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Neuroendocrine Consequences of Prenatal Androgen Exposure in the Female Rat: Absence of Luteinizing Hormone Surges, Suppression of Progesterone Receptor Gene Expression, and Acceleration of the Gonadotropin-Releasing Hormone Pulse Generator[1]

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ABSTRACT

Preovulatory GnRH and LH surges depend on activation of estrogen (E_2)-inducible progesterone receptors (PGRs) in the preoptic area (POA). Surges do not occur in males, or in perinatally androgenized females. We sought to determine whether prenatal androgen exposure suppresses basal or E_2-induced *Pgr* mRNA expression or E_2-induced LH surges (or both) in adulthood, and whether any such effects may be mediated by androgen receptor activation. We also assessed whether prenatal androgens alter subsequent GnRH pulsatility. Pregnant rats received testosterone or vehicle daily on Embryonic Days 16–19. POA-hypothalamic tissues were obtained in adulthood for *PgrA* and *PgrB* (*PgrA+B*) mRNA analysis. Females that had prenatal exposure to testosterone (pT) displayed reduced *PgrA+B* mRNA levels ($P < 0.01$) compared with those that had prenatal exposure to vehicle (pV). Additional pregnant animals were treated with vehicle or testosterone, or with 5α-dihydrotestosterone (DHT). In adult ovariectomized offspring, estradiol benzoate produced a 2-fold increase ($P < 0.05$) in *PgrA+B* expression in the POA of pV females, but not in pT females or those that had prenatal exposure to DHT (pDHT). Prenatal testosterone and DHT exposure also prevented estradiol benzoate-induced LH surges observed in pV rats. Blood sampling of ovariectomized rats revealed increased LH pulse frequency in pDHT versus pV females ($P < 0.05$). Our findings support the hypothesis that prenatal androgen receptor activation can contribute to the permanent defeminization of the GnRH neurosecretory system, rendering it incapable of initiating GnRH surges, while accelerating basal GnRH pulse generator activity in adulthood. We propose that the effects of prenatal androgen receptor activation on GnRH neurosecretion are mediated in part via permanent impairment of E_2-induced *PgrA+B* gene expression in the POA.

androgen receptor, gene regulation, gonadotropin-releasing hormone, luteinizing hormone, progesterone receptor

INTRODUCTION

In female rats, ovarian estrogen (E_2) evokes preovulatory gonadotropin surges through its combined actions in the brain and pituitary gland. In preoptic-hypothalamic tissues, E_2 couples a daily neural signal to the neural systems governing GnRH neurosecretion, thereby permitting the timed release of the GnRH surge during the late afternoon hours of proestrus. In gonadotropes, E_2 directs a robust increase in responsiveness to GnRH stimulation. The net result of these parallel actions of E_2 is the secretion of a massive proestrus LH surge, which in turn, triggers ovulations on the following morning of estrus.

Estrogen treatments have long been known to stimulate expression of progesterone receptors (PGRs) in preoptic and mediobasal hypothalamic tissues [1, 2]. In this study, we have proposed that the ability of E_2 to evoke a GnRH surge depends on the capacity of the steroid to induce PGRs, particularly in the anteroventral periventricular (AVPv) nucleus. The AVPv, a sexually dimorphic nucleus [3, 4], contains a dense population of steroid receptor-expressing neurons, and it has been shown to function as a critically important integrative locus controlling ovulatory cyclicity and release of gonadotropin surges [5, 6]. Several of our recent findings have supported the idea that E_2-induced PGRs in the AVPv are obligatory in the GnRH surge-generating process, including observations that PGR knockout (PRKO) mice are incapable of releasing spontaneous or E_2-induced gonadotropin surges [7], and that PGR antagonism and knockdown of *Pgr* gene expression in the AVPv and adjacent preoptic tissues can block E_2-induced GnRH and LH surges in female rats. On the basis of these findings, we have proposed a model for release of the GnRH surge that holds that E_2-induced PGRs in the AVPv can be activated by neural signals in a ligand-independent manner. We hypothesize that through this mechanism, E_2 acts to couple the daily neural signal to the surge-releasing mechanism, and thereby prompts appropriately timed release of the preovulatory GnRH surge.

The same E_2 treatments that induce LH surges in female rats, however, are incapable of evoking appreciable LH surges in male rats [8–11]. This is likely due to the early organizational actions of prenatal and perinatal androgen secretions, which may program male preoptic and hypothalamic tissues to exhibit refractoriness to the surge-inducing actions of E_2 in adulthood. Because the ability of E_2 to induce PGRs in preoptic tissue is also attenuated in males compared to females [12–14], and given the critical dependence of GnRH surges on PGR activation, it is possible that early androgen exposure may render males incapable of releasing GnRH surges in response to E_2 by permanently conferring resistance to PGR induction by E_2 in preoptic and hypothalamic target neurons. Early findings that neonatal androgenization confers resistance to progesterone (P_4) would also be explained on this basis [15, 16]. The present experiments tested this idea by examining the ability of E_2 to induce PGRs and LH surges in adult prenatally

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androgen-exposed female rats. We reasoned that androgen exposure—specifically an exposure that mimics the prenatal androgen surge in the male fetus—would defeminize both the LH surge mechanism and responsiveness to PGR induction by E_2. We also assessed whether prenatal androgen exposure leads to any permanent changes in the basal activity of the GnRH pulse generator, as deduced from pulsatile LH patterns. To assess the specific importance of androgen receptor (AR) activation in prenatal programming, we compared the effects of prenatal testosterone (T) and the nonaromatizable androgen, dihidrotestosterone (DHT), on both the surge and pulse generating mechanisms in the adult female rat. Our hormonal treatments consisted of free T and free DHT injections on Gestational Days 16–19, so as to limit the duration and magnitude of the fetal exposure to the steroid; the free steroids were used because they have short half-lives and are metabolized more rapidly than their esters, particularly propionate [17]. Therefore, the effects induced by these hormones are specific to the days of administration to the pregnant animal [18]. Gestational Days 16–19 were chosen because the endogenous prenatal T surge in male rats starts on Day 16 and remains elevated throughout Day 19 [19, 20].

Our results suggest that there exists a prenatal period during which androgens, at least in part via AR activation, can permanently render females resistant to the PGR-inducing actions of estrogen, and thereby defeminize the LH surge mechanism. Androgen actions during this same period also appears to direct development of a hyperactive GnRH pulse generator.

MATERIALS AND METHODS

Animals and Hormone Treatments

Virgin female rats (experiment 1), and pregnant rats and their female offspring (experiments 2–5) were used in these studies (Sprague-Dawley, Charles River Laboratories, Portage, WI). Animals in all experiments were provided rat chow and water ad libitum, and housed in a temperature-controlled (23–25°C) and light-controlled (14L:10D) environment. All animal procedures were completed in accordance with protocols specifically approved by the Animal Care and Use Committee at Northwestern University (Evanston, IL).

Prenatal hormone exposures were accomplished by treatment of pregnant dams from Embryonic Day 16 to Embryonic Day 19 with daily injections (i.p., s.c.) of either free T or DHT, or sesame oil/benzyl benzoate vehicle (V) solution. This hormonal paradigm mimics the endogenous T surge that is observed in male rats. Plasma T levels are elevated in male rats between Gestational Days 16 and 19 compared to females rats [19, 20]. Free T and DHT were used because they have relatively short half-lives compared to T propionate and DHT propionate [17], and therefore, the effects induced by these androgens can be assumed to be specific to these gestational days. Injections were composed of 5 mg of either T or DHT dissolved in a 1:4 solution of benzyl benzoate:sesame oil. This dose of T was selected on the basis of previous experiments demonstrating that the administration of the free steroid at this dose is capable of partially masculinizing the anal-genital distance and sex behavior [18]. Our own experiments revealed that a similar dose of free DHT produced the same degree of masculinization of the anal-genital distance as did the free T treatments. The anal-genital distance was significantly larger in both the female rats exposed prenatally to testosterone (pT; 1.96 ± 0.02 cm) and in those exposed prenatally to DHT (pDHT; 1.98 ± 0.01 cm) compared with female rats exposed prenatally to vehicle (pV; 1.51 ± 0.01 cm; $P < 0.001$; $n = 7$ for all groups). The duration of pregnancy was similar among animals of all three treatment groups (21–22 days). Postweaning body weights were not significantly different among animals in each treatment group, and maternal care was not observed to be grossly disrupted in any of the treated mothers. All experiments were conducted on prenatal-exposed female rats between Postnatal Days 60–70, an age that is after the onset of puberty in pV rats. Estrous cyclicity was determined by daily inspection of vaginal cytology. Because pT and pDHT females exhibited partial masculinization of the external genitalia, vaginal smears could not be obtained from these animals; however, they were subject to similar handling procedures for experimental consistency.

