revealing the responsible connections between suprachiasmatic nucleus neurons and GnRH and estradiol-receptive areas. Recent investigations show that GnRH and pituitary cells express circadian clock genes that might play a role in the release and reception of the GnRH signal. Analysis of the circadian regulation of the LH surge may provide a model for understanding how multiple neural oscillators function within other neuroendocrine axes. (Endocrinology 147: 1148–1153, 2006)

A Circadian Window of Secretion

A circadian mechanism is responsible for timing gonadotropin activation to a specific phase of the 24-h day. Administering barbiturates to rats (5) and hamsters (6–8) during a critical period (of a few hours duration) on the afternoon of proestrus not only blocks the expected LH surge and ovulation but then delays these processes for 24 h or multiples of 24 h if additional injections are given on successive days. Likewise, the time of maximal sensitivity of LH release to electrical stimulation of the GnRH-rich medial preoptic area is also on the afternoon of proestrus (9, 10). In ovariectomized (OVX) females primed with implants or successive injections of E2 (OVX + E2), the circadian-based trigger for LH secretion is revealed by the occurrence of a daily LH surge in the late afternoon, with release still blocked by barbiturate during the critical period (11–13). Daily LH surges are also present in female hamsters exposed to a short daylength, even after ovariectomy and in the absence of exogenous E2 (14, 15). Both the preovulatory LH surge in intact animals and the daily LH surge in OVX + E2 animals are correlated with GnRH secretion (16, 17).

Circadian regulation of the LH secretory surge parallels that of behavioral rhythmicity. Thus, when animals are housed in constant light and exhibit circadian locomotor activity rhythms with free-running periods much greater than 24 h, the frequency of their cyclic LH surges continues to maintain a 4-fold multiple of the circadian period (6, 18, 19). When animals are instead entrained to a 24-h light-dark cycle, the phase of the surge is stably coupled to the onset of locomotor activity and lordosis behavior; this phase relationship is maintained after phase shifts of the activity rhythm are induced (6, 19–22). Finally, there is a remarkable circumstance in hamsters known as “splitting” in constant light, in which the single daily bout of locomotor activity dissociates into two components stably coupled 180 degrees (~12 h) apart (Fig. 1A). In OVX + E2 female hamsters, splitting also includes an approximately
12-h LH secretory surge before each of the split locomotor activity bouts (23).

Recently, a set of circadian clock genes has been identified by analyses of induced and spontaneous mutations, gene sequence homologies, and protein-protein interactions. These clock genes are believed to lie at the core of the circadian oscillatory mechanism and function as autoregulatory feedback loops, with oscillating levels of nuclear proteins negatively regulating the transcription of their own mRNAs (for review, see Ref. 24). Circadian LH regulation is affected in the two clock gene mutants thus far investigated for such a defect. Rhythmic locomotor activity and LH secretion are both altered in parallel in females of the short-period hamster phenotype (\( \tau \)) caused by a mutation of the casein kinase 1 gene (25). Female homozygous clock mutant mice—which become behaviorally arrhythmic in constant light (26)—also show irregular estrous cycles and anomalous proestrus LH release, fail to express LH surges after OVX + E\(_2\) treatment, and exhibit a lower rate of successful pregnancy, probably due to a disruption in the circadian profile of prolactin secretion.

**A Preeminent Role for Neural Efferents from the Suprachiasmatic Nucleus (SCN)**

The master circadian pacemaker in mammals is located in the SCN of the hypothalamus (for review, see Ref. 27), and SCN lesions do abolish estrous cyclicity, the preovulatory LH surge in intact females, and the daily LH surge in OVX + E\(_2\) females (28–31). It is important to note, however, that large complete SCN lesions may also disrupt GnRH axons of passage or even encroach upon parts of the preoptic and periventricular areas critical for GnRH release. Small partial electrolytic lesions of the SCN have generally been ineffective in causing arrhythmicity, although a recent report claims that a lesion targeted to a specific SCN subregion (marked by a population of calbindin-labeled cells) abolishes circadian rhythms of behavior, physiology, and melatonin and cortisol secretion in hamsters (32). Effects on LH secretion and reproductive rhythmicity have not yet been investigated after such localized lesions.

It is believed that humoral factor(s) released by SCN nerves drive the circadian locomotor rhythm, because this rhythm is restored in arrhythmic, SCN-lesioned animals by transplantation of SCN tissue encased in semipermeable capsules (33). On the other hand, circadian LH secretion and reproductive cycles are abolished by discrete knife cuts dorsocaudal to the SCN in rats (34), and they are not restored by transplants in hamsters (35). These data raise the possibility that specific neural efferents from the SCN might carry the output signal for this function, perhaps via a known, direct, and predominantly ipsilateral projection to preoptic GnRH neurons in rats and hamsters (36, 37). Evidence for this interpretation includes the re-
sults of a recent study exploiting the “splitting” phenomenon in female hamsters (Fig. 1). Because splitting is believed to represent the activity of two circadian oscillators cycling oppositely in antiphase, and because these oscillators appear to correspond to the left and right sides of the bilaterally paired SCN (38), de la Iglesia and colleagues (39) hypothesized that each circa-12 h LH surge in split females might reflect the alternating activation of either left- or right-sided GnRH neurons. Their demonstration of a marked left-right asymmetry in immunoreactive c-Fos expression in activated GnRH neurons of split O VX + E2 hamsters is consistent with this view. Of note, guinea pigs (40, 41) and rhesus monkeys (42), in which GnRH cells are present within the hypothalamus, are able to sustain LH surges after knife cut isolation of the mediobasal hypothalamus, suggesting that hypothalamic GnRH cells caudal to the knife cut can sustain LH surges after de-efferentating the SCN.

