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Neurosteroids, trigger of the LH surge

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ABSTRACT

Recent experiments from our laboratory are consistent with the idea that hypothalamic astrocytes are critical components of the central nervous system (CNS) mediated estrogen positive feedback mechanism. The "astrocrine hypothesis" maintains that ovarian estradiol rapidly increases free cytoplasmic calcium concentrations ($[Ca^{2+}]_i$) that facilitate progesterone synthesis in astrocytes. This hypothalamic neuroprogesterone along with the elevated estrogen from the ovaries allows for the surge release of gonadotropin-releasing hormone (GnRH) that triggers the pituitary luteinizing hormone (LH) surge. A narrow range of estradiol stimulated progesterone production supports an "off-on-off" mechanism regulating the transition from estrogen negative feedback to estrogen positive feedback, and back again. The rapidity of the $[Ca^{2+}]_i$ response and progesterone synthesis support a non-genomic, membrane-initiated signaling mechanism. In hypothalamic astrocytes, membrane-associated estrogen receptors (mERs) signal through transactivation of the metabotropic glutamate receptor type 1a (mGluR1a), implying that astrocytic function is influenced by surrounding glutamatergic nerve terminals. Although other putative mERs, such as mERβ, STX-activated mER-$G\alpha_q$, and G protein-coupled receptor 30 (GPR30), are present and participate in membrane-mediated signaling, their influence in reproduction is still obscure since female reproduction be it estrogen positive feedback or lordosis behavior requires mERα. The astrocrine hypothesis is also consistent with the well-known sexual dimorphism of estrogen positive feedback. In rodents, only post-pubertal females exhibit this positive feedback. Hypothalamic astrocytes cultured from females, but not males, responded to estradiol by increasing progesterone synthesis. Estrogen autoregulates its own signaling by regulating levels of mERα in the plasma membrane of female astrocytes. In male astrocytes, the estradiol-induced increase in mERα was attenuated, suggesting that membrane-initiated estradiol signaling (MIES) would also be blunted. Indeed, estradiol induced $[Ca^{2+}]_i$ release in male astrocytes, but not to levels required to stimulate progesterone synthesis. Investigation of this sexual differentiation was performed using hypothalamic astrocytes from post-pubertal four core genotype (FCG) mice. In this model, genetic sex is uncoupled from gonadal sex. We demonstrated that animals that developed testes (XYM and XXM) lacked estrogen positive feedback, strongly suggesting that the sexual differentiation of progesterone synthesis is driven by the sex steroid environment during early development.

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1. Role of neuroprogesterone in female reproduction

Ovulation is a critical event in mammalian female reproduction. In rodents and primates, maturing ovarian follicles synthesize and secrete estrogens. Circulating estrogen levels increase until they activate the hypothalamic-pituitary axis producing a surge release of luteinizing hormone (LH). This is the estrogen positive feedback that triggers ovulation. During positive feedback, the same estrogens that inhibited the hypothalamus and pituitary gland now stimulate these cells [1]. Many of the steps in the positive feedback cascade have been elucidated. In particular, rising estrogen levels induce the synthesis of hypothalamic progesterone receptors (PRs), which are required for the LH surge [2–6]. Specifically, Chappel and Levine demonstrated that both transcription and activation of PRs in the hypothalamus are obligatory events in the stimulation of the gonadotropin-releasing hormone (GnRH) and LH surges in estradiol-primed, ovariectomized (OVX) rats [7]. Studies with PR knockout mice *in vivo* demonstrated that PR-A in the hypothalamus, but not PR-B, mediates the LH surge and sexual receptivity in estrogen-primed female mice [8]. Therefore, not only is a pre-ovulatory increase in peripheral estradiol required, but an increase in progesterone synthesis

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and activation of PRs are all essential for inducing the LH surge.

In the intact rat, both the ovary and the adrenal cortex are highly steroidogenic organs capable of producing the pre-ovulatory rise in progesterone needed for the LH surge [9]. However, no significant rise in progesterone has been detected in the systemic circulation prior to the LH surge, indicating that the progesterone required for the LH surge may not be synthesized peripherally [10–12]. Consistent with this idea is that neither the adrenals nor the ovaries are necessary for an estrogen-induced LH surge [13,14]. Indeed, OVX and adrenalectomized (ADX) rats injected with 17β-estradiol, but not progesterone, have been shown to produce a robust LH surge [13,15].

The source of this progesterone appears to be from the brain. The steroidogenic capacity of the brain has been well established [16–26]. Neuroprogesterone, progesterone synthesized *de novo* in the brain, can be induced by estradiol. Neurons, astrocytes, and oligodendrocytes have been demonstrated to possess all the steroidogenic enzymes and associated proteins required to convert cholesterol directly to progesterone within the brain, including cytochrome P-450 side-chain cleavage (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), steroidogenic acute regulatory protein (StAR), and 18 kDa translocator protein (TSPO), formerly known as peripheral-type benzodiazepine receptor (PTBR) [27,28]. Estradiol treatment of OVX/ADX female rats increased hypothalamic neuroprogesterone levels and induce a physiological relevant LH surge, indicating that the source of progesterone was neither the ovary nor adrenal gland [15]. Furthermore, treatment with trilostane, a blocker of the enzyme 3β-HSD that catalyzes the conversion of pregnenolone to progesterone, inhibited the LH surge, indicating that neuroprogesterone synthesis is critical for estrogen positive feedback in OVX/ADX female rats [15]. In gonadally intact rats with normal four-day estrous cycles, blocking hypothalamic steroidogenesis with aminoglutethemide (AGT), a P450scc enzyme inhibitor, on the morning of proestrus eliminated the LH surge, ovulation and luteinization [29]. After several days, the effects of AGT wore off, hypothalamic progesterone synthesis recovered, and the treated rats resumed their estrous cycles. These data strongly suggest that estrogen stimulates neuroprogesterone synthesis locally within the hypothalamus, which is essential (directly or through its metabolites) in mediating the positive feedback regulation of the LH surge.

