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Luteinizing hormone secretion as affected by hypophyseal stalk transection and estradiol-17β in ovariectomized gilts☆

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Abstract

The objectives were to determine hypothalamic regulation of pulsatile luteinizing hormone (LH) secretion in female pigs and the biphasic feedback actions of estradiol-17β (E_2-17β). In the first 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. In the second study, ovariectomized gilts were assigned randomly on day 0 to treatments: (a) hypophyseal stalk transection (HST), (b) cranial sham-operated control (SOC), and (c) unoperated control (UOC). On day 3, gilts from each group received a single i.m. injection of either E_2-17β (20 μg/kg body weight) or sesame oil. Blood was collected from an indwelling jugular cannula at 15 min intervals for 3 h before (day −2) and after treatment (day 2) from HST, SOC and UOC gilts. On day 3, blood was collected at 2 h intervals for 12 h after E_2-17β or sesame oil injection and at 4 h intervals thereafter for 108 h. Pulsatile LH secretion in all gilts 2 days after ovariectomy exhibited a frequency of 0.9 ± 0.06 peaks/h, amplitude of 1.3 ± 0.13 ng/ml, baseline of 0.8 ± 0.07. Serum LH concentrations from SOC and UOC gilts

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were similar on day 2 and profiles did not differ from those on day −2. In HST gilts pulsatile LH release was abolished and mean LH concentration decreased compared with controls (0 versus 0.9 ± 0.06 peaks/h and 0.77 ± 0.03 versus 1.07 ± 0.07 ng/ml, respectively; $P < 0.05$). E_2-17β or sesame oil did not affect serum LH concentration in HST gilts, and LH remained constant throughout 120 h (0.7 ± 0.07 ng/ml). In SOC and UOC control gilts, E_2-17β induced a 60% decrease ($P < 0.05$) in LH concentration within 12 h, and LH remained low until 48 h, then increased to peak values ($P < 0.05$) by 72 h, followed by a gradual decline to 120 h. Although pituitary weight decreased 31% in HST gilts compared with controls (228 versus 332 mg, $P < 0.05$), an abundance of normal basophils was evident in coronal sections of the adenohypophysis of HST comparable to that seen in control gilts. The third and fourth studies determined that hourly i.v. infusions of LHRH (2 μg) and a second injection of E_2-17β 48 h after the first had no effect on the positive feedback action of estrogen in UOC. However, in HST gilts that received LHRH hourly, the first injection of E_2-17β decreased ($P < 0.05$) plasma LH concentrations while the second injection of E_2-17β failed to induce a positive response to estrogen. These results indicate that both pulsatile LH secretion and the biphasic feedback action of E_2-17β on LH secretion depend on hypothalamic regulatory mechanisms in the gilts. The isolated pituitary of 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 gonadotropes are supported by exogenous LHRH, but E_2-17β at high concentrations will not induce positive feedback in isolated pituitaries. Thus, the direct effect of E_2-17β on the pituitary of monkeys cannot be mimicked in pigs.

Keywords: Luteinizing hormone; Hypothalamic regulation; Estradiol-17β; Pig

1. 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. The patterns of LH secretion in intact prepubertal gilts indicate few changes evident in mean serum concentration during 10–25 week of age (Diekman et al., 1983). Weekly sequential bleedings indicated that the frequency and magnitude of LH pulses remained similar during this period. Ovariectomy in gilts produces increases in LH secretion with a characteristic episodic rhythm (Berardinelli et al., 1984).

