Brain Regulation of Hormones Affecting Reproduction in Pigs

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Summary and Implication

Brain hormones exquisitely regulate the secretion of hormones secreted by the pituitary gland that affect onset of puberty and reproductive function in pigs. Luteinizing hormone releasing hormone (LHRH) is a hypothalamic 10-amino acid peptide produced by a small placode of LHRH neurons (~1,500) that is crucial for gonadotropin (LH and follicle stimulating hormone, FSH) release by the pituitary gland. Neurosurgical interventions obliterate LHRH release thus interfering with episodic LH and FSH secretion required for continued reproductive function in male and female pigs. The hypothalamus at the base of the brain regulates episodic LH and FSH secretion from the pituitary in part by its endogenous release of LHRH and by feedback effects of gonadal protein (i.e., inhibin) and steroid (i.e., estrogen, progesterone, androgen) hormones. The objectives were to determine hypothalamic regulation of episodic LH secretion in female pigs and the biphasic feedback actions of estradiol-17 beta (E_2-17β). Ovariectomy of gilts eliminates negative feedback effects of estrogen on the brain and thus allows an elevated level of episodic LH secretion compared with intact control animals. Neurosurgical disconnection of the neurohypophyseal link between the hypothalamus and pituitary (hypophyseal stalk transection, HST) blocks hypothalamic LHRH secretion to the pituitary gland, eliminating episodic LH secretion that renders the animal acyclic. In this study, the minimum effective dosage of E_2-17β that would induce estrus in ovariectomized gilts was determined to be 20 μg/kg body weight. Then, ovariectomized gilts were assigned randomly to HST, cranial sham operation control (SOC), and unoperated control (UOC). In HST gilts, episodic LH release was abolished and average LH blood concentration decreased compared with controls. E_2-17β or sesame oil vehicle did not affect blood LH concentration in HST gilts, and LH remained constant throughout 120 hours (0.7 ng/ml). In contrast in the SOC and UOC gilts, E_2-17β induced a 60% decrease in LH concentration within 12 hours, and LH remained low until 48 hours, then increased to peak values by 72 hours, and was followed by a decline to 120 hours. These results indicate that both episodic LH secretion and the biphasic feedback action of E_2-17β on LH secretion depend on hypothalamic regulatory mechanisms in the gilt. The isolated pituitary in HST gilts is capable of autonomous secretion of LH, E_2-17β will elicit direct negative feedback action on the isolated pituitary gland if the gonadotropin-producing cells in that gland are supported by exogenous LHRH, but E_2-17β at high concentrations will not induce positive feedback in isolated pituitaries.

Introduction

Ovarian steroids modulate luteinizing hormone (LH) secretion in the rat, sheep, rhesus monkey, and pig. After ovariectomy, the elevated and pulsatile profiles of LH secretion are caused by LH-releasing hormone (LHRH) from hypothalamic secretory neurons that is secreted episodically into hypophyseal portal vessels. Hypothalamic destruction or drug-induced inhibition of secretory neurons within the hypothalamus eliminates pulsatile LH secretion in ovariectomized rats, rhesus monkeys, and ewes. Ovariectomy in gilts produces increases in LH secretion with the characteristic episodic rhythm.

In gilts, estrogens, but not progesterone, are the major ovarian steroids that regulate LH secretion; estrogens cause biphasic LH secretion in ovariectomized gilts. Initially peripheral blood LH concentrations decrease in response to negative feedback of estrogen; and then, if estrogen levels increase above a critical “threshold” a subsequent preovulatory LH rise occurs in response to positive feedback. Similarly, a sequence of negative and positive feedback of estrogen on LH secretion occurs in rhesus monkeys, ewes, and female rats.

In contrast to rats and sheep, estrogen in monkeys can mediate its biphasic, feedback effects on LH secretion directly at the level of the pituitary gland. Thus, the two models proposed for regulation of LH secretion by estrogen include regulation primarily at the level of either the hypothalamus or the pituitary (hypophysis). The current study in gilts examined dosages of estrogen to determine if the effects of E_2-17β on pulsatile LH secretion are regulated primarily by 1) the pituitary gland independent of hypothalamic control, or 2) the biphasic feedback of estrogen at the hypothalamus.

Materials and Methods

Animals. Postpubertal gilts (n = 39) were ovariectomized and treated with either E_2-17β or estradiol benzoate (EB) at varying dosages to determine minimum effective dosages for induction of behavioral estrus. Estrogens (5, 10 or 20 μg of E_2-17β or 1.25, 2.5, or 5 μg of EB per kg body weight) were given in oil as a single intramuscular injection. Gilts were observed for behavioral estrus twice daily in the presence of mature boars; proportion that exhibited estrus and latency to estrus were determined.