2. Estrogen effects on astrocytes

Our understanding of astrocytes in regulating nervous system function has evolved from providing structural support to regulating metabolic events [30] and synaptic function in adjacent neurons [31,32]. Astrocytes respond to numerous transmitters, peptides, and steroids [33–36]. It is now well accepted that estradiol acts on astrocytes [37]. Similar to granulosa cells of the ovary, astrocytes have been shown to express estrogen receptor-alpha (ERα) and estrogen receptor-beta (ERβ), which provides a mechanism for estradiol regulation [33,38–42]. Estradiol profoundly influences astrocyte morphology and function [43], glial fibrillary acidic protein (GFAP) distribution [44], sexual differentiation [45], and steroidogenesis [46,47]. The presence of surrounding neurons further enhances the changes in astrocytic shape induced by estradiol [48]. Astrocytes have been described to play an important role in estrogen-mediated neuroprotection [49]. Astrocytes also regulate numerous hypothalamic processes including regulation of releasing factors [50–54] and synthesis of neurosteroids [54–57].

Although enriched cultures of neurons and oligodendrocytes are capable of synthesizing progesterone, enzymatic activity studies indicate that astrocytes are the most steroidogenically active cells in the brain [57]. Not only do astrocytes contain ERs and interact with neurons in response to estradiol, but astrocytes are the main source of the essential neuroprogesterone produced within the hypothalamus [15,37,46,57]. Thus, hypothalamic astrocytes are critical for the central nervous system (CNS) response mediating estrogen positive feedback [58,59]. This increase in hypothalamic neuroprogesterone activates the progesterone receptors in the neuronal circuit that regulates the activity of GnRH neurons, resulting in greater release of GnRH that triggers the pituitary LH surge leading to ovulation – the critical event in female reproduction [6,7,15,29,37].

3. mER signaling

As in neurons, estradiol can influence cell signaling in astrocytes, which express ERα and ERβ both intracellularly and in the plasma membrane [33,40–42]. Therefore, estradiol can activate nuclear-initiated and/or membrane-initiated signaling mechanisms. Classic nuclear-initiated estradiol action is well established and mediated through activation of ERα and ERβ located in the nucleus to behave as ligand-activated transcription factors. Evidence suggests that these same receptors can mediate both nuclear- and membrane-initiated signaling. Although long-studied, it is only more recently that membrane-initiated estradiol action has been widely accepted [33,42,60–76].

Activation of membrane-associated estrogen receptors (mERs) with estradiol or a membrane impermeable construct E-6–BSA (estradiol-coupled to bovine serum albumin) initiates a rapid $[Ca^{2+}]_i$ increase *via* activation of the phospholipase C/inositol trisphosphate (PLC/IP_3) pathway that releases intracellular stores of calcium from the smooth endoplasmic reticulum in neurons and astrocytes [33,46,77]. This rise in $[Ca^{2+}]_i$ stimulates the *de novo* synthesis of progesterone in post-pubertal female hypothalamic astrocytes within 5 min [15,46,78]. Confirmation of this idea was obtained through the use of thapsigargin, a potent Ca^{2+}-ATPase inhibitor that rapidly releases IP_3-sensitive Ca^{2+} stores from the smooth endoplasmic reticulum. This massive release of Ca^{2+}, which was similar in magnitude to estradiol stimulation, resulted in progesterone synthesis by itself [46]. Although these studies were done with nanomolar doses of estradiol, subsequent experiments demonstrated that subnanomolar doses of estradiol were sufficient to induce $[Ca^{2+}]_i$ release in cultured hypothalamic astrocytes [62]. Estradiol induction of progesterone synthesis had a half-maximal effector concentration (EC_{50}) of 0.82 nM, which may be related to the extent of the $[Ca^{2+}]_i$ increase [78]. Thus, the estradiol facilitation of progesterone synthesis appears to be a "step function" responding to physiological levels of estradiol that are reached during the proestrus surge [79–81]. Both estradiol and progesterone stimulation of the hypothalamus are essential for estrogen positive feedback, ultimately leading to the LH surge [7,15,37,59]. The threshold response to estradiol is consistent with the idea that stimulation of neuroprogesterone synthesis is part of an "off-on-off" mechanism regulating the transition from estrogen negative feedback to estrogen positive feedback, and back again [78]. For example, as estradiol rises with developing ovarian follicles, gradually increasing levels of $[Ca^{2+}]_i$ release will be stimulated by the estradiol. However, only with physiologically peak estradiol levels, consistent with mature follicles ready for ovulation, does the $[Ca^{2+}]_i$ release reach a critical threshold allowing for progesterone synthesis. Otherwise, hypothalamic progesterone may rise too early, resulting in a premature LH surge before ovarian follicles are fully mature and ready to ovulate.

Membrane-impermeable E-6–BSA–FITC (estradiol–bovine serum albumin–fluorescein isothiocyanate conjugate) and E-6–biotin (estradiol–biotin conjugate) constructs also bind to and