In gilts, estrogens, but not progesterone, are the major ovarian steroids that regulate LH secretion (Brinkley, 1981; Van de Wiel et al., 1981); estrogens cause biphasic LH secretion in ovariectomized gilts. Initially peripheral blood LH concentrations decrease in response to negative feedback of estrogen; then, if estrogen levels increase above a critical 'threshold' a subsequent preovulatory LH rise occurs in response to positive feedback (Berardinelli et al., 1984). Similarly, a sequence of negative and positive feedback of estrogen on LH secretion occurs in rhesus monkeys (Hotchkiss and Knobil, 1994), ewes (Scaramuzzi et al., 1971), and female rats (Sherwood et al., 1976). The negative feedback of estrogen on LH secretion involves both hypophyseal and hypothalamic components in

rats (Halász and Gorski, 1967; Blake, 1977). Furthermore, in rats and sheep, interruption of afferent signals from nuclei rostral to the arcuate nucleus eliminates preovulatory- as well as E_2-17β-induced, surges of LH (Halász and Pupp, 1965; Blake, 1977; Jackson et al., 1978).

In contrast to rats and sheep, estrogen in monkeys can mediate its biphasic, feedback effects on LH secretion directly at the pituitary gland (Nakai et al., 1978; Ferin et al., 1979; Pavasuthipaisit et al., 1981). Thus, the two models proposed for regulation of LH secretion by estrogen include regulation primarily at either the hypothalamus or the hypophysis. However, the amount of estrogen required to induce positive feedback is greater in hypophyseal stalk-transected (HST) than in control monkeys (Karsch et al., 1973a; Ferin et al., 1979; Pavasuthipaisit et al., 1981). Likewise, estrogen produces positive feedback on LH secretion in gonadectomized male monkeys, but the dosage must be greater than in females (Yamaji et al., 1971; Karsch et al., 1973b). In HST gilts estrogen failed to induce a positive feedback effect on LH secretion (Kesner et al., 1989); however, this study used a single dosage of estrogen. The current study in gilts used greater dosages of estrogen to determine if the effects of E_2-17β on pulsatile LH secretion are regulated primarily by (a) the pituitary gland independent of hypothalamic control or (b) the biphasic feedback of estrogen at the hypothalamus.

2. Materials and methods

2.1. Animals

2.1.1. Minimum effective dosage of E_2-17β

Thirty-nine white-composite, postpubertal gilts 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 E_2-17β or 1.25, 2.5 or 5 μg EB per kg body weight (BW)) 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.

2.1.2. LH secretion after hypophyseal stalk transection and E_2-17β treatment

For the second study, 23 purebred Yorkshire gilts, averaging 211 ± 5 (±S.E.) days of age and weighing 122 ± 7 kg, that had exhibited at least one estrus were used. Before treatment assignment (day −3), each gilt was laparotomized via a midventral incision and bilaterally ovariectomized, and an indwelling catheter (Tygon microbore tubing, 1.27 mm i.d., Fisher Scientific, Pittsburgh, PA) was inserted into a jugular vein by a modified nonsurgical technique (Ford and Maurer, 1978). Anesthesia was induced by i.v. injection of thiamylal sodium (0.8–1 g; Surital, Parke–Davis, Morris Plaines, NJ) and maintained by closed-circuit inhalation of halothane (4–9%; Ayerst, Rouses Point, NY) and O_2 (500–1000 cc/min). Treatment assignments on day 0 were hypophyseal stalk transection (HST, $N = 11$), cranial sham-operated control (SOC, $N = 6$), or unoperated control (UOC, $N = 6$). Three days after assignment to treatments, an equal number of animals from each treatment group was assigned randomly for i.m. injection of either E_2-17β in sesame oil (E_2-17β; 20 μg/kg