For the second study, 23 Yorkshire gilts that had exhibited at least one estrus were used. Before treatment assignment, each gilt was laparotomized via a midventral incision and bilaterally ovariectomized, and an indwelling

catheter was inserted into a jugular vein for repeat blood sampling. Treatment assignments were hypophyseal stalk transection (HST, n = 11), cranial sham-operated control (SOC, n = 6), or unoperated control (UOC, n = 6).

The third study evaluated the effect of exogenous, pulsatile LHRH on the biphasic effect that E_2-17β has on LH secretion. Indwelling jugular catheters were inserted into gilts that had been ovariectomized postpubertally. Beginning a minimum of 72 hours after placement of catheters (day -2), gilts were assigned to receive hourly intravenous injections of 2 µg of LHRH for 144 hours and other two received diluent intravenously.

In the fourth study, postpubertal, ovariectomized, Yorkshire gilts received indwelling jugular catheters as described above. Treatments were HST (n = 6) and UOC (n = 5). The HST gilts were given hourly LHRH infusion as described in the third study beginning on the morning after HST (day -2). E_2-17β (20 µg/kg body weight) was given on days 0 and 2 to HST gilts and UOC gilts. The UOC gilts did not receive hourly injections of LHRH.

RIA of LH and E_2-17β. LH concentrations were determined by a double-antibody technique in duplicate aliquots of 200 µl. Assay sensitivity was 0.08 ng per tube. Pools of sera containing high (1.03 ± 0.03 ng/ml, mean ± SE) or low (0.43 ± 0.07 ng/ml) concentrations of LH were included in each assay and had inter- and intra-assay coefficients of variation of 18 ± 2.5 and 8 ± 0.6%, respectively.

Serum E_2-17β was assayed in 0.5-ml duplicate aliquots that had been extracted twice with diethyl ether (10:1 v/v). The antibody was specific for E_2-17β and had no cross-reactivity with estrone or estradiol-17β (<0.05%). Assay sensitivity was 10 pg/ml serum, and interassay coefficient of variation, determined from a pool of boar serum (42 ± 8 pg/ml) was 15%. Extraction recoveries ranged from 91–98%.

Statistical analysis of experimental data. Characterization of pulsatile secretion of LH on day -2 and day 2 for HST and SOC gilts, or 2 and 5 days after ovariectomy in UOC gilts, were analyzed by a split-plot analysis using a one-way ANOVA, and Student's *t* test for continuous variables was used for comparisons between groups. Mean number of peaks, mean amplitude, baseline, and LH values were analyzed with day and treatment within day as variables. Fisher's LSD test was used to determine differences among means.

Results

Proportion of ovariectomized gilts that exhibited behavioral estrus in the first study was 0/9, 5/9, and 9/9 for E_2-17β dosages of 5, 10, and 20 µg/kg body weight, respectively. This compared to 2/4, 4/4, and 4/4 for EB dosages of 1.25, 2.5, and 5 µg/kg body weight. Latency to estrus was 1.9 ± 0.5 days and did not differ ($P > 0.10$) for the two forms of estrogen.

In the second study, pulsatile LH secretion on day -2 for the 20 HST gilts was characterized by a frequency of 0.9 ± 0.06 peaks/hour, amplitude of 1.3 ± 0.13 ng/ml, baseline of 0.8 ± 0.07 ng/ml, and an overall average of 1.0 ± 0.07 ng/ml. After HST, pulsatile releases of LH were abolished and mean LH concentration decreased ($P < 0.05$) compared with control gilts. Baseline LH concentration tended to be lower in HST gilts compared with controls (0.7 vs. 1.0 ng/ml, respectively; $P < 0.10$).

E_2-17β or sesame oil treatment did not affect LH concentration during a 120-hour sampling period in HST gilts; LH remained relatively constant (0.7 ± 0.07 ng/ml). E_2-17β, but not sesame oil, treatment affected LH concentration over time in E_2-17β-treated controls. LH decreased 60% 12 hours after E_2-17β injection (1.4 ± 0.1 and 0.8 ± 0.03 ng/ml, respectively; $P < 0.05$). LH remained low in E_2-17β-treated gilts for 48 hours and then increased to peak concentration (1.9 ± 0.13 ng/ml; $P < 0.05$) by 72 hours, and decreased thereafter to 1.4 ± 0.13 ng/ml at 120 hours.