Semiquantitative Reverse Transcription-Polymerase Chain Reaction Analysis of Progesterone Receptor mRNA

In all experiments, tissues were obtained between 1130 and 1200 h for subsequent reverse transcription-polymerase chain reaction (RT-PCR) analysis of *Pgr* mRNA as previously described [21]. Animals were first anesthetized via halothane inhalation, decapitated, and tissues were rapidly dissected and processed. In initial experiments, blocks of tissue that isolate the mediobasal hypothalamus and preoptic area (MBH/POA) were obtained as previously described [22]. In other experiments, the POA was isolated and analyzed alone, as indicated. Tissues were homogenized in Trizol reagent (Invitrogen, Carlsbad, CA) using a Polytron homogenizer (Brinkmann Kinematica, Westbury, NY). Subsequently, all RNA samples were treated with RQ1 RNase-free DNase (Promega, Madison, WI) and purified with a phenol/chloroform extraction. Approximately 4–5 mg of total RNA was reverse transcribed into cDNA using 10× buffer (Mg-free; Perkin-Elmer), 10 mM dNTPs, 25 mM $MgCl_2$, random hexamer primers (450 μg/ml), RNasin (33 U/μl; Promega), and Maloney-murine leukemia virus reverse transcriptase (10 U/μl; Promega). The reaction mixture was incubated at 21°C for 10 min, held at 42°C for 75 min, and followed by 5 min at 95°C. Two separate PCR protocols were employed to detect the combination of both isoforms of PGR mRNA, *PgrA+B*, or the *PgrB* isoform alone. Primers for the first PCR, 5′-CCCACAGGAGTTTGTCAAGCTC-3′ (sense) and 5′-TAACTTCAGACATCATTTCCGG-3′ (antisense), were initially designed by Park and Mayo [23] to amplify the ligand binding domain, which is common to both A and B isoforms. The second PCR amplified a fragment with the primers 5′-GTGTGAGGATTCTGCCTTTC-3′ (sense) and 5′-CGCTCTCAGGACTTCTTACG-3′ (antisense), binding to the 5′ untranslated region of rat *Pgr* cDNA that is unique to the B isoform whose sequence was originally published by Park-Sarge and Mayo [24] and retrieved from GenBank (accession numbers L16921 and L16922). One of us had previously validated this semiquantitative PCR method [21] with samples normalized to the amplified product of the housekeeping gene RPL19 as an internal control. Signal intensities were quantified by PhosphorImaging with the Image Quant program (Molecular Dynamics, Sunnyvale, CA).

Experiment 1: Analysis of EB Induction of PgrA+B *and* PgrB *mRNA in the MBH/POA*

At random stages of the estrous cycle, rats were bilaterally ovariectomized under metophane anesthesia at 0900–1100 h. The day of surgery was designated as Day 0. At 1100 h on Days 6 and 7, animals were injected (s.c.) with either vehicle (sesame oil) or estradiol benzoate (EB; 10μg/rat; n = 6/treatment). Rats were killed by decapitation at 1100 h on Day 9, and MBH/POA tissues were collected for RNA extraction. All tissues were analyzed by semiquantitative RT-PCR for the combined *PgrA+B* mRNA expression, and for *PgrB* mRNA expression alone.

Experiment 2: Expression of PgrA+B *in the MBH/POA of Females Prenatally Exposed to V or T*

MBH/POA tissues were dissected from cycling pV females at 1130–1200 h on metestrus or proestrus. Because vaginal smears were unattainable in the rats exposed prenatally to testosterone (pT), they were age-matched to the pV animals, and their tissues were gathered randomly on days coinciding with dissections of the pV female tissues. *PgrA+B* mRNA expression was detected in all tissues via semiquantitative RT-PCR.

Experiment 3: Effects of EB on LH Secretion and PgrA+B *mRNA Expression in Females Prenatally Exposed to V, T, or DHT*

A group that had been exposed prenatally to DHT was included to ascertain the role of AR versus estrogen receptor (ER) activation in utero on EB-induced *PgrA+B* mRNA expression levels in the adult animal. Females exposed prenatally to vehicle were ovariectomized at 0900 h on diestrus, while the age-matched pT and pDHT rats were ovariectomized at the same time. All treatment groups were subsequently injected with 30 μg EB or with oil vehicle. At 1200 h on the following day, the POA, defined by a block extending 2 mm rostrally from the caudal border of the optic chiasm, 2 mm dorsal to the ventral surface of the forebrain, and 1 mm to each side of the sagittal midline, was dissected and *PgrA+B*

expression was determined by semiquantitative RT-PCR. The POA section dissected included the AVPv, which is known to be a center for the GnRH/LH surge mechanism, and as such it was analyzed apart from MBH/POA tissue as described in experiment 1. A second cohort of females was similarly ovariectomized and treated with EB, and catheters were surgically implanted into the left jugular vein of these females to permit blood sampling the following day. A twice-hourly blood sampling paradigm was used to assess the effects of prenatal androgen treatments on the ability of EB to induce LH surges. Blood samples were obtained from 1200 h to 2100 h, 24 h after EB injection, to assess the potential release of LH surges that are normally induced using this treatment regimen [7]. Serum was collected and subsequently analyzed by LH radioimmunoassay.

Experiment 4: LH Pulsatility in Females Prenatally Exposed to V, T, or DHT

Animals that had been prenatally exposed to vehicle were ovariectomized at diestrus, and the pT and pDHT females were ovariectomized at similar days of age. On the seventh day after ovariectomy, rats were fitted with jugular catheters to facilitate blood sample collection on the following day. Blood samples were collected every 5 min for 3 h from 1200 h to 1500 h, and serum LH levels were determined via LH radioimmunoassay.

Experiment 5: Pituitary Responsiveness to GnRH in Females Prenatally Exposed to V, T, or DHT

To assess the locus of prenatal androgen effects on LH pulsatility, LH responses to GnRH challenges were determined in pV, pT, and pDHT rats. Metestrus pV rats and age-matched pT and pDHT females were fitted with jugular catheters. On the following day, animals were bled every 5 min for 3 h from 1200 h to 1500 h. Samples were collected for 1 h before GnRH treatment to provide baseline measurements for each animal. At 60 and 120 min, rats were injected with 25 ng and 100 ng GnRH in 0.9% saline, respectively. All serum samples were subsequently analyzed by LH radioimmunoassay.

Radioimmunoassays

LH and FSH standards, RP-3 and RP-2, respectively, were generously provided by the National Institute on Diabetes and Digestive and Kidney Diseases (Bethesda, MD). The sensitivity of the LH and FSH radioimmunoassays (RIAs) were 0.01 and 0.05 ng/tube, respectively. The intraassay coefficients of variance (CV) for the LH RIA was 14.8% and the FSH RIA was 4.4%. P_4 and T serum levels were determined using reagents provided by RIA kits from ICN Pharmaceuticals (Costa Mesa, CA). Sensitivities for these kits are 0.15 ng/nl for P_4 and 20 pg/ml for T. The intraassay CV for P_4 was 3.1%, and it was 7.5% for T. Serum E_2 concentrations were determined by RIA kits from Diagnostic Products Corp. (Los Angeles, CA). The sensitivity and intraassay CV were 2 pg/ml and 1.00%, respectively. All data are presented as means ± SEM.

