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Neurobiological mechanisms underlying oestradiol negative and positive feedback regulation of gonadotropin-releasing hormone (GnRH) neurones

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Abstract

The feedback actions of ovarian oestradiol during the female reproductive cycle are among the most unique in physiology. During most of the cycle, oestradiol exerts homeostatic, negative feedback upon the release of gonadotropin-releasing hormone (GnRH). Upon exposure to sustained elevated oestradiol levels, however, there is a switch in the feedback effects of this hormone to positive, resulting in induction of a surge in the release of GnRH that serves as a neuroendocrine signal to initiate the ovulatory cascade. Here we review recent developments stemming from studies in an animal model exhibiting daily switches between positive and negative feedback that have probed the neurobiological mechanisms, including changes in neural networks and intrinsic properties of GnRH neurones, underlying this switch in oestradiol action.

Introduction

Once each female reproductive cycle, the normal pattern of steroid negative feedback upon gonadotropin-releasing hormone (GnRH) neurones is interrupted by a positive feedback response to the sustained elevation of oestradiol at the end of the follicular phase (1–5). This positive feedback initiates a surge of GnRH release, which in combination with oestradiol action at the pituitary activates the luteinizing hormone (LH) surge and thus triggers ovulation. As a result of this switch, the pattern of GnRH release is altered from one that is strictly episodic, with little secretion in between pulses, to one that produces a continuous elevation of GnRH levels in the pituitary portal blood that lasts for many hours (6,7). Oestradiol also has feedback actions at the pituitary, notably increasing responsiveness to GnRH during positive feedback (8–11), but here we will focus on changes at the level of the central nervous system, in particular at the GnRH neurone and its afferents.

The negative and positive feedback actions of oestradiol have been known for decades. It is only recently, however, that advances in methodology have allowed us to begin to probe the types of neurobiological mechanisms that oestradiol engages to bring about these changes in GnRH release. Primary among these was the development of reporter gene transgenic animals to allow identification and measurement of the biophysical properties of GnRH neurones (12–14). Use of these transgenic models has substantially increased our understanding of the mechanisms of oestradiol positive and negative feedback.

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An endocrine model for the study of oestradiol feedback actions

The study of oestradiol action is complicated by the dose and duration dependence of negative and positive feedback. During the early follicular phase, lower levels of oestradiol provide negative feedback, largely reducing the amplitude of GnRH pulses (5). As the follicular phase progresses, GnRH pulses accelerate due both to the effects of oestradiol and further withdrawal from progesterone negative feedback of the luteal phase. This drives increases in both LH levels and subsequent steroidogenesis, causing a sustained elevation of circulating oestradiol levels. Although circulating oestradiol levels actually decline during the surge, ample evidence indicates this sustained exposure to elevated oestradiol is the trigger during the cycle for the switch from negative to positive feedback (1,15).

An argument can be made that the best experiments would examine how regulation of GnRH neurones changes during this normal cycle. This would indeed provide valuable insight, but this approach is complicated by several factors. First, oestrous cycle detection is not as reliable in mice as in other rodents, making the identification of cycle stage problematic in most of the available transgenic models. Second, GnRH neurones from different cycle stages would be exposed to different levels of oestradiol for different durations. Third, other ovarian hormones also change in a cyclical manner, further adding to the number of variables that must be accounted for in data interpretation. Finally, some genetic models that are of interest for understanding the mechanisms of feedback do not cycle, precluding studying the natural cycle and mandating an endocrine treatment to induce the surge.

For these reasons, we chose to use a model of surge induction. In rodents, there have been three main approaches to this problem. The first two involve ovariectomy, treatment with a basal level of oestradiol for approximately one week followed by injections of oestrogen alone, or oestrogen followed by progestin one day later (16–19). These models induce surges, but they do not avoid the problem of a changing endocrine milieu. Further the levels of steroids achieved by injection are often supraphysiological.