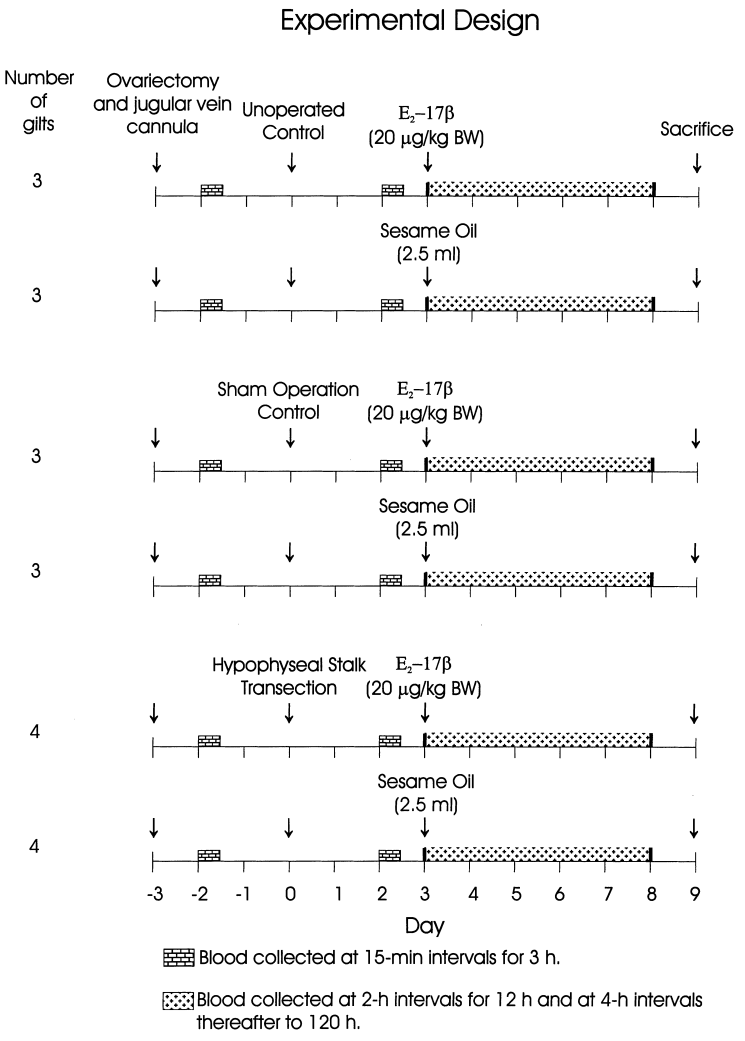

Fig. 1. Description of experimental and control groups for determination of effects of estradiol-17β on neuroendocrine regulation of luteinizing hormone secretion in ovariectomized Yorkshire gilts.

BW) or vehicle (2.5 ml sesame oil). Fig. 1 depicts the experimental regimen and shows the number of gilts per treatment.

Blood (5 ml) was collected at 15 min intervals during 3 h on the afternoon of day −2 and day 2 for radioimmunoassay (RIA) of LH. To evaluate effects of E_2-17β, blood was collected immediately before (0 h) E_2-17β or vehicle injection, at 2 h intervals for 12 and 4 h intervals thereafter for 108 h. Blood was allowed to clot at 4°C for 18 h, then centrifuged ($2000 \times g$, 4°C), and serum was decanted into labeled, glass culture tubes, frozen, and stored at −20°C for hormone assays.

2.1.3. Effect of pulsatile LHRH infusion and E_2-17β treatment on LH secretion

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 four white composite gilts that had been ovariectomized postpubertally, BW = 115 + 2 kg. Beginning a minimum of 72 h after placement of catheters (day −2), two gilts were assigned to receive hourly i.v. infusions of 2 μg LHRH for 144 h and the other two received diluent i.v. The LHRH (Sigma Chemical Co., St. Louis, MO) was diluted (1 μg/ml) in 0.1% BSA and administered by infusion pumps connected to timers, 2 ml/min for 1 min each hour. All gilts received i.m. injections of E_2-17β (20 μg/kg BW) on days 0 and 2. Blood samples were collected at 0, 6, 12, 24, 36, 48, 53, 60, 72, 78, 84, 90, 96, 108 and 120 h (day 7 of LHRH infusion) after initiation of the first E_2-17β injection. Samples were taken just before the coincident hourly infusion. Additionally, on day 0 before E_2-17β treatment, blood samples were obtained from all four gilts at 0.5, 10, 20, 30, 45 and 60 min after an i.v. infusion of 2 μg of LHRH. Hourly LHRH infusions stopped on day 7, and 1 h after the last infusion all four gilts received 25 μg of LHRH i.v. with blood collected at 0, 7, 15, 30, 45, 60 and 90 min after this bolus treatment. Seven days after the 25 μg bolus of LHRH, treatment of these gilts was reversed with respect to hourly infusions of LHRH and diluent, and the protocol repeated.