Statistical Analysis

To compare *Pgr* mRNA expression and hormone levels among treatment groups in experiments 1–4, separate one-way analyses of variance (ANOVAs) and Newman-Keuls multiple comparison posthoc tests were used. In experiment 4, LH pulses were identified and pulse characteristics were determined by the ULTRA software program originated by Dr. Van Cauter, University of Chicago, Chicago, IL [25]. A threshold of 2 times the CV in the corresponding ranges of values in the LH radioimmunoassay was used in the algorithm program to identify significant LH pulses. LH pulse amplitude (difference in value from peak to trough), LH pulse frequency, and mean LH concentration were determined for 6 pV and pT rats, and for 3 pDHT females. The average for each treatment group was calculated and significant differences were assessed using separate one-way ANOVAs with the Newman-Keuls multiple comparison posthoc test. LH hormone levels in the GnRH challenge experiment were statistically analyzed using NCSS 97 (Number Cruncher Statistical Systems, Kaysville, UT). Two-way ANOVA with repeated measures was used for comparisons of hormone levels between the groups. Posthoc comparisons were made with the Duncan multiple-comparisons test. For all posthoc statistical analyses, $P < 0.05$ was used to judge significance between treatment groups.

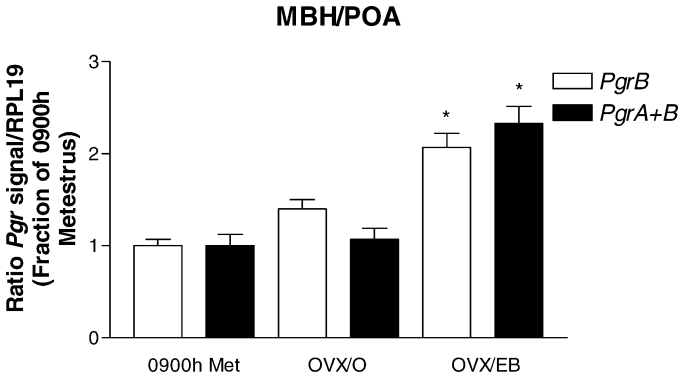

FIG. 1. Effects of ovariectomy and EB replacement (10 μg EB s.c.) on *PgrB* and *PgrA+B* mRNA levels in the MBH/POA. Semiquantitative RT-PCR results show a 1.5-fold and 2.2-fold induction (*$P < 0.05$) of *PgrB* and *PgrA+B,* respectively, in ovariectomized females treated with EB compared with those females with ovariectomy and vehicle treatment. No induction was seen in the ovariectomized animals treated with vehicle. These data are expressed as a fraction of the 0900 h metestrus control to easily display the difference in fold induction and are represented as mean ± SEM.

RESULTS

Experiment 1: Effects of EB on PgrA+B *and* PgrB *mRNA in MBH/POA*

An initial experiment explored the ability of EB to induce *PgrA+B* and *PgrB* mRNA expression in the MBH/POA tissue. Hypothalamic tissues of ovariectomized females treated with oil vehicle were compared with those that had been ovariectomized and injected with EB. Ovariectomy for 8 days did not significantly alter mRNA levels for either *PgrA+B* or *PgrB* in the MBH/POA compared with intact metestrus females (Fig. 1). Treatment with EB on Days 6 and 7 after ovariectomy caused a significant 1.5-fold and 2.2-fold induction of *PgrA+B* and *PgrB* mRNA levels in the MBH/POA (*$P < 0.05$ compared with metestrus females and those that had been ovariectomized and treated with vehicle). Because the induction of *PgrA+B* and *PgrB* mRNA by EB were not significantly different from each other, we assessed only alterations in *PgrA+B* mRNA as a representative total PGR change by EB in all subsequent experiments. In contrast to the MBH/POA, the mRNA levels for both *Pgr* isoforms were not significantly increased in the hippocampus or the amygdala of animals that were treated with EB (data not shown).

Experiment 2: PgrA+B *mRNA Expression and Basal Hormone Levels in Androgenized Rats*

To test the hypothesis that prenatal androgen exposure influences *PgrA+B* expression in adulthood, the MBH/POA tissues of adult pV animals in metestrus (pV-Met) and proestrus (pV-Pro) and age-matched pT females were dissected at 1200 h. Semiquantitative RT-PCR revealed, as depicted in Figure 2, a significant ($P < 0.01$; $F_{2,11} = 8.231$) decrease in *PgrA+B* mRNA expression in the MBH/POA of the pT rats ($n = 6$) compared with the pV-Pro females ($n = 5$). Statistical analysis of *Pgr* mRNA expression levels in pV-Met animals ($n = 4$) approached but did not reach a statistically significant difference from levels in pT females.

Hormone levels were measured from serum obtained from prenatally vehicle-exposed females at 1200 h on metestrus and proestrus. Serum was similarly collected from age-matched pT and pDHT females. Table 1 represents a

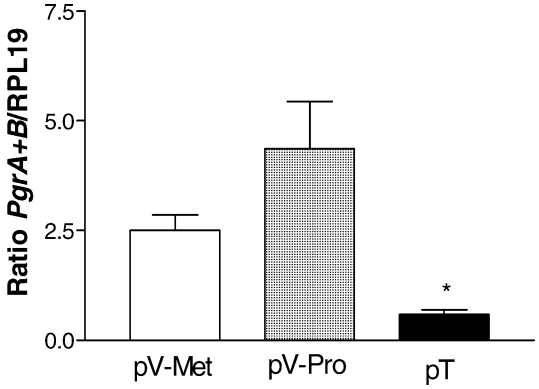

FIG. 2. Effects of prenatal testosterone-exposure on adult female *Pgr* expression in the MBH/POA. Semiquantitative RT-PCR demonstrates a significant (*$P < 0.01$) reduction in *Pgr* expression in the pT (n = 6) females compared with prenatal vehicle-exposed rats whose tissues were collected at proestrus (pV-Pro; n = 5). The data are normalized for total RNA to the housekeeping gene RPL19, and are represented as mean ± SEM.

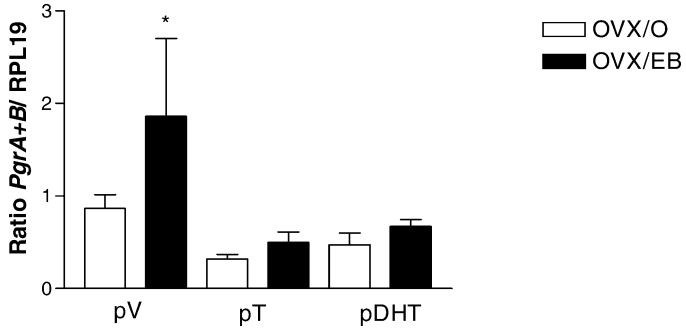

FIG. 3. Effects of EB on *Pgr* mRNA in prenatally androgenized female rats. EB (30 μg s.c.) can significantly induce *Pgr* mRNA expression in the POA of ovariectomized pV rats compared with those pV rats that were ovariectomized and given a oil vehicle (*$P < 0.05$; n = 6 for both ovariectomy/oil and ovariectomy/EB). No induction of *Pgr* mRNA expression was seen by EB over the ovariectomy and oil-treated group in either pT (n = 7 for both ovariectomy/oil and ovariectomy/EB) or pDHT (ovariectomy/oil; n = 7, ovariectomy/EB; n = 9) rats. The data are normalized for total RNA to the housekeeping gene RPL19, and are represented as mean ± SEM.

combination of basal hormone profile of animals from multiple experiments; therefore, sample size varied between hormones with a range from n = 6 to n = 15. The hormone levels dropped in the normal range for the pV females, and within the range of constant estrus for the pT and pDHT rats, as would be predicted by previous studies [26]. No significant differences were observed among treatment groups for serum progesterone, estradiol, LH, FSH, and testosterone levels.

