Published in final edited form as:
Horm Metab Res. 2012 July ; 44(8): 587–591. doi:10.1055/s-0032-1306301.

Hormonal Regulation of Female Reproduction

A. Christensen[1,2], **G. E. Bentley**[3,4], **R. Cabrera**[5], **H. H. Ortega**[6,7], **N. Perfito**[3,4], **T. J. Wu**[8], and **P. Micevych**[1,2]

[1]Department of Neurobiology, University of California, Los Angeles, USA

[2]Laboratory of Neuroendocrinology of the Brain Research Institute, University of California, Los Angeles, USA

[3]Department of Integrative Biology, University of California, Berkeley, USA

[4]Helen Wills Neuroscience Institute, University of California, Berkeley, USA

[5]Instituto de Investigaciones Biodmédicas-IMBECU-CONICET, Universidad de Mendoza, Argentina

[6]Morphological Sciences Department, Universidad Nacional de Litoral, Santa Fe, Argentina

[7]Argentine National Research Council, Buenos Aires, Argentina

[8]Department of Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Abstract

Reproduction is an event that requires the coordination of peripheral organs with the nervous system to ensure that the internal and external environments are optimal for successful procreation of the species. This is accomplished by the hypothalamic-pituitary-gonadal axis that coordinates reproductive behavior with ovulation. The primary signal from the central nervous system is gonadotropin-releasing hormone (GnRH), which modulates the activity of anterior pituitary gonadotropes regulating follicle stimulating hormone (FSH) and luteinizing hormone (LH) release. As ovarian follicles develop they release estradiol, which negatively regulates further release of GnRH and FSH. As estradiol concentrations peak they trigger the surge release of GnRH, which leads to LH release inducing ovulation. Release of GnRH within the central nervous system helps modulate reproductive behaviors providing a node at which control of reproduction is regulated. To address these issues, this review focuses on several critical questions. How is the HPG axis regulated in species with different reproductive strategies? What internal and external conditions modulate the synthesis and release of GnRH? How does GnRH modulate reproductive behavior within the hypothalamus? How does disease shift the activity of the HPG axis?

Keywords

HPG axis; GnRH; estradiol; LH surge

Correspondence: ***A. Christensen***, Department of Neurobiology, University of California, Los Angeles, 650 Charles E Young Drive South, Los Angeles, CA 90095, USA, Tel.: +1/310/206 0533, Fax: +1/310/825 2224, achristensen@mednet.ucla.edu.

Introduction

The complexity of controlling the hypothalamo-pituitary-gonadal (HPG) axis is apparent on several levels including peripheral hormonal regulation and the extensive network of neuronal and glial cells that mediate this function. While there is a single neuroendocrine output, gonadotropin releasing hormone (GnRH), its regulation depends on the precise coordination of internal and external signals. The relative influence of each environmental signal varies between organisms; some organisms are highly responsive to changes in day length, whereas others are more sensitive to fluctuations in temperature, olfactory input, food availability, or acoustic stimuli. In other vertebrates, this coordination of reproduction is dependent on internal signals such as steroid feedback and protein hormone modulation. In general, though, there is a complex interaction between external environmental cues and a number of internal signaling processes that converge to elicit regulation of the GnRH system, the final common pathway for transduction of these cues into a reproductive output. This physiological regulation of reproduction to induce ovulation via GnRH needs to be coordinated to regulate behavioral outcome for successful reproduction.

Female mammals exhibit an internal hormonal milieu that is in a daily state of flux and can be perturbed by disturbances in external inputs. Regardless of whether the organism is more internally or externally biased in terms of its reproductive control, estradiol produced by developing follicles in the ovaries has a central role. The ovarian synthesis of estradiol is a carefully regulated system that is coordinated by feedback mechanisms between the hypothalamus, anterior pituitary, and ovaries. It is this estradiol signal that regulates the neural network either inhibiting or facilitating the release of GnRH to act on the pituitary causing the synthesis and release of FSH or LH, ovulation, and reproductive behaviors.

A circuit that includes hypothalamic and limbic nuclei coordinating olfactory, hormonal and environmental information controls female sexual behavior in rodents, which is precisely timed to coincide with ovulation. Estradiol initially activates the circuit by acting in the arcuate nucleus of the hypothalamus (ARH), sending a projection to the medial preoptic nucleus (MPN) where olfactory and limbic system inputs are integrated and then activates the final common output of the hypothalamus ventromedial nucleus (VMH). Projections of the VMH descend to the periaqueductal gray, the vestibular complex and eventually directly and indirectly innervate motoneurons mediating the reproductive behavior.

There are many reproductive strategies that exist that evolved based on their environmental cues. Whatever the strategy, these animals are submitted to stressors and disease. This review examines the regulation and dysregulation of the LH surge that triggers ovulation, hypothalamic control of reproductive behavior and how these circuits are affected by photoperiod and disease.