The third model arose from observations of Everett and Sawyer in the middle of the past century that ovulation in rats could be delayed for 24 hrs by general anaesthesia if this was timed appropriately relative to the day-night cycle (20). These observations lead to the postulate that the central nervous system generates a daily signal that initiates the ovulatory process, and serve as the underpinning of our current understanding of the importance of the interaction between circadian and oestradiol signals in generating the switch between negative and positive feedback. Two decades after these studies, work in the rat and hamster demonstrated that ovariectomy and replacement with a constant high physiological level of oestradiol generated daily surges in LH levels reminiscent of negative and positive feedback (21,22). A few years ago, we extended this model to the mouse to take advantage of the ability to identify GnRH neurones for electrophysiological recording (23).

An advantage of this “daily surge” approach in studying the effects of oestradiol feedback is that the level of oestradiol remains constant and time of day is the only variable. This replicates to some extent the sustained elevation in oestradiol at the end of the follicular phase, when the neural response switches from negative to positive, and provides a basis for understanding how neurobiological processes change independent of a change in circulating oestradiol level. There are, of course, also limitations to this model. We cannot be certain that surges observed on subsequent days are mechanistically the same, although thus far no variation in measured parameters among surge days has been observed. Further, important changes caused by variations in other ovarian hormones, such as progesterone, are not taken into account and will require additional investigation. Nonetheless, we feel the positives of

this approach outweigh the negatives for establishing a baseline of neurobiological mechanisms; all the data reviewed below were gathered utilizing this model.

Daily surges *in vivo* and changes in GnRH neurone activity in brain slices

To generate daily surges, we ovariectomised (OVX) adult mice (42–90 days of age) on experimental day 0 and implanted a capsule producing a high but physiological level of oestradiol (OVX+E) (23). OVX mice without further treatment serve as controls. This oestradiol treatment generates daily surges in LH from days 2–5, with low levels of LH indicative of negative feedback in the morning (AM) and high levels of LH indicative of positive feedback in the late afternoon (PM). OVX controls not treated with oestradiol show no time-of-day-dependent effects. After day 5, the LH surges decline in amplitude, but can be restored by replacement of the oestradiol capsule. All measures were conducted on days 2–4, when oestradiol levels and surge response are stable.

GnRH neurone activity in brain slices prepared at different times of day was examined using extracellular recordings. This is a critical step as the mechanisms underlying the increase in GnRH release that leads to the surge in species in which this hormone has been directly measured (2–5) may be interrupted by removal of the brain and slicing for recording. Similar to the lack of change in LH levels, there were no time-of-day differences in activity of GnRH neurones from OVX mice. In contrast, oestradiol suppressed GnRH neurone firing during negative feedback and increased firing during positive feedback.

Monitoring the activity of a single GnRH neurone in a brain slice and relating it to LH levels measured *in vivo* omits the measurement of GnRH itself. Although this limitation is commonly encountered, particularly in small species, it is important to strengthen the correlation between these parameters. To do so, we again returned to the classic work of Everett and Sawyer (20), and adapted the pentobarbital sedation model to mice (24). Sedation of mice for one hour beginning 8.5 hours after lights on (14L:10D) blocked generation of the LH surge that day, just as it had blocked ovulation in the rat. No oestradiol-induced increase in GnRH neurone activity during the normal time of positive feedback was observed in brain slices from these mice. Together these data indicate that oestradiol-induced diurnal changes in activity of GnRH neurones persist in the brain slice, and that they correlate with changes in LH release in at least two experimental paradigms. This suggests that at least minimal circuitry required for generating oestradiol negative and positive feedback is preserved in this preparation, and that investigations into underlying neurobiological mechanisms can provide insight into this question.