Experiment 3: Prenatal Androgen Effects on E_2-Induced PgrA+B *mRNA Expression in POA and E_2-Induced LH Release*

The effects of prenatal androgen on *PgrA+B* mRNA expression in the POA were explored to more specifically assess alterations in *Pgr* expression that may be relevant to GnRH surge production [27, 28]. By 24 h, EB treatment in ovariectomized rats produced a significant, 2-fold ($P < 0.05$; n =6 for each group; $F_{5,36} = 2.960$) increase in *PgrA+B* expression in the POA of the pV rats compared with those treated with oil (Fig. 3). By contrast, EB was without effect on *PgrA+B* expression in the POA of pT (n = 7 for both groups) and pDHT (ovariectomized/oil, n = 7; ovariectomized/EB, n = 9) rats.

As depicted in Figure 4, prenatal androgen exposure resulted in a complete refractoriness to LH surge induction by EB. Robust LH surges were observed in all pV females (n = 4), and were absent in the pT (n = 3) and pDHT (n = 3) rats. The area under the LH curves (data not shown) confirms the significant increase in LH secretion in the pV animals ($F_{2,7} = 4.511$; $P < 0.05$).

Experiment 4: Effects of Prenatal Androgens on GnRH Pulsatility

To further investigate the effects of prenatal androgen exposure on GnRH secretion, GnRH pulse generator activity as characterized by LH pulse frequency and amplitude in ovariectomized females were examined. Examples of the LH pulse profiles for pV, pT, and pDHT females are shown in Figure 5, a, b, and c, respectively. A summary of the LH pulse characteristics from these animals is presented in Figure 6. Pulse frequency ($F_{2,14} = 5.445$), pulse amplitude ($F_{2,17} = 4.054$), and mean LH ($F_{2,14} = 4.575$) secretion are increased in the pDHT rats ($P < 0.05$, n = 3) compared with the pV females (n = 6). Pulse frequency was significantly increased in pT animals ($P < 0.05$; n = 6) compared with the pV females; however, pulse amplitude and mean LH values from the pT animals were not significantly different from corresponding values in either pV or pDHT females.

Experiment 5: Pituitary Responsiveness to a GnRH Challenge

Figure 7 presents the mean baseline LH, and the LH responses after injection with 25 ng or 100 ng GnRH. Analysis of serum LH levels revealed a modest increase after treatment with 25 ng GnRH and a significant effect of 100 ng GnRH on LH levels in all 3 treatment groups ($P < 0.05$ for pV and pT; $P < 0.01$ for pDHT; $F_{2,9} = 10.29$). There were, however, no differences in the magnitudes of the responses observed among the treatment groups. These results indicate that the effect of prenatal androgen on the GnRH pulse generator is at the level of the hypothalamic

TABLE 1. Serum hormone profiles from pV, pT, and pDHT rats.[a]

Hormone	pV-Metestrus	pV-Proestrus	pT	pDHT
E_2 (pg/ml)	16.3 ± 4.4	38.0 ± 10.3	22.4 ± 0.94	23.9 ± 6.8
P_4 (ng/ml)	32.1 ± 2.8*	14.6 ± 1.8	24.7 ± 5.0	25.6 ± 4.8
LH (ng/ml)	1.23 ± 0.18	0.86 ± 0.10	0.77 ± 0.14	0.73 ± 0.28
FSH (ng/ml)	6.4 ± 1.3	5.1 ± 0.6	5.9 ± 0.8	7.1 ± 1.2
T (ng/ml)	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01

[a] Data are represented as mean ± SEM (*$P<0.05$ as compared to pV-Pro rats).

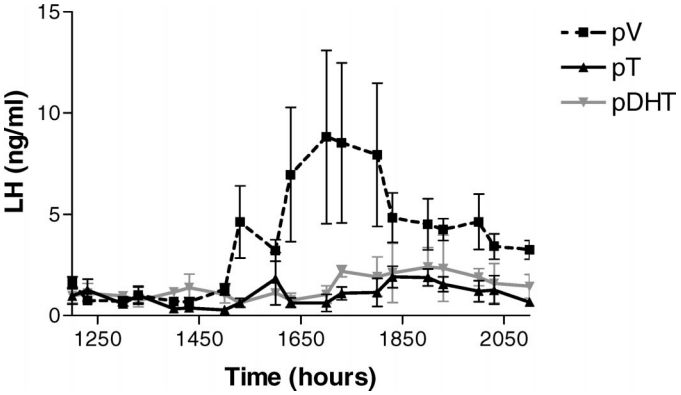

FIG. 4. Effects of prenatal exposure androgen exposure on EB-induced preovulatory LH surges. Prenatal exposure to both T and DHT blocks the preovulatory LH surge in adulthood. Statistically significant LH surges were released in prenatally vehicle-exposed rats (n = 4) the day following ovariectomy at 0900 h and EB treatment (30 μg s.c.), but no LH surges were seen in pT (n = 3) or pDHT (n = 3) females under the same conditions. The data are represented as mean ± SEM.

secretion of GnRH, and is not due to an alteration in sensitivity of the pituitary to GnRH.

DISCUSSION

Sex differences in gonadotropin secretory patterns are established during fetal and perinatal development, when testicular androgen secretions appear to render the developing male brain incapable of producing preovulatory gonadotropin surges in adulthood [8–11]. In the absence of this androgen exposure, the female POA and hypothalamus acquire the ability to sustain normal, female-typical cyclic gonadotropin secretions as adults [5, 6]. It is thus generally held that the default developmental pathway for neurons controlling the GnRH surge is female, and that early androgen action in the brain represents a defeminization of this pathway. This idea is strongly supported by the observation that female rodents exposed to exogenous androgens in fetal or perinatal development exhibit male-typical, acyclic gonadotropin secretion patterns [18, 29]. The mechanisms by which early androgen exposure can suppress gonadotropin secretion in adulthood have remained unclear, and were a major focus of the present studies. We provide new evidence that androgens can exert these organizational effects by 1) prenatally activating the AR, and thereby 2) programming permanent refractoriness of POA neurons to the PGR-inducing actions of E_2.

In female rodents, preovulatory GnRH surges are dependent on the ability of ovarian E_2 to couple a daily neural signal [28, 30, 31] to the neuronal circuitry that governs GnRH release. This coupling process appears to be mediated by the ability of the steroid to induce *Pgr* expression in the AVPv, a key preoptic tissue locus [27, 28]. We have proposed that these E_2-induced PGRs are activated in a ligand-independent manner by the daily neural signal, thereby initiating timely release of a GnRH surge into the hypophysial portal vessels. Supporting this hypothesis are studies showing that the absence of *Pgr* expression [7], diminishment of *Pgr* expression in the AVPv [27, 28], or pharmacological antagonism of PGRs [32–36] block E_2-induced GnRH and LH surges. The present studies demonstrate that prenatal androgen exposure blocks E_2-induced *Pgr* expression in the POA, while at the same time, these same treatments abolish spontaneous and E_2-induced LH