Control of the LH Surge

The surge release of GnRH is triggered by a positive feedback loop involving the rapid increase in plasma estradiol concentration. In rats and humans, both estradiol and progesterone are involved in stimulating the LH surge. In the early stages of the estrus cycle, estradiol levels are low and the release of GnRH is inhibited. As estradiol levels surge at proestrus, GnRH is released and causes the release of LH, which stimulates the production of progesterone in the ovaries. This GnRH release is not directly through the action of estradiol on GnRH cells, but rather through intermediate cells which contain the neuropeptide kisspeptin. Kisspeptin has been shown to be vital for the release of GnRH, and its receptor, GPR54, is located on GnRH cells (reviewed in [1]). According to Micevych and co-workers (recently reviewed in [2]), estradiol has 2 main effects on the neural-glial network mediating the GnRH system in females. As estradiol rises during diestrus, it

induces the expression of progesterone receptors (PRs) in kisspeptin neurons, which strongly stimulate GnRH neurons. When estradiol levels peak on proestrus, membrane estrogen receptors (ERs) on astrocytes are activated, which transactivate metabotropic glutamate receptor type 1a (mGluR1a) and initiate the rapid synthesis of neuroprogesterone (neuroP; [2, 3]). The local neuroP then stimulates the kisspeptin cells to release kisspeptin onto GnRH cells and causes the release of GnRH onto the pituitary. Cabrera and his colleagues suggest a different hypothesis. They observed that GnRH release may be modified by the neurosteroid allopregnanolone, which is synthesized from progesterone. Allopregnanolone stimulates the release of glutamate activating NMDA receptors on GnRH neurons [4]. The results in this work indicate that allopregnanolone strongly stimulates GnRH release. Neurosteroids have been proposed to interact both directly and indirectly; they bind with their classical receptors and with ionic channels, such as the $GABA_A$ [5] and the glutamic acid receptors [6]. Such interactions could produce fast modifications of neuronal excitability. Further evidence suggests that gamma-aminobutyric acid (GABA) containing neurons may enhance LH release [7]. The GABAergic system regulates LH release via modulation of catecholaminergic systems that control GnRH secretion. In addition, Cabrera et al. present results suggesting that the neurosteroid allopregnanolone might have an important physiologic function due to its possible action on NMDA receptors [4]. They show an acute response to allopregnanolone in an in vitro system on GnRH release. These results suggest that allopregnanolone effects on GnRH release could be specifically mediated by NMDA receptors since co-administration of AP-7, an NMDA antagonist, decreased the stimulatory action of the neurosteroid. The reversion observed in the effect of allopregnanolone on glutamate release suggests that this action is also mediated by NMDA receptors. One possible explanation could be the existence of presynaptic NMDA receptors regulating glutamate release in glutamatergic terminals. These competing theories of estrogen positive feedback through neuroP and allopregnanolone are not mutually exclusive because neuroP may be converted to allopregnanolone by 5-alpha reductase and 3-alpha hydroxysteroid oxide reductase, both of which are present in astrocytes [5]. Indeed, both mechanisms are probably involved.

Wu and colleagues focus on the complexity of GnRH function in regulating reproductive function and behavior. They argue that GnRH-(1–5), a metabolite of the decapeptide GnRH, composed of the first 5 amino acids, may be bioactive [8-12]. Previous studies in the laboratory showed that this metabolite stimulates GnRH mRNA expression in the neuronal GT1–7 cell line [12] as well as increasing secreted GnRH pulse amplitude from hypothalamic explants [13]. GnRH treatment, on the other hand, had a negative autoregulatory effect on its own gene expression and peptide secretion, indicating that these effects are not mediated through the GnRH-R. In addition, this pentapeptide is also capable of regulating reproductive behavior [11].

GnRH-(1–5) is produced by the 75-kDa EP24.15 (also known as thimet oligopeptidase). This endopeptidase was identified in the soluble fraction of rat brain homogenates [14] and is widely distributed in a variety of cell and tissue types [15-19]. The enzyme is regulated by protein kinase A [19] and as a thermolysin-like metalloendopeptidase, the enzyme is dependent on zinc [20]. Substrate specificity is dependent upon peptide size (< 17 amino acids), without preference for the amino acid sequence [21]. However, a hydrophobic amino acid residue in the P1 and P2 positions along with a bulky hydrophobic residue in the P3′ position increases specificity [22]. The results of studies show that GnRH-(1–5) is biologically active and supports EP24.15's role as an extracellular processing peptidase and converting enzyme. Processing of GnRH by EP 24.15 to GnRH-(1–5) presents itself as an additional layer of regulation of this already complex system.