Role of changes in fast synaptic transmission to GnRH neurones in oestradiol feedback

To elicit feedback regulation of GnRH neurones, oestradiol may act via classical oestrogen receptors (ER) α and β , which can act either as transcription factors or to initiate membrane-associated signaling cascades, and/or via more recently described membrane receptors (25–28). Several lines of evidence suggest ER α is critical for a substantial portion of feedback (29). Mice with ER α knocked out are infertile and do not exhibit positive or negative feedback, whereas ER β knockouts can exhibit reproductive cycles, although they are subfertile (30–32). In the daily surge model, mice with mutations of ER α that prevent only its association with oestrogen response elements but leave other genomic and non-genomic signaling modalities intact do not exhibit the oestradiol-induced changes in GnRH neurone activity that wild-type mice do (33,34). These data, in combination with the failure of the vast majority of studies to detect ER α in native GnRH neurones, strongly suggest oestradiol-sensitive afferents are critical for negative and positive feedback.

Fast synaptic transmission mediated via the ionotropic receptors for GABA and glutamate underlies a large proportion of neural communication. Both GABA and glutamate neurones express ER α and project to GnRH neurones, and GnRH neurones express functional GABA_A, AMPA and NMDA receptors making these modes of communication likely candidates for conveying the feedback effects of oestradiol (12,35–40). Glutamatergic transmission is excitatory to cells, whereas the direction of response to GABA depends on the cell's membrane potential and the chloride equilibrium potential (41). Although still debated, considerable evidence suggests GnRH neurones maintain elevated intracellular chloride levels in adulthood and therefore can be excited by activation of GABA_A receptors (42–47). We thus hypothesized that oestradiol would reduce fast synaptic transmission by GABA and/or glutamate during negative feedback, and would increase fast synaptic transmission by GABA and/or glutamate during positive feedback.

GABA

Spontaneous GABAergic postsynaptic currents (p.s.c.s) were recorded from GnRH neurones using whole-cell voltage-clamp at -60 mV with an isotonic chloride pipette solution after pharmacologically blocking glutamatergic currents (48). There were no diurnal changes in GABAergic p.s.c. frequency in cells from OVX mice. In contrast, in cells from OVX+E mice, p.s.c. frequency and amplitude varied diurnally in a manner that depended upon the orientation that brain slices were cut. In coronal sections, p.s.c. frequency was low during negative feedback relative to OVX controls, increased at surge onset, and was somewhat reduced relative to surge onset levels at the surge peak. In sagittal sections, p.s.c. frequency was similarly suppressed during negative feedback and increased at surge onset, but unlike in coronal sections, the increase in GABAergic transmission to GnRH neurones was maintained during the surge peak. These data suggest oestradiol-induced changes in GABAergic transmission may be involved in multiple aspects of negative and positive feedback. First, the oestradiol-induced reduction in p.s.c. frequency during negative feedback provides one mechanism for reduced GnRH neurone activity at this time: a reduction in excitatory drive. Second, the activity of at least two different populations of GABAergic afferents increases during positive feedback. The increase at surge onset may help drive the increase in GnRH neurone activity, whereas the increased transmission during the peak of the surge may help maintain activation of this system.

The marked effects of oestradiol on GABA transmission suggested further investigation would reveal additional insight. The overall increase in GABA transmission could be broken down into two subgroups. P.s.c. frequency increased substantially in about one third of GnRH neurones examined during the time of surge onset and surge peak, whereas the rate of transmission remained similar to that observed in cells from OVX mice in two thirds of cells. This dichotomy could be due to subpopulations of GnRH neurones that arise either due to their involvement in generating the positive feedback response, or in the signal they receive (GABA vs. another) to increase their activity during the surge. We favor the latter explanation as the increase in GnRH neurone activity occurs in the majority of GnRH neurones, not just one-third of cells, suggesting additional signals may be involved (23). That said, the GnRH neurones receiving increased GABA transmission were located more medially and largely in the vicinity of the organum vasculosum of the lamina terminalis (OVLT), a region implicated in knife-cut studies to be involved in positive feedback.