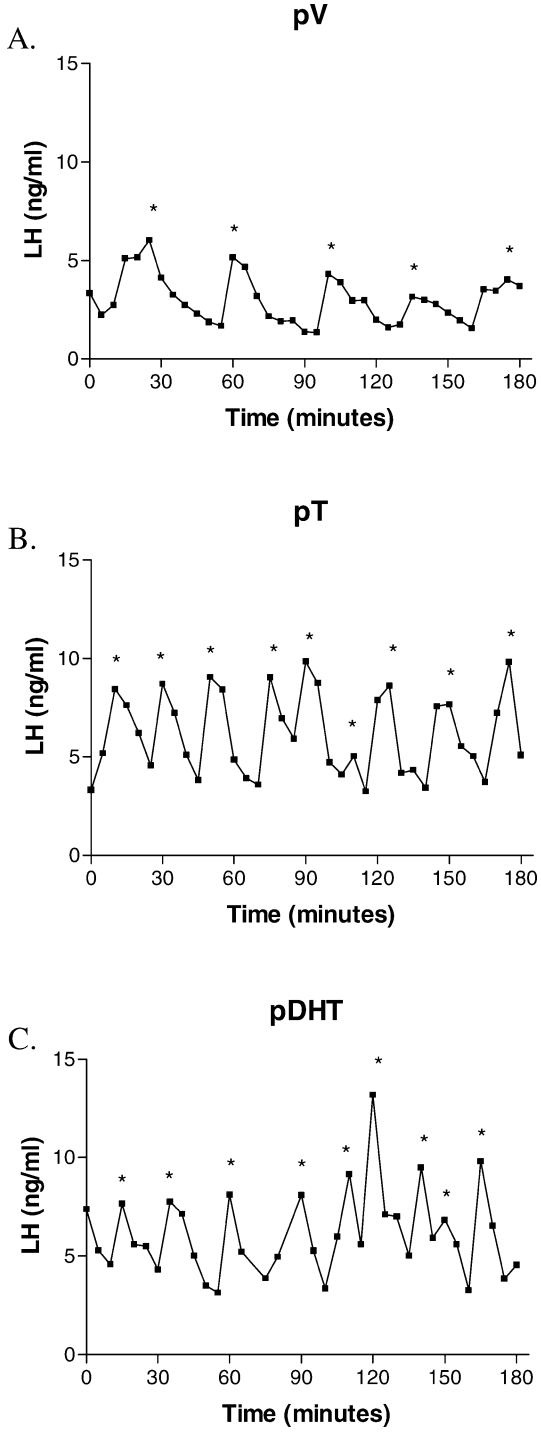

FIG. 5. Effects of prenatal androgen exposure on GnRH pulse generator activity. Representative LH pulsatility profiles demonstrating an acceleration in pulse generator activity in pT (**B**) and pDHT (**C**) rats compared with pV females (**A**). *Significant pulse by ULTRA pulse analysis program.

surges. Taken together, the present and previous findings argue that E_2-induced *Pgr* expression in the POA is obligatory for release of GnRH and LH surges, and that the ability of prenatal androgen treatments to permanently render this molecular process inoperative mediates their capacity to prevent LH surges in adulthood.

The defeminizing organizational effects of androgens have been believed to be mediated in large part by aromatization of T to E_2, and subsequent activation of POA and hypothalamic ERs by E_2. A large body of evidence,

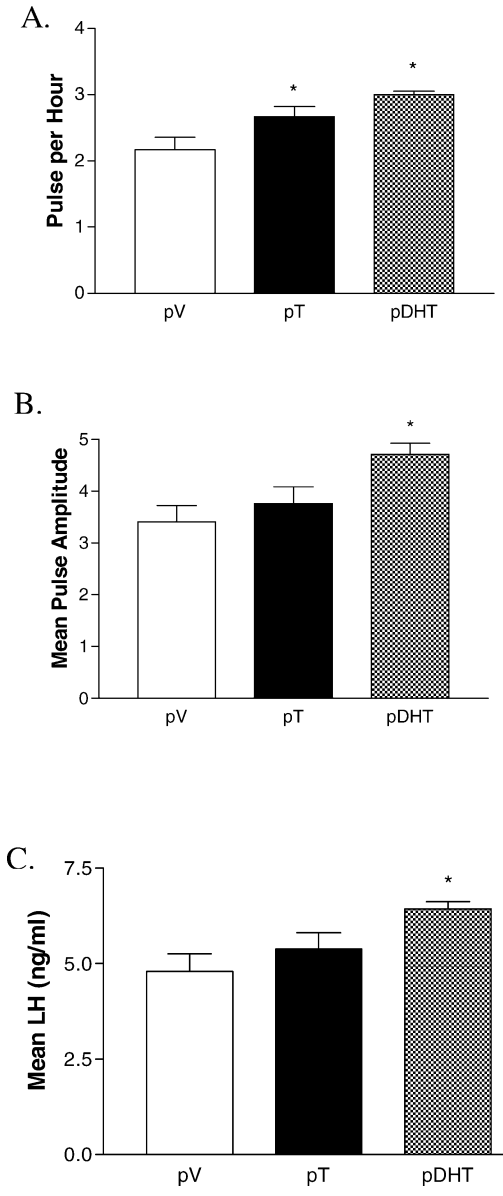

FIG. 6. LH pulse characteristics in prenatally androgenized females. Prenatal exposure to DHT (n = 3) and T (n = 6) results in a significant increase (*$P < 0.05$) in LH pulse frequency (**A**) compared with pV (n = 6) animals. Mean pulse amplitude (**B**) and mean LH levels (**C**) were significantly increased in the pDHT rats compared with pV animals. The data are represented as mean ± SEM.

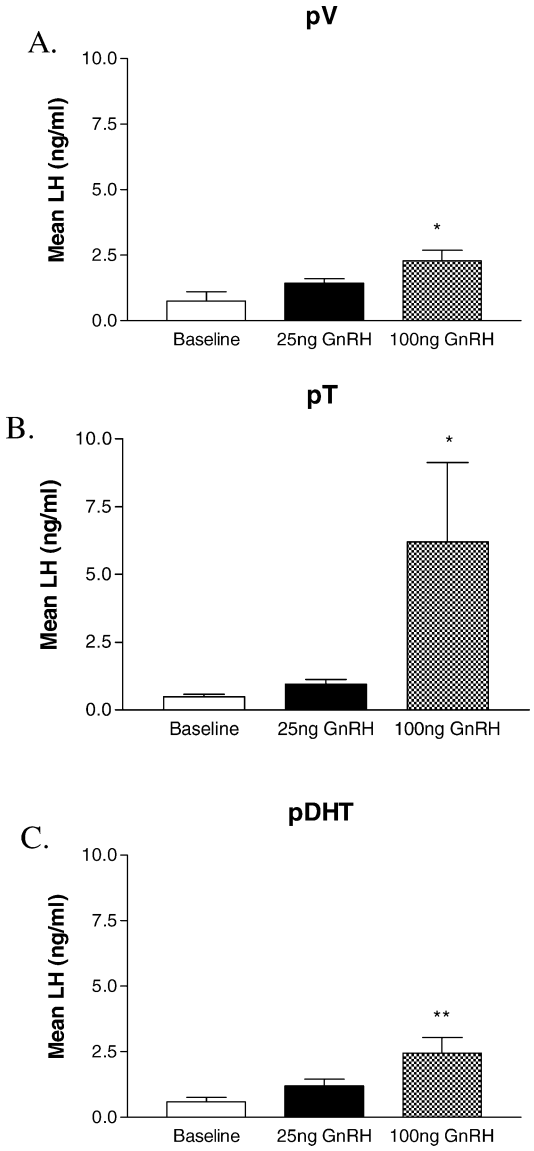

FIG. 7. Effects of prenatal androgen exposure on pituitary responsiveness. Pituitary responsiveness to GnRH is not altered in the pV rats (**A**; n = 3) compared with pT (**B**; n = 5) or pDHT females (**C**; n = 3). A significant increase in LH secretion was seen in response to a bolus of 100 ng of GnRH in the pV, pT, and pDHT animals (*$P < 0.05$, **$P < 0.01$) compared to their baselines. The data represent the response within 30 min of the GnRH bolus and are represented as mean ± SEM.

principally derived from studies employing postnatal steroid injections, supports this idea [1, 37, 38]. The absence of male sexual behaviors in ERα and ERαβ knockout mice is also consistent with this hypothesis [39–41], at least as it may pertain to the organizational actions of T on circuitries controlling sexual behavior. Recent studies in AR gene knockout mice, however, have prompted a reconsideration of the importance of the AR in mediating both organizational and activational effects of T on behavior [42].