Putative neurotransmitters for SCN efferents have been implicated (43), especially two neuroactive peptides. Arginine vasopressin (AVP) V1a receptor mRNA is expressed in the female rat preoptic area (44), AVP peptide appears to induce an LH surge when it is administered intracerebroventricularly to O VX + E2 rats (45, 46), and AVP rhythmic release may drive GnRH secretion in SCN/preoptic cocultures in vitro (47). Vasoactive intestinal polypeptide (VIP)-containing SCN axons innervate GnRH neurons (48, 49), GnRH neurons express the VPAC2 receptor (50), and central administration of antisense (51) or antiserum (52) to VIP delays and diminishes the LH surge in O VX + E2 rats, an effect similar to that seen in aging (53). The differential roles that AVP and VIP might play in LH secretion are not known, but it is noteworthy that the two peptides are synthesized in separate dorsal and ventral subdivisions of the SCN, respectively (for review, see Ref. 54). Afferents to the two subdivisions differ, and circadian oscillations between subdivisions can be functionally disassociated (55–59), hinting that AVP and VIP might represent nonredundant, perhaps differentially timed, neural signals to the hypothalamo-pituitary-gonadal axis.

Even though the SCN (60, 61) and GnRH neurons (for review, see Ref. 3) both express estrogen receptor (ER) β, the anterointerventricular nucleus (AVPV)—a sexually dimorphic region interposed between the SCN and medial preoptic area—has been proposed as a candidate point for convergent E2 and circadian signals (3). ERα is highly abundant in this nucleus and appears to be the ER critical for the positive feedback effects of estrogens and the occurrence of the LH surge (Herbison, A., University of Otago, Dunedin, New Zealand, personal communication). Because ERα cells in the AVPV are both innervated by (36, 63) and project to (64) SCN neurons, the functional circuitry of this network remains to be completely defined.

Clock Genes in GnRH Neurons

Although extra-SCN oscillators have been suspected in mammals for some time, it is only recently that a variety of cultured brain regions (65), peripheral tissues (66), and even immortalized fibroblasts after serum shock (67) have been shown to exhibit circadian oscillations in the expression of clock genes. In vitro oscillations are sustained (68, 69), including within the pituitary gland, although overall amplitude may dampen as the rhythms of individual cells desynchronize (70, 71).

GnRH neurons show immunoreactivity for PERIOD1 (72), the protein product of a core clock gene, and the GT1–7 immortalized GnRH cell line exhibits circadian rhythms of clock gene expression after the cells are synchronized by serum shock or other treatments known to induce such gene expression in fibroblasts (72–74). Importantly, serum shock also induces a circadian rhythm of GnRH mRNA expression in GT1–7 cells (73), and GnRH pulsatility is disrupted after transient transfection of the

![Schematic sagittal view of the neuroendocrine network leading to the preovulatory surge of LH](image-url)
cells with the dominant-negative mutant CLOCK protein from homozygous clock mutant mice (74). Although the role of extra-SCN cycling of clock genes in the physiological synthesis and secretion of GnRH and LH in vivo is not known, the data hint that GnRH cells and pituitary gonadotropes could be “slave” oscillators, that is autonomous oscillators whose phase is set by the master oscillator within the SCN. Thus, the SCN might time gonadotropin surges by entraining the endogenous rhythmicity of slave oscillators within GnRH neurons and the pituitary. Speculatively, such a mechanism might allow for the independent adjustment of the phase of reproductive rhythmicity relative to other SCN-regulated circadian behaviors. For example, speeding up or slowing down the endogenous circadian period of the GnRH slave oscillator could advance or delay, respectively, the phase relationship of the SCN-entrained GnRH surge to the light-dark cycle. Indeed, such a multioscillator organization might account for the observed dissociation of estrus and locomotor activity onsets in hamsters entrained to non-24-h light-dark cycles (75).

More Questions, More Opportunities

Figure 2 outlines our current concept of the neuroendocrine circuits for generating the preovulatory LH surge, but our understanding of the functional connectivity of this network is far from complete. How does a diurnally active rodent (like the grass rat, Arvicanthis) activate its GnRH neurons before lights-on rather than before lights-off (76, 77), whereas photic responsiveness and clock gene expression in the SCN, as well as SCN efferent projections to GnRH and ER-containing cells, appear similar to those in nocturnal rodents (77–79)? Is the sexual dimorphism of the SCN (80–82) critical for the induction of LH surges? Neonatal androgen exposure renders the male hypothalamus unable to sustain GnRH surges and, therefore, LH surges. Although most of the focus has been on the sexual dimorphism of the AVPV (3, 83, 84), it is still unknown whether the female SCN is differentially able to decode and generate signals leading to the LH surge. Is the modulation of the circadian system by ovarian steroids (85–88) relevant for the induction of the LH surge, and is this modulation secondary to an E2 action on SCN gene expression (89, 90) and/or possible intercellular coupling (62, 91)?

The discovery of the first hypothalamic releasing factors revealed the design by which the hypothalamic-anterior pituitary axis integrates complex neural signals to control diverse physiological and behavioral processes. The signals that time these processes to the 24-h day begin within the circadian pacemaker in the SCN, and the study of the hypothalamic-pituitary-ovarian axis will not only unravel the pathways involved in timing reproductive processes but also likely unlock common mechanistic threads in the circadian regulation of other neuroendocrine systems as well.

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