In seasonal breeders, several nonreproductive hormones affect the release of LH. In birds, it has been proposed that changing day length results in an increase in type 2 iodothyronine deiodinase (*Dio2*), which activates thyroid hormone locally in the mediobasal hypothalamus (MBH). Some potentially exciting findings on the role of thyroid hormones in the avian photoperiodic response have been published in recent years. The first of these findings was that long day lengths induce the gene for *Dio2*, an enzyme which activates thyroid hormone [23]. Long days increase conversion of T_4 into its more bioactive form T_3. Under long-day conditions, the hypothalamic content of T_3 was about 10-fold higher than under short-day conditions. In addition, the ICV infusion of T_3 induced testicular growth in quail held under non-stimulatory short days. The second finding was that there is high expression of *Dio3* [type 3 deiodinase, an enzyme which inactivates thyroid hormone via conversion of T_4 and T_3 to reverse (r) T_3 and T_2, respectively] and low expression of *Dio2* under short-day conditions. Conversely, there was low expression of *Dio3* and high expression of *Dio2* under long-day conditions, indicating increased activation and decreased inactivation of thyroid hormone in response to long days. These findings indicate that thyroid hormone gene switching is one of the earliest events detected in the photoperiodic cascade and that it must occur at or before hour 16 of a long day, as a single 16-h day can induce LH secretion [24, 25]. These opposite effects on gene activation are thought to amplify the localized action of thyroid hormones and lead to neuroendocrine changes that cause GnRH secretion a few hours later (although no study on this putative mechanism implying this action on GnRH has measured GnRH synthesis or release – but see below). Exactly how signals from this local regulation of thyroid hormones are processed and transferred to the GnRH system is not yet known, but there is possible involvement of mechanical actions of glia in the median eminence [26]. More recently, a wave of gene expression was identified at hour 14 of a single long day, and included increased thyrotropin (TSH) beta-subunit expression in the pars tuberalis [27]. Central administration of TSH to short-day quail stimulated gonadal growth and expression of *Dio2*. Thus, there appears to be a role for TSH in the regulation of the avian photoperiodic response. However, it is entirely possible that the increase in *Dio* 2 is not involved in the avian photoperiodic response and is instead a system that co-varies with day length. In support of this, thyroidectomized birds continue to exhibit a long-day gonadal response, despite having no circulating thyroxine for *Dio2* to act on and produce T_3 in the MBH [28]. Furthermore, in a species of wild songbird, exposure to a single long day resulted in an increase in LH without an increase in *Dio2* [29]. This difference was population specific with the more northern population of the same species showing the expected gene switching (i. e., an increase in *Dio2* and decrease of *Dio3* mRNA) while a more southern population did not. Yet both populations show an ultimate increase in FSH mRNA expression in the anterior pituitary and in LH secretion to plasma. These findings suggest that that regulation of *Dio2/Dio3* expression is not required for GnRH and/or gonadotropin stimulation. Current data cannot yet determine whether the differences observed are due to evolutionary or experiential effects. However, they open up new avenues for future work examining ecological and evolutionary variation in the physiological organization of reproductive timing.

Ovulation and Disease

Dysregulation of ovulation can occur during disease. One variable is an increase in the secretion of LH associated with the development of cystic ovarian disease in cattle (COD). In this disease, variable hormone levels reflect the hypothalamic-pituitary-gonadal axis attempting to maintain homeostatic control of circulating hormones.

COD in cattle is characterized by disruptions of folliculogenesis and the formation of cysts in the ovaries. These changes are correlated with alterations in the expression of ERα, ERβ, androgen receptor (AR), and PR. High expression of ERα has been described in granulosa

and theca cells of cystic follicles, concomitant with a decrease in the expression of ERβ in cattle [30, 31], rats [32], and women [33]. Gonadotrophins and estrogen downregulate granulosa expression of the ERβ isoforms [34, 35] and that both ERs show a tendency to be upregulated together as estrogen levels increase in the follicular fluid. This correlates with the upregulation in the LH and FSH receptors [36]. In mice, ERα over expression produces downregulation of the ERβ gene and subfertility [37], which raises the possibility that the decrease in ERβ observed in cystic follicles may be secondary to ERα upregulation. In this sense, studies with ERα-knockout mice that were anovulatory also indicate that ERα is not required for follicular recruitment or early differentiation but is necessary for subsequent follicular growth [38], an important component of COD pathogenia in cattle.

PRs are mainly expressed in bovine granulosa cells [39, 40]. But, cystic follicles show a higher expression in the thecal cells compared with healthy follicles [31]. Multiple PR isoforms may partly explain the intracellular processes involved in progesterone activation of a target cell. The 116 kDa B-receptor (PRb) and the 94 kDa A-receptor (PRa) can have very different transcriptional functions that are cell specific and promoter specific. Although the PRb and PRa isoforms have similar ligand-binding affinities and similar DNA-binding affinities [41], the different isoforms have different functions and PRb significantly increases in the granulosa of cystic follicles [31]. While selective ablation of PRb does not affect ovarian function, double PR (PRa and PRb) knockout mice fail to ovulate [42].