2.1.4. Effect of pulsatile LHRH infusion and E_2-17β treatment on LH secretion after hypophyseal stalk transection

In the fourth study, postpubertal, ovariectomized, Yorkshire gilts (BW = 106 ± 3 kg) 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 BW) was given on days 0 and 2 to HST gilts and UOC gilts. The UOC gilts did not receive hourly infusions of LHRH. Blood samples were collected at the intervals described for the third study. Plus, all gilts received 2 μg LHRH i.v. on day 0, before E_2-17β treatment, with blood collected at 0, 5, 10, 20 and 30 min after LHRH. Hourly LHRH infusions of HST gilts stopped on day 7, and 1 h after the last infusion all gilts received 25 μg of LHRH with blood collected at 0, 15, 30, 45, 60, 75, 90, 105 and 120 after the LHRH bolus infusion.

2.2. Hypophyseal stalk transection (HST)

Anesthesia was induced with thiamylal sodium and maintained with halothane/O_2 administered after endotracheal intubation. A modified transfrontal supraorbital approach for visualization of the porcine hypophyseal stalk, developed by du Mesnil du Buisson et al. (1964), was used. The hypophyseal stalk was severed by blunt dissection with platinum-tipped probes. A nylon disc (6.0–8.0 mm diameter and 0.45 mm thickness) was inserted between severed ends of the stalk to prevent vascular regeneration of the hypothalamus and pituitary gland (Anderson et al., 1967). Gel-foam (Pharmacia & Upjohn Co., Kalamazoo, MI), soaked in topical thrombin (bovine origin, Parke–Davis), was inserted near the disc to arrest hemorrhage of severed vessels.

2.3. Sham operation control (SOC)

Gilts were subjected to all cranial surgical procedures as HST, with the exception that the hypophyseal stalk was not severed.

2.4. Unoperated control (UOC)

Gilts were not subjected to cranial surgery.

2.5. Postoperative care and hormone treatment

During each study, all gilts were placed in individual pens. After HST or SOC, gilts were placed in a room maintained at 28–30°C; respiration, heart rate and rectal temperature were monitored and recorded every 4 h for 48 h. Intramuscular injection of cortisone acetate (50 mg/ml; Merck & Co. Inc., West Point, PA) or i.v. injection of cortisone succinate (Solu-Cortif, 100 mg/ml; Merck & Co.) was administered within 24 h after surgery. Water and food intake usually returned to normal by 12–24 h postsurgery. UOC gilts were penned individually in a room separate from gilts subjected to cranial surgery.

2.6. Postmortem

Experimental and control gilts in studies two and four were sacrificed by an overdose of thiamylal sodium and decapitated. The frontal bone and calvaria were removed to expose the brain. Cranial nerves were severed, and the brain was raised to expose the hypophyseal stalk and sella turcica. The hypophyseal stalk was examined to determine completeness of stalk transection and position of the nylon disc in HST gilts. The pituitary gland was excised from the sella turcica, weighed, cut transversely into three blocks and placed into Susa's fixative for histological evaluation. Coronal sections of the glands were cut at 6 μm and stained (Heath, 1965), whereas other sections were stained with hematoxylin and eosin. Thyroid and adrenal glands were excised, connective tissue was removed, and glands were weighed. The glands were cut at 6 μm and stained with hematoxylin and eosin. One set of sections from thyroid glands was stained with Mallory triple stain for identification of basophilic and acidophilic colloid.