Our findings, together with observations in rats [43], hamsters [44], and mice [29] also implicate ARs in the organizational effects of T on gonadotropin control systems in rodents. In the present studies, prenatal injections of either T or the nonaromatizable T metabolite, DHT, rendered female offspring incapable of mounting LH surges in response to E_2 treatments. These results indicate that prenatal androgen actions, at least in part via AR activation, can contribute to the defeminization of the neuroendocrine mechanisms governing release of gonadotropin surges. We cannot exclude the possibility that some of the effects of prenatal DHT on subsequent neuroendocrine function were due to the metabolism of this androgen to 3βAdiol, and subsequent activation of ER by this metabolite [45]. However, previous studies have employed even more sustained and robust DHT treatment paradigms than those used in these studies, and have unequivocally revealed no apparent ER-mediated alterations in GnRH content or gonadotropin secretion even as these variables were concurrently altered via unambiguous AR activation [46]. At present, the most straightforward interpretation of these data holds that the activation of AR mediates at least some of the prenatal actions of DHT on *Pgr* expression and the release of gonadotropin surges in response to E_2.

These observations also suggest that the critical period

of sensitivity to AR activation in this regard includes Embryonic Days 16–19. Notably, a previous study found no effect of brief prenatal androgen exposure (1 day) late in gestation on subsequent gonadotropin surge release in female rats [18]. Thus, the ability of prenatal androgens to disrupt adult gonadotropin secretory capacity in the female may depend on exposures that more closely mimic the 3-day duration of endogenous androgen production in the male fetus. In the male rat fetus, androgens are produced in a biphasic manner. Prenatal endogenous androgen exposure from the fetal testes begins on Embryonic Day 16 and remains elevated throughout Embryonic Day 19 [20]. A secondary bout of secretion peaks 2 h after birth and extends to Postnatal Day 5 [47]. Our androgen exposure paradigm was designed to extend the duration of androgen exposure through all 4 days of the initial bout of androgen secretions. We speculate that the efficacy of our steroid exposure regimen—specifically by blocking gonadotropin surge induction in adulthood—is a consequence of a close physiological mimicry of the duration of normal male prenatal T secretion.

The present results suggest that prenatal AR activation in the female rat can prevent release of gonadotropin surges in adulthood, as has previously been shown in female sheep [48–51] and female monkeys [52]. These findings do not exclude the possibility that ER activation, consequent to the aromatization of T, may also normally play a role in the masculinzation of the gonadotropin surge induction mechanism. We could not test the possibility that prenatal ER activation may likewise mediate masculinizing effects on this system, because prenatal estrogen administration before Embryonic Day 19 produces abortions in pregnant rats [38]. Previous studies, however, have demonstrated that perinatal E_2 treatments can block LH surge induction in adulthood, and it remains unclear why this additional mechanism may operate [15, 53, 54]. We speculate that both AR and ER stimulation during prenatal and perinatal critical periods can activate separate sets of signaling pathways, any of which may interfere with the acquisition of competency to release gonadotropin surges. Accordingly, it is possible that endogenous T in male fetuses may normally activate both mechanisms in an additive, or redundant manner to induce permanent hormone acyclicity. It may also be the case that AR-mediated signals predominate during the prenatal period, while T effects are disproportionately mediated by ER activation during the perinatal period. That both prenatal and postnatal mechanisms are required for full defeminization is supported by previous findings that castration of newborn male pups, viz., elimination of the secondary T surge, results in only partial feminization of the responsiveness to E_2-positive feedback in adulthood [55, 56]. Indeed, initial AR activation may be required to induce aromatase activity [37, 57], which would subsequently confer greater signaling via T-derived E_2 at the ER.

These experiments also reveal that prenatal AR activation results in permanent refractoriness of the POA to PGR-inducing actions of E_2. We hypothesize that this effect mediates the ability of prenatal androgens to permanently incapacitate the gonadotropin surge induction mechanism. Where and how may androgens produce refractoriness to E_2? Several studies point to the importance of the AVPv as a likely locus of androgen action, because it is a preoptic nucleus that is 1) sexually dimorphic [4, 58]; 2) larger in females than in males [58]; 3) rich in AR [59], ER [3, 59], and *Pgr* [3] expression throughout development and adulthood; 4) critically important in the integration of signals leading to release of gonadotropin surges [5, 60]; and 5) expresses PGRs whose activation appears to be obligatory for release of gonadotropin surges [61, 62]. Collectively, this evidence suggests one possible scenario for androgen actions, in which AR activation in the AVPv leads to a reduction in the number of steroid-responsive neurons that remain viable into adulthood, which, ultimately, incapacitates both the E_2-induced PGR and E_2-induced surge mechanisms that would otherwise be operative in that structure.

Our findings also reveal that prenatal AR activation produces an acceleration of pulsatile LH secretion. Prenatal androgen treatments have been found to produce accelerations of LH pulsatility in sheep [63], and elevations in LH secretion in female mice [29] and monkeys [64]. Our findings reveal a similar response in rats, as prenatal DHT exposure was found to increase LH pulse frequency in the adult ovariectomized animal; prenatal exposure to T produced a trend toward enhanced pulse frequency that did not reach statistical significance. Because prenatal DHT exposure produced an enhancement of LH pulse frequency, yet did not alter pituitary responsiveness to GnRH stimulation, we conclude that these effects are mediated via acceleration of the GnRH pulse generator. Our measurements were made in animals devoid of gonadal hormone feedback. This suggests that the acceleration of GnRH pulsatility would appear to be a consequence of programming of the basal pulse-generating mechanism to operate at a higher frequency in adulthood, rather than an alteration in the responsiveness of the pulse generator to negative feedback regulation; while the latter may also be true, the present experiments provide no information toward this end. We hypothesize that an accelerated pulse generator may represent a more masculinized functional phenotype, as the frequency of GnRH/LH pulsatility has often been found to be greater in males than in females in the gonadectomized state. The maximum frequencies of LH pulsatility in gonadectomized male and female rats have been reported to be a 3.3 pulses/hour [65] and 1.9 [66] to 2.2 pulses/hour (as seen in our study), respectively, at 8 days following removal of the gonads. The functional significance of relatively faster GnRH pulsatility in males or androgenized females remains unknown. Hyperandrogenic disorders in women, such as polycystic ovarian syndrome (PCOS), are often characterized by LH excess, which in turn, appears to be driven by accelerated GnRH pulse generator activity. Findings of accelerated pulsatility in prenatally androgenized animals [63] are clearly consistent with the hypothesis that this symptom arises in women with PCOS as a consequence of prenatal androgen excess and hence, defeminizing the GnRH pulse generator mechanism.

It has remained unclear how prenatal AR activation may program hyperactivity of the GnRH pulse generator in adulthood. Prenatal androgenization of female sheep was found to reduce synaptic contacts to GnRH neurons to lower levels observed in males [67], suggesting that effects of androgens on GnRH pulsatility may be mediated by alterations in synaptic connectivity. A recent study by Sullivan and Moenter [29], however, has provided compelling evidence suggesting that AR activation may produce specific alterations in the drive from gamma-aminobutyric acid (GABA)-releasing neurons to GnRH neurons, perhaps by altering synaptic connectivity between the two neuronal phenotypes. The cellular locus of AR action could be the GnRH neuron itself, as ARs have been detected in immortalized GnRH cells, GT1-7 [68], but not in GnRH neurons [69]. More likely, AR activation in GABAergic or other

afferent neuronal groups may direct alterations in synaptic inputs to GnRH neurons, and thereby influence the basal state of activity of the GnRH pulse generator. The molecular and cellular consequences of early AR activation in GABA neurons remain to be fully elucidated.

Our studies provide evidence that prenatal androgens can defeminize gonadotropin secretions in the female fetus by rendering POA tissues insensitive to PGR-inducing actions of E_2. On the basis of these findings and previous observations, we hypothesize that refractoriness of *Pgr* gene expression to E_2 stimulation is the cause of the gonadotropin acyclicity that is characteristic of these prenatally androgenized females. These findings, along with the observation that prenatal androgen exposure produces accelerated GnRH pulse generator activity in adulthood, may have important implications for the understanding of accelerated GnRH pulse generator activity and oligomenorrhea or amenorrhea commonly observed in PCOS and other hyperandrogenic syndromes in women.