The mechanisms by which oestradiol increased the frequency of GABAergic p.s.c.s was examined by recording miniature (m) p.s.c.s. Miniature currents are recorded after blockade of action potential firing and are thus independent of the activity of the presynaptic network. If an increase in spontaneous transmission is due to increased activity of that network, this increase should be eliminated by blocking action potential firing. If, however, the increase in spontaneous transmission is due to an increase in the number of contacts made by the

presynaptic network on the postsynaptic GnRH neurone or a change in release probability from the synaptic terminal, then blockade of firing activity should not eliminate the increased transmission as the frequency of m.p.s.c.s is proportional to the amount of synaptic contact, which would not be affected by a short-term block of firing activity. These mechanisms are not exclusive of one another; oestrogens have been reported to alter both neuronal activity and parameters that can alter synaptic connectivity such as the number of dendritic spines and neuronal-glial apposition (49–51). In GnRH neurones in coronal sections, the frequency of GABA transmission during positive feedback was not altered by action potential blockade, suggesting an action potential-independent mechanism. In GnRH neurones in sagittal slices, however, blockade of action potential firing reduced GABA transmission in many cells, suggesting oestradiol-induced increases in the activity of GABAergic afferents underlie some of the increased transmission observed in sagittal sections.

GnRH neurons are surrounded by many populations of GABAergic neurons that could remain within either coronal or sagittal slices. Although the origin of populations providing increased transmission in coronal slices is not known, the sagittal brain slice preparation has the potential to preserve connections from at least two regions of high interest with regard to positive feedback: the suprachiasmatic nuclei (SCN) and the anteroventral periventricular region (AVPV). LH surges are blocked by lesion of either of these regions and both contain GABAergic neurones (52,53). Anatomical evidence exists for a direct path to GnRH neurones from both the SCN and AVPV, and also an indirect path from the SCN via the AVPV to GnRH neurones (54,55). The SCN is the primary circadian oscillator in the brain, and although it does not appear to express ER α , it receives oestradiol-sensitive inputs, including from the AVPV (56,57). The AVPV expresses ER α and has been postulated by many to be an integration center for circadian and steroid cues in regulating GnRH neurones (55). To begin to examine the sources of the GABAergic input to GnRH neurones, brain slices were prepared for recording at surge peak and a cut was placed in sagittal brain slices caudal to the AVPV but rostral to the SCN, extending approximately 0.5 cm from the ventral surface of the brain (48). This cut would sever SCN-to-GnRH neurone projections, as well as those to GnRH neurones arising more caudally. In the most medial sections, which would contain the SCN, the frequency of GABA transmission was reduced in cut slices, indicating the SCN as a potential source. In more lateral sections, however, the cut had no effect, suggesting the inputs arise from areas rostral to the cut, possibly the AVPV. In these lateral sections, blockade of action potential firing did not reduce the high frequency of GABA transmission to GnRH neurones. Together these data suggest the AVPV may be a source of an action potential-independent increase in GABA transmission to GnRH neurones, such as a change in innervation pattern, and that at least a portion of the activity-dependent increase may arise from near the SCN.

Glutamate

To assess the role of glutamatergic transmission in conveying oestradiol-dependent signals to GnRH neurones similar studies were performed, beginning with an initial characterization of AMPA vs. NMDA-mediated transmission. Whole-cell voltage-clamp recordings were made under conditions to isolate glutamatergic currents, including blockade of GABA $_A$ receptors (58). The membrane potential of the cell was held at either -70 mV, or $+40$ mV to remove the Mg $^{2+}$ blockade of NMDA receptors. Excitatory postsynaptic currents (e.p.s.c.s) were observed at both potentials, with those observed at -70 mV being blocked by the AMPA/kainate receptor antagonist CNQX and those at $+40$ being blocked by the NMDA receptor antagonist APV. Interestingly, unlike GABA transmission, which is observed in essentially 100% of studied GnRH neurones, approximately a quarter of GnRH neurones do not exhibit glutamatergic e.p.s.c.s that are detectable by recordings at the cell body. Further,

the frequency of glutamatergic transmission is much lower than that of GABAergic transmission. Although a small percentage of cells did exhibit NMDA receptor-mediated e.p.s.c.s, a much higher proportion exhibited AMPA/kainate receptor-mediated currents, consistent with a prior report of functional glutamatergic receptor expression by GnRH neurones (12,59).