Animals with COD usually present high levels of circulating estrogen and LH [43], and this could cause alterations in PR expression. One possible scenario is that changes in the ratio of PR isoform expression could regulate the biological activity of progesterone and result in functional hormone withdrawal in the absence of changes in serum concentrations or total progesterone-binding activities of the reproductive tissues [44-47]. Ovaries from animals with COD exhibited an altered steroid receptor expression compared with ovaries from control animals and numerous studies provide evidence that an altered steroid signalling system may be present in the bovine cystic follicles. Therefore, it is reasonable to suggest that in conditions characterized by altered ovulation, such as COD, changes in the expression of ovarian steroid receptors could play a fundamental role in the pathogeny of this disease.

Reproductive Behavior

Reproductive behavior in female mammals is timed to coincide with ovulation. Since reproduction in many cases requires some risk to the well-being of the animal, it is only during ovulation, when pregnancy could result, that the risk is acceptable. Estradiol from developing ovarian follicles begins to activate neural circuits that control receptive behavior. In the ARH, estradiol rapidly activates mERα and transactivates the mGluR1 [48]. In both the VMH and the ARH, estradiol increases spine density [49, 50]. This increase in dendritic spines is important for the regulation of female sexual behavior [50]. The growth of estradiol-induced spines may play a role in the timing of sexual behavior. A bolus of estradiol given to an ovariectomized rat initially produces immature filapodial spines, which are thought to be insufficient for synaptic transmission. However, at later time points, when the female is sexually receptive, the spines mature to functional mushroom-shaped spines. This change in synaptic morphology underlies critical changes in neuronal connections within the lordosis regulating circuit. When spinogenesis is pharmacologically inhibited in the ARH, females do not display full sexual receptivity.

The interaction between ERα and mGluR1, which has been shown to be critical for reproductive behavior [48], was shown to be important for the growth of estradiol-mediated spinogenesis as well [50]. The actin depolymerizing factor, cofilin, is rapidly

phosphorylated and inactivated during spinogenesis. Estradiol causes the phosphorylation of cofilin. However, when mGluR1 is inhibited, estradiol is unable to increase the phosphorylation of cofilin and the growth of new spines is decreased.

Conclusions

The regulation of reproduction is multi-faceted. It begins with the regulation of the ovaries. The level of estradiol released by these structures ultimately determines the readiness of the system to advance to the next stage of fertility. The release of LH is then regulated in several ways. Not only must the level of estradiol increase beyond some critical point, but progesterone and allopregnanolone must begin to be synthesized by astrocytes in the MBH. These neurosteroids have several effects, but ultimately lead to the release of GnRH and finally LH. As another level of regulation, GnRH level is controlled by an enzyme that cleaves it. In this way its own concentration of release is increased.

Diseases of the ovary which perturb the secretion of sex steroids disrupt the entire axis. Changes in the secretion of estradiol initiate disruptions in ability of the animal to produce the LH surge required for regulation of the rest of the HPG axis. Indeed, dysregulation of any part of the axis will result in a loss of normal reproductive behavior.

Because ovulation is so closely tied to reproductive behavior, the estradiol that causes the release of LH also has other roles within the reproductive circuit. Importantly, it is able to alter the synaptic connections of the hypothalamus to increase the sexual receptivity of the female. It is only when the entire system is healthy and ready for reproduction that sexual behavior is able to occur successfully.

Seasonal breeders must be able to detect the appropriate time of year for reproduction. In order to do this some part of their internal milieu must change in response to changing day length. It is possible that an increase in the thyroid hormone activating enzyme, *Dio2*, is one method used by some seasonal breeders to prepare for the mating season.

The regulation of the reproductive system is complex and requires the coordination of multiple central and peripheral systems and the input of internal and external environments. There is much known about the neurosteroids and enzymes that are involved centrally in regulating the release of GnRH as well as the roles of steroids and their receptors peripherally. Many questions remain to be answered such as: how are the neuroP and allopregnanolone systems coordinated together to regulate GnRH release? Is the loss of steroid receptors seen in COD the cause or result of the disease? And can this change in receptor levels be observed with other diseases or reproductive senescence? And what is the timing in these cases?

Although neuroendocrinology has unraveled a variety of physiological processes that require the coordination of the brain and body to respond to the external environment to ensure reproductive success, a large number of unresolved questions remain to be answered. Answering these questions will provide the basis for understanding the integration that is regulated by the reproductive neuroendocrine axis.

Acknowledgments

Supported by NSF IOS#1052288 (TJW) and DoD RO85FN (TJW).

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