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REFERENCES

1. Parsons B, Rainbow TC, McEwen BS. Organizational effects of testosterone via aromatization on feminine reproductive behavior and neural progestin receptors in rat brain. Endocrinology 1984; 115: 1412–1417.
2. Romano GJ, Krust A, Pfaff DW. Expression and estrogen regulation of progesterone receptor mRNA in neurons of the mediobasal hypothalamus: an in situ hybridization study. Mol Endocrinol 1989; 3: 1295–1300.
3. Simerly RB, Carr AM, Zee MC, Lorang D. Ovarian steroid regulation of estrogen and progesterone receptor messenger ribonucleic acid in the anteroventral periventricular nucleus of the rat. J Neuroendocrinol 1996; 8:45–56.
4. Orikasa C, Kondo Y, Hayashi S, McEwen BS, Sakuma Y. Sexually dimorphic expression of estrogen receptor beta in the anteroventral periventricular nucleus of the rat preoptic area: implication in luteinizing hormone surge. Proc Natl Acad Sci U S A 2002; 99:3306–3311.
5. Wiegand SJ, Terasawa E. Discrete lesions reveal functional heterogeneity of suprachiasmatic structures in regulation of gonadotropin secretion in the female rat. Neuroendocrinology 1982; 34:395–404.
6. Petersen SL, Barraclough CA. Suppression of spontaneous LH surges in estrogen-treated ovariectomized rats by microimplants of antiestrogens into the preoptic brain. Brain Res 1989; 484:279–289.
7. Chappell PE, Schneider JS, Kim P, Xu M, Lydon JP, O'Malley BW, Levine JE. Absence of gonadotropin surges and gonadotropin-releasing hormone self-priming in ovariectomized (OVX), estrogen (E2)-treated, progesterone receptor knockout (PRKO) mice. Endocrinology 1999; 140:3653–3658.
8. MacLusky NJ, McEwen BS. Oestrogen modulates progestin receptor concentrations in some rat brain regions but not in others. Nature 1978; 274:276–278.
9. MacLusky NJ, McEwen BS. Progestin receptors in rat brain: distribution and properties of cytoplasmic progestin-binding sites. Endocrinology 1980; 106:192–202.
10. Crowley WR. Sex differences in the responses of hypothalamic luteinizing hormone-releasing hormone and catecholamine systems to ovarian hormones and naloxone: implications for sexual differentiation of luteinizing hormone secretion in rats. Brain Res 1988; 461: 314–321.
11. Horvath TL, Garcia-Segura LM, Naftolin F. Lack of gonadotropin-positive feedback in the male rat is associated with lack of estrogen-induced synaptic plasticity in the arcuate nucleus. Neuroendocrinology 1997; 65:136–140.
12. Brown TJ, Clark AS, MacLusky NJ. Regional sex differences in progestin receptor induction in the rat hypothalamus: effects of various doses of estradiol benzoate. J Neurosci 1987; 7:2529–2536.
13. Scott RE, Wu-Peng XS, Pfaff DW. Regulation and expression of progesterone receptor mRNA isoforms A and B in the male and female rat hypothalamus and pituitary following oestrogen treatment. J Neuroendocrinol 2002; 14:175–183.
14. Lauber AH, Romano GJ, Pfaff DW. Sex difference in estradiol regulation of progestin receptor mRNA in rat mediobasal hypothalamus as demonstrated by in situ hybridization. Neuroendocrinology 1991; 53:608–613.
15. Gorski RA. Modification of ovulatory mechanisms by postnatal administration of estrogen to the rat. Am J Physiol 1963; 205:842–844.
16. Barraclough CA, Gorski RA. Evidence that the hypothalamus is responsible for androgen-induced sterility in the female rat. Endocrinology 1961; 68:68–79.
17. James KC, Nicholls PJ, Roberts M. Biological half-lives of [4-14C]testosterone and some of its esters after injection into the rat. J Pharm Pharmacol 1969; 21:24–27.
18. Rhees RW, Kirk BA, Sephton S, Lephart ED. Effects of prenatal testosterone on sexual behavior, reproductive morphology and LH secretion in the female rat. Dev Neurosci 1997; 19:430–437.
19. Ward IL, Ward OB, Affuso JD, Long WD 3rd, French JA, Hendricks SE. Fetal testosterone surge: specific modulations induced in male rats by maternal stress and/or alcohol consumption. Horm Behav 2003; 43:531–539.
20. Weisz J, Ward IL. Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 1980; 106:306–316.
21. Szabo M, Kilen SM, Nho SJ, Schwartz NB. Progesterone receptor A and B messenger ribonucleic acid levels in the anterior pituitary of rats are regulated by estrogen. Biol Reprod 2000; 62:95–102.
22. Urban JH, Das I, Levine JE. Steroid modulation of neuropeptide Y-induced luteinizing hormone releasing hormone release from median eminence fragments from male rats. Neuroendocrinology 1996; 63: 112–119.
23. Park OK, Mayo KE. Transient expression of progesterone receptor messenger RNA in ovarian granulosa cells after the preovulatory luteinizing hormone surge. Mol Endocrinol 1991; 5:967–978.
24. Park-Sarge OK, Mayo KE. Regulation of the progesterone receptor gene by gonadotropins and cyclic adenosine 3′,5′-monophosphate in rat granulosa cells. Endocrinology 1994; 134:709–718.
25. Van Cauter E. Estimating false-positive and false-negative errors in analyses of hormonal pulsatility. Am J Physiol 1988; 254:E786–E794.
26. Hutter HS, Gibson MJ. Effect of neonatal androgenization on positive feedback in female mice. Biol Reprod 1988; 38:636–638.
27. Chappell PE, Levine JE. Stimulation of gonadotropin-releasing hormone surges by estrogen. I. Role of hypothalamic progesterone receptors. Endocrinology 2000; 141:1477–1485.
28. Levine JE. New concepts of the neuroendocrine regulation of gonadotropin surges in rats. Biol Reprod 1997; 56:293–302.
29. Sullivan SD, Moenter SM. Prenatal androgens alter GABAergic drive to gonadotropin-releasing hormone neurons: implications for a common fertility disorder. Proc Natl Acad Sci U S A 2004; 101:7129–7134.
30. Everett JW, Sawyer CH. A 24-hour periodicity in the ''LH-release apparatus'' of female rats, disclosed by barbiturate sedation. Endocrinology 1950; 47:198–218.
31. Chappell PE, Lee J, Levine JE. Stimulation of gonadotropin-releasing hormone surges by estrogen. II. Role of cyclic adenosine 3′5′-monophosphate. Endocrinology 2000; 141:1486–1492.
32. Collins RL, Hodgen GD. Blockade of the spontaneous midcycle gonadotropin surge in monkeys by RU 486: a progesterone antagonist or agonist? J Clin Endocrinol Metab 1986; 63:1270–1276.
33. Bauer-Dantoin AC, Tabesh B, Norgle JR, Levine JE. RU486 administration blocks neuropeptide Y potentiation of luteinizing hormone (LH)-releasing hormone-induced LH surges in proestrous rats. Endocrinology 1993; 133:2418–2423.
34. Batista MC, Cartledge TP, Zellmer AW, Nieman LK, Loriaux DL, Merriam GR. The antiprogestin RU486 delays the midcycle gonadotropin surge and ovulation in gonadotropin-releasing hormone-induced cycles. Fertil Steril 1994; 62:28–34.
35. Bellido C, Gonzalez D, Aguilar R, Sanchez-Criado JE. Antiprogestins RU486 and ZK299 suppress basal and LHRH-stimulated FSH and LH secretion at pituitary level in the rat in an oestrous cycle stage-dependent manner. J Endocrinol 1999; 163:79–85.
36. Szabo M, Knox KL, Ringstrom SJ, Perlyn CA, Sutandi S, Schwartz NB. Mechanism of the inhibitory action of RU486 on the secondary follicle-stimulating hormone surge. Endocrinology 1996; 137:85–89.
37. Roselli CE, Klosterman SA. Sexual differentiation of aromatase ac-