Because most currents were AMPA/KA receptor-mediated, we focused on these (60). As with GABAergic transmission, there was no diurnal change in glutamatergic transmission in OVX mice. In oestradiol-treated mice, AMPA/KA receptor-mediated glutamatergic transmission to GnRH neurones was reduced during negative feedback compared to that in OVX controls. During positive feedback, however, glutamatergic transmission to GnRH neurones was not elevated relative to OVX controls, suggesting a more important role for oestradiol-induced changes in glutamate may be during negative feedback, reducing glutamatergic transmission and thus further reducing excitatory drive to GnRH neurones.

Is fast synaptic transmission critical for oestradiol-induced changes in GnRH neurone activity?

The above studies show correlation between oestradiol-induced changes in GnRH neurone activity and fast synaptic transmission, but they do not demonstrate that fast synaptic transmission is critical for generating negative or positive feedback. To approach this, both ionotropic GABA and glutamate transmission were blocked (“blockade”) in the sagittal slice preparation and GnRH neurone activity was monitored (24). Under blockade conditions, GnRH neurones from oestradiol-treated animals recorded in the AM showed elevated, rather than suppressed activity compared to OVX controls, and those recorded in the PM showed suppressed rather than elevated activity typical of GnRH neurons from OVX+E mice. Interestingly, the normally low GnRH neurone activity observed in the PM in pentobarbital-sedated mice was elevated by blockade. Thus the increases in GABAergic and glutamatergic transmission to GnRH neurones that were observed in this model are an important component in elevating GnRH neurone activity during positive feedback. We postulate pentobarbital sedation either prevents these increases or alters their timing and thereby blocks the neural signal for the GnRH surge from occurring during that day.

Together these data indicate that oestradiol and the diurnal cycle interact to generate changes in neurotransmission to GnRH neurones. These neurobiological changes constitute a part of the mechanisms underlying the switch in oestradiol feedback action that produces the changes in GnRH neurone firing activity needed to generate the GnRH surge and ultimately trigger ovulation.

Neuromodulators and oestradiol feedback

There is considerable evidence that in addition to fast synaptic transmission, neuromodulators that act via metabotropic receptors, such as vasoactive intestinal polypeptide (VIP), kisspeptin, and vasopressin, may also be involved in the regulation of GnRH neurones by oestradiol (61–64). Indeed, the data from the neurotransmitter receptor “blockade” experiment discussed above point to an important role for neuromodulation in the circuitry for establishing a negative feedback state. Specifically, observed transmission from either GABA or glutamate directly to GnRH neurones during negative feedback was very low and one wouldn’t expect that solely blocking this low level of transmission, which would be excitatory in any case, would lead to the marked increase in GnRH neurone activity that was observed. Because the blockade cocktail was applied to the entire slice, and because several layers of synaptic connection can be preserved in brain slices, we interpret this to indicate that an “upstream” blockade of fast synaptic transmission altered neuromodulation directly at the GnRH neurones, likely reducing release of inhibitory

neuromodulator(s), to produce the increase in GnRH firing rate observed during the normal time of negative feedback.