2.7. RIA of LH and E_2-17β

LH concentrations were determined by a double-antibody technique in duplicate aliquots of 200 μl as described by Niswender et al. (1970). Assay sensitivity was 0.40 ng/ml. Pools of sera containing high (1.03 ± 0.03 ng/ml) 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. Detectable concentrations of LH in serum of HST gilts prompted revalidation of the LH RIA. Effect of assay volume (200 and 400 μl) on content and concentration of LH from serum samples of HST gilts obtained on either day 2 or 10 h after E_2-17β injection were evaluated. Doubling the assay serum volume doubled the quantity of LH measured per assay tube, independent of gilt or time of sample collection.

Serum E_2-17β was assayed in 0.5 ml duplicate aliquots that had been extracted twice with diethyl ether (10 : 1 v/v; Redmer et al., 1984). The antibody was specific for E_2-17β and had no cross-reactivity with estrone or estradiol-17α (<0.05%). Assay sensitivity was 10 pg/ml of serum. Interassay coefficient of variation, determined from a pool of boar serum (42 ± 8 pg/ml) was 15% and intra-assay coefficient of variation was 12%. Extraction recoveries ranged from 91–98%.

2.8. Statistical analysis of experimental data

In the second study, 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*-tests for continuous variables was used for comparisons between groups (SAS, 1990). Fisher's LSD test was used to determine differences among mean number of peaks, mean amplitude, baseline, and LH values with day and treatment within day as variables (Snedecor and Cochran, 1980). LH concentrations after E_2-17β or sesame oil treatment were compared by analysis of variance for split-plot, with treatment as main plot and time as the subplot. Pituitary, adrenal, and thyroid gland weights were analyzed by analysis of variance using treatment as the single classification.

For the third and fourth studies, LH profiles were evaluated using Mixed Model procedures for repeated measures (SAS; Littell et al., 1996). The model for the third study included treatment group, time, the interaction of these two variables and treatment within gilt. The model for the fourth study was treatment group, time and the interaction of these two variables. Specific comparisons were made with the PDIFF option.

3. Results

3.1. Minimum effective dosage of E_2-17β

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

3.2. LH secretion after hypophyseal stalk transection and E_2-17β treatment

In the second study, three HST gilts died within 60 h after surgery from hemorrhage or elevated temperature. In the eight other HST gilts, the nylon disc was in the proper location and had prevented vascular regeneration of the hypophyseal stalk.

Pulsatile LH secretion on day −2 for the 20 gilts in this study was characterized by a frequency of 0.9±0.06 peaks/h, 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 (Fig. 2a). LH concentrations on day 2 for SOC gilts, or 5 days after ovariectomy in UOC gilts, did not differ significantly and were pooled. After HST, pulsatile releases of LH were abolished (Fig. 2b) and mean LH concentration decreased ($P < 0.05$) compared with control gilts (Table 1). Baseline LH concentration