In the third study, LH secretion in response to 2 µg of LHRH intravenously was greater ($P < 0.01$) in ovariectomized, control gilts than in gilts that had received LHRH hourly for 48 hours. The overall means were 2.9 vs. 2.0 ng LH/ml for controls and LHRH infused gilts, respectively; the time by group interaction approached significance ($P < 0.08$). At initiation of E_2-17β treatment, LH concentrations were similar in both groups ($P > 0.10$), and the responses to the two intramuscular injections of E_2-17β did not differ ($P > 0.10$). Clearly, the negative response to the first E_2-17β injection preceded the positive response. After 7 days of hourly LHRH infusions and the E_2-17β regimen, LH concentrations were similar in the two groups, but the response to an intravenous bolus of 25 µg LHRH was greater ($P < 0.01$) in controls than in the infused group with the greatest difference at 15 min after LHRH.

Concentrations of LH were less ($P < 0.01$) in the four HST gilts that had received 48 hours of hourly LHRH than in UOC. Moreover, these HST gilts achieved a lower concentration ($P < 0.01$) of LH in response to 2 µg of LHRH than the UOC gilts. Initial injection of E_2-17β reduced ($P < 0.05$) LH in both HST and UOC gilts, but after the second injection of E_2-17β, LH concentrations increased in UOC's and were greater than in LHRH-infused gilts from 72 to 96 hours after initial E_2-17β ($P < 0.05$). The time by group interaction approached significance ($P < 0.06$).

Discussion

The main findings were that 1) high circulating concentrations of E_2-17β are incapable of inducing an ovulatory release of LH in HST gilts, 2) autonomous secretion of LH continues in ovariectomized gilts with a pituitary gland isolated by HST, and 3) pulsatile LH secretion occurs in ovariectomized gilts in response to hypothalamic stimulation. We established that a single intramuscular injection of E_2-17β into ovariectomized gilts induced biphasic LH secretion (negative followed by positive feedback) after cranial sham operation and in unoperated animals. However, in HST gilts, estrogen treatment elicited a negative feedback response only

if gilts received hourly pulses of exogenous LHRH, and in neither of the two estrogen protocols did estrogen evoke a positive feedback response. These observations extend the findings that estrogen manifests its positive feedback on LH secretion primarily through the central nervous system, and estrogen at excessively elevated concentrations was no more effective than concentrations observed during the estrous cycle.

Administration of central nervous system depressants to female pigs and rats, electro-lesion of the hypophysiotropic area in female rhesus monkeys and ewes, or interruption of hypophyseal blood circulation in female rhesus monkeys, beef calves and female pigs (current study) eliminates pulsatile LH secretion. This pulsatile pattern reflects a basic rhythm of the secretory neurons of the hypophysiotropic area in the hypothalamus that secretes LHRH into hypophyseal portal vessels for transport to the anterior pituitary gland (adenohypophysis).

In ovariectomized prepubertal gilts, neural stimuli originating or traversing the neural areas rostral to the median eminence are required for LH secretion. Interruption of the neural processes between the anterior hypothalamus and the median eminence by hypothalamic deafferentation abolished episodic release of LH; however, basal concentrations of the hormone were maintained at reduced levels. Episodic LH release was abolished after anterior hypothalamic or complete hypothalamic deafferentation, but not after posterior hypothalamic deafferentation or in sham-operated gilts. A robust peak LH release within 15 minutes after LHRH challenge in anterior hypothalamic deafferentated and complete hypothalamic deafferentated animals indicated that the pituitary gland of immature gilts is capable of secreting LH after disconnection of the anterior neural links of the hypothalamus. Additionally, this current study clearly indicated that the neural stimuli originating or traversing in the anterior hypothalamus are required for the episodic release of LH.

After HST, circulating LH concentration did not decrease to nondetectable values, but remained consistently lower than in control gilts, indicating that the isolated pituitary gland in gilts secretes LH autonomously. The consistently low level of LH secretion in HST gilts depends upon basal function of differentiated basophils of the pituitary gland, and E_2-17β may exert its actions on these isolated gonadotrophs. In this study, E_2-17β caused a 60 percent decrease in serum LH concentration in ovariectomized gilts; LH decreased to a level comparable to that in HST gilts. E_2-17β did not decrease serum LH concentration in stalk-transected gilts unless they were receiving pulsatile LHRH treatment. Estrogen may affect LH secretion only when gonadotrophs function at levels above a basal rate in this species.

This study indicates that pulsatile secretion of LH in gilts is regulated by neuroendocrine mechanisms and that the positive feedback action of E_2-17β is mediated via the central nervous system. It seems that regulation of LH secretion in gilts resembles more closely that observed in the ewe and rat than the rhesus monkey. Furthermore, the isolated pituitary gland of the gilt is capable of autonomous secretion of LH, and estrogen does not influence this hormone secretion except when the isolated pituitary gland is exposed to pulsatile LHRH.