Because VIP is produced by the SCN, and VIP-containing fibers project to GnRH neurones expressing receptors for it, this peptide is of particular interest with regard to generating a response that has a diurnal dependence, like oestradiol feedback in rodents. The effects of VIP were examined in the daily surge model. VIP had no effect on GnRH neurone firing activity in OVX mice regardless of time of day (65). VIP also had no effect on GnRH neurone activity during the normal time of negative feedback in slices from OVX+E mice. In contrast, VIP increased activity of about half of GnRH neurones from OVX+E mice at the time of surge onset, similar to the percentage of these cells reported to express the type 2 VIP receptor (66). Curiously, VIP had no effect during the peak of the surge. To test the hypothesis that the lack of effect of VIP during surge peak was due to occlusion by endogenous peptide, the effects of a VIP antagonist were examined at this time. Antagonizing VIP receptors reduced GnRH neurone firing activity in about half of cells during surge peak, corresponding remarkably well with the percent responding to the exogenous peptide during the onset of the surge. This suggests the response of GnRH neurones to VIP is dependent both upon oestradiol and time of day.

Intrinsic changes in GnRH neurones

Because of the importance of ER α in generating the positive feedback response, much of the focus has been on transsynaptic mechanisms. Given the likely role of neuromodulators, as demonstrated above, and possible modulatory roles of ER β , it is important to also consider changes in the intrinsic properties of GnRH neurones in surge generation. These studies are more limited, but changes in the intrinsic properties of GnRH neurones induced by oestradiol treatment *in vivo* have been found. Oestradiol increases an inward sodium current that is activated after a GnRH neurone fires an action potential (67). This current underlies generation of a slow afterdepolarization potential (sADP) in GnRH neurones. This sADP depolarizes GnRH neurones towards threshold and additional spontaneous action potentials often occur during this event, which lasts for about one second in these cells. Indeed there is a significant increase in spontaneous firing activity during the sADP in GnRH neurones from oestradiol-treated mice in the daily surge model. This is accompanied by a reduction in the afterhyperpolarizing potential and a hyperpolarization of the threshold for action potential firing (68). All three of these are intrinsic properties and the direction of oestradiol-induced changes for all would lead to an increase in GnRH neurone excitability. Interestingly, unlike synaptic changes monitored thus far, there is no time of day dependence; that is, oestradiol increases the intrinsic excitability of GnRH neurones during both negative and positive feedback. The low activity of GnRH neurones during negative feedback is thus likely due to a combination of low excitatory drive from GABAergic and glutamatergic fast synaptic transmission, and an inhibitory neuromodulatory tone. Based on the studies thus far, it is tempting to speculate that synaptic changes are modified by both the circadian clock and oestradiol, whereas intrinsic changes are only sensitive to oestradiol. Preliminary evidence on calcium currents, another intrinsic property, however, suggest these are sensitive to both oestradiol and time of day (69).

Conclusions

The phenomena of oestradiol negative and positive feedback have intrigued neuroendocrinologists for decades. By establishing a model system in which both ends of the oestradiol feedback spectrum are generated while minimizing variables, we have begun to make inroads into the neurobiological processes that are altered to bring about these oestradiol-dependent changes in GnRH neurone function (Figure 1). As the picture from this

model system crystallizes, additional variables that accompany this response during the normal cycle can be incorporated to understand how these variables modulate the fundamental oestradiol-driven switch from negative to positive feedback.

Abbreviations

GnRH	gonadotropin-releasing hormone
OVX	ovariectomised
LH	luteinizing hormone
TTX	tetrodotoxin
ADP	afterdepolarization
sADP	slow ADP
AHP	afterhyperpolarization
DPN	2,3-bis(4-hydroxyphenyl)-propionitrile
PPT	propylpyrazoletriol
APV	D(-)-2-amino-5-phosphonovaleric acid
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione

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Figure 1.

Model depicting possible feedback actions of estradiol upon the GnRH neuronal network. Oestradiol interacts with the circadian clock to produce diurnal changes in the regulatory network afferent of GnRH neurons, including changes in GABA and glutamate transmission and neuromodulation of GnRH neurons, resulting in a net negative afferent signal during negative feedback in the AM and positive feedback as the time of lights-out approaches in the PM. This produces an alteration between a strictly episodic GnRH signal and one that is elevated in surge mode for several hours. Oestradiol may also act directly on GnRH neurons in a positive manner that is independent of the circadian clock to modulate the output of these cells.