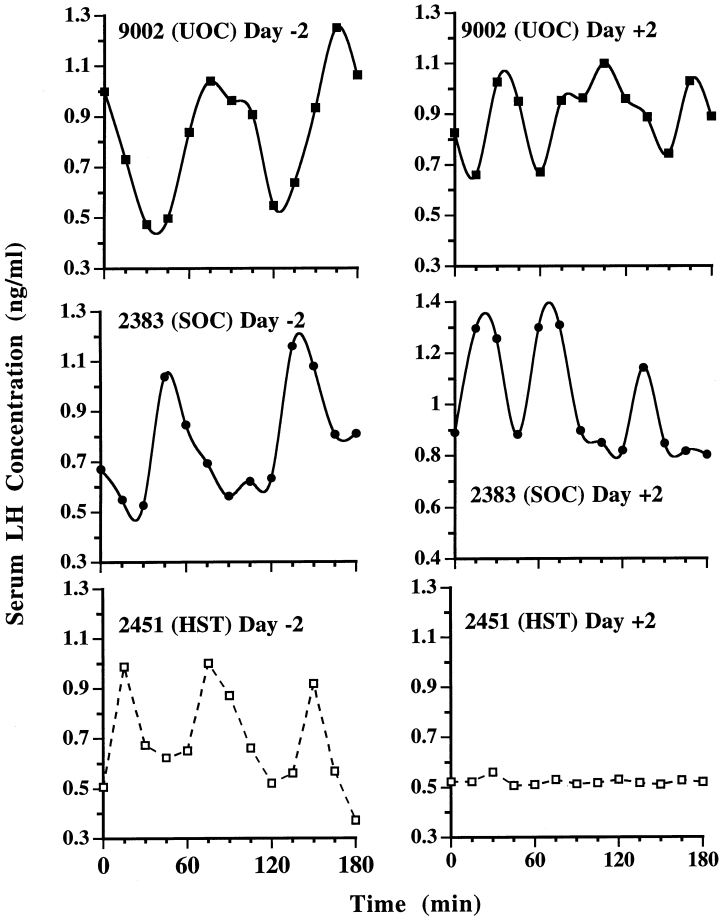

Fig. 2. Sequential profiles of luteinizing hormone concentrations in peripheral blood serum during 3 h at (a) 2 days before cranial surgery and (b) 2 days after sham operation (○) or hypophyseal stalk transection (●), as compared with those in unoperated control (▲) gilts. There was a total of eight HST, six SOC, and six UOC. All gilts were ovariectomized 3 days before cranial surgery.

tended to be lower in HST gilts compared with controls (0.7 versus 1.0 ng/ml, respectively; $P < 0.10$). Frequency and amplitude of LH pulsatile release and mean concentration in controls 5 days after ovariectomy (day 2 of the study) did not differ significantly from those observed 2 days after ovariectomy (day 0).

E_2-17β or sesame oil treatment did not affect LH concentration during a 120 h sampling period in HST gilts; LH remained relatively constant (0.7 ± 0.07 ng/ml; Fig. 3). E_2-17β, but not sesame oil, treatment affected LH concentration over time in E_2-17β-treated controls (Fig. 3). LH decreased 60% by 12 h 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 h and then increased to peak concentration (1.4 ± 0.17 ng/ml; $P < 0.05$) by 72 h, and decreased thereafter to 1.0 ± 0.07 ng/ml at 120 h.

Table 1
Effect of hypophysial stalk transection (HST), sham operation control (SOC), and unoperated control (UOC) on pulsatile secretion of luteinizing hormone in ovariectomized gilts[a]

Hormone characteristic[b]	LH in peripheral serum (ng/ml)[b]		
	HST	SOC	UOC
Mean	0.77 ± 0.03	1.07 ± 0.13	1.07 ± 0.07
Baseline	0.77 ± 0.03	0.97 ± 0.10	1.0 ± 0.07
Amplitude	NA	1.33 ± 6.13	1.23 ± 0.07
Frequency[c]	O*	0.9 ± 0.06	0.9 ± 0.07

[a] Values are mean ± S.E.M.
[b] Anterior vena cava blood samples were obtained at 15 min intervals throughout a period of 180 min 2 days after cranial surgery.
[c] Peaks/h.
* $P < 0.05$.

Circulating blood concentration of E_2-17β followed a similar sequential pattern ($P > 0.05$) among HST, SOC and UOC gilts. E_2-17β increased from <10 to 2500 ± 400 pg/ml by 4 h after E_2-17β injection ($P < 0.01$), decreased to 100 ± 25 pg/ml by 24 h and declined thereafter to <10 pg/ml by 96 h. E_2-17β was <10 pg/ml after sesame oil treatment of ovariectomized HST, SOC and UOC gilts.

Pituitary, adrenal and thyroid gland weights remained similar ($P > 0.05$) in SOC and UOC gilts and were pooled (Table 2). By 9 days after surgery, pituitary glands were 31% smaller ($P < 0.05$) in HST than SOC and UOC gilts; however, thyroid and adrenal gland