tivity in the rat brain: effects of perinatal steroid exposure. Endocrinology 1998; 139:3193–3201.
38. Quadros PS, Pfau JL, Goldstein AY, De Vries GJ, Wagner CK. Sex differences in progesterone receptor expression: a potential mechanism for estradiol-mediated sexual differentiation. Endocrinology 2002; 143:3727–3739.
39. Wersinger SR, Sannen K, Villalba C, Lubahn DB, Rissman EF, De Vries GJ. Masculine sexual behavior is disrupted in male and female mice lacking a functional estrogen receptor alpha gene. Horm Behav 1997; 32:176–183.
40. Ogawa S, Washburn TF, Taylor J, Lubahn DB, Korach KS, Pfaff DW. Modifications of testosterone-dependent behaviors by estrogen receptor-alpha gene disruption in male mice. Endocrinology 1998; 139: 5058–5069.
41. Ogawa S, Chester AE, Hewitt SC, Walker VR, Gustafsson JA, Smithies O, Korach KS, Pfaff DW. Abolition of male sexual behaviors in mice lacking estrogen receptors alpha and beta (alpha beta ERKO). Proc Natl Acad Sci U S A 2000; 97:14737–14741.
42. Sato T, Matsumoto T, Kawano H, Watanabe T, Uematsu Y, Sekine K, Fukuda T, Aihara K, Krust A, Yamada T, Nakamichi Y, Yamamoto Y, Nakamura T, Yoshimura K, Yoshizawa T, Metzger D, Chambon P, Kato S. Brain masculinization requires androgen receptor function. Proc Natl Acad Sci U S A 2004; 101:1673–1678.
43. Snyder BW, Beecham GD, Winneker RC. Danazol suppression of luteinizing hormone in the rat: evidence for mediation by both androgen and estrogen receptors. Proc Soc Exp Biol Med 1990; 194:54–57.
44. Donham RS, Stetson MH. Neonatal androgen abolishes clock-timed gonadotrophin release in prepubertal and adult female hamsters. J Reprod Fertil 1985; 73:215–221.
45. Lund TD, Munson DJ, Haldy ME, Handa RJ. Dihydrotestosterone may inhibit hypothalamo-pituitary-adrenal activity by acting through estrogen receptor in the male mouse. Neurosci Lett 2004; 365:43–47.
46. Lindzey J, Wetsel WC, Couse JF, Stoker T, Cooper R, Korach KS. Effects of castration and chronic steroid treatments on hypothalamic gonadotropin-releasing hormone content and pituitary gonadotropins in male wild-type and estrogen receptor-alpha knockout mice. Endocrinology 1998; 139:4092–4101.
47. Corbier P, Kerdelhue B, Picon R, Roffi J. Changes in testicular weight and serum gonadotropin and testosterone levels before, during, and after birth in the perinatal rat. Endocrinology 1978; 103:1985–1991.
48. Herbosa CG, Dahl GE, Evans NP, Pelt J, Wood RI, Foster DL. Sexual differentiation of the surge mode of gonadotropin secretion: prenatal androgens abolish the gonadotropin-releasing hormone surge in the sheep. J Neuroendocrinol 1996; 8:627–633.
49. Masek KS, Wood RI, Foster DL. Prenatal dihydrotestosterone differentially masculinizes tonic and surge modes of luteinizing hormone secretion in sheep. Endocrinology 1999; 140:3459–3466.
50. Sharma TP, Herkimer C, West C, Ye W, Birch R, Robinson JE, Foster DL, Padmanabhan V. Fetal programming: prenatal androgen disrupts positive feedback actions of estradiol but does not affect timing of puberty in female sheep. Biol Reprod 2002; 66:924–933.
51. Robinson JE, Birch RA, Foster DL, Padmanabhan V. Prenatal exposure of the ovine fetus to androgens sexually differentiates the steroid feedback mechanisms that control gonadotropin releasing hormone secretion and disrupts ovarian cycles. Arch Sex Behav 2002; 31:35–41.
52. Zeleznik AJ, Little-Ihrig L, Ramasawamy S. Administration of dihydrotestosterone to rhesus monkeys inhibits gonadotropin-stimulated ovarian steroidogenesis. J Clin Endocrinol Metab 2004; 89:860–866.
53. McCarthy MM, Schlenker EH, Pfaff DW. Enduring consequences of neonatal treatment with antisense oligodeoxynucleotides to estrogen receptor messenger ribonucleic acid on sexual differentiation of rat brain. Endocrinology 1993; 133:433–439.
54. Pinilla L, Trimino E, Garnelo P, Bellido C, Aguilar R, Gaytan F, Aguilar E. Changes in pituitary secretion during the early postnatal period and anovulatory syndrome induced by neonatal oestrogen or androgen in rats. J Reprod Fertil 1993; 97:13–20.
55. Diaz DR, Fleming DE, Rhees RW. The hormone-sensitive early postnatal periods for sexual differentiation of feminine behavior and luteinizing hormone secretion in male and female rats. Brain Res Dev Brain Res 1995; 86:227–232.
56. Handa RJ, Corbier P, Shryne JE, Schoonmaker JN, Gorski RA. Differential effects of the perinatal steroid environment on three sexually dimorphic parameters of the rat brain. Biol Reprod 1985; 32:855–864.
57. Resko JA, Connolly PB, Roselli CE, Abdelgadir SE, Choate JV. Differential effects of aromatase inhibition on luteinizing hormone secretion in intact and castrated male cynomolgus macaques. J Clin Endocrinol Metab 1993; 77:1529–1534.
58. Simerly RB, Swanson LW, Handa RJ, Gorski RA. Influence of perinatal androgen on the sexually dimorphic distribution of tyrosine hydroxylase-immunoreactive cells and fibers in the anteroventral periventricular nucleus of the rat. Neuroendocrinology 1985; 40:501–510.
59. Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J Comp Neurol 1990; 294:76–95.
60. Gu GB, Simerly RB. Projections of the sexually dimorphic anteroventral periventricular nucleus in the female rat. J Comp Neurol 1997; 384:142–164.
61. Hagihara K, Hirata S, Osada T, Hirai M, Kato J. Distribution of cells containing progesterone receptor mRNA in the female rat di- and telencephalon: an in situ hybridization study. Brain Res Mol Brain Res 1992; 14:239–249.
62. Simerly RB, Young BJ, Carr AM. Co-expression of steroid hormone receptors in opioid peptide-containing neurons correlates with patterns of gene expression during the estrous cycle. Brain Res Mol Brain Res 1996; 40:275–284.
63. Matwijiw I, Faiman C. Control of gonadotropin secretion in the ovine fetus. IV. Male-specific entrainment of the hypothalamic control of luteinizing hormone secretion by testosterone in the female ovine fetus. Endocrinology 1991; 129:1447–1451.
64. Dumesic DA, Abbott DH, Eisner JR, Goy RW. Prenatal exposure of female rhesus monkeys to testosterone propionate increases serum luteinizing hormone levels in adulthood. Fertil Steril 1997; 67:155–163.
65. Ellis GB, Desjardins C, Fraser HM. Control of pulsatile LH release in male rats. Neuroendocrinology 1983; 37:177–183.
66. Leipheimer RE, Bona-Gallo A, Gallo RV. Influence of estradiol and progesterone on pulsatile LH secretion in 8-day ovariectomized rats. Neuroendocrinology 1986; 43:300–307.
67. Kim SJ, Foster DL, Wood RI. Prenatal testosterone masculinizes synaptic input to gonadotropin-releasing hormone neurons in sheep. Biol Reprod 1999; 61:599–605.
68. Belsham DD, Evangelou A, Roy D, Duc VL, Brown TJ. Regulation of gonadotropin-releasing hormone (GnRH) gene expression by 5alpha-dihydrotestosterone in GnRH-secreting GT1-7 hypothalamic neurons. Endocrinology 1998; 139:1108–1114.
69. Huang X, Harlan RE. Absence of androgen receptors in LHRH immunoreactive neurons. Brain Res 1993; 624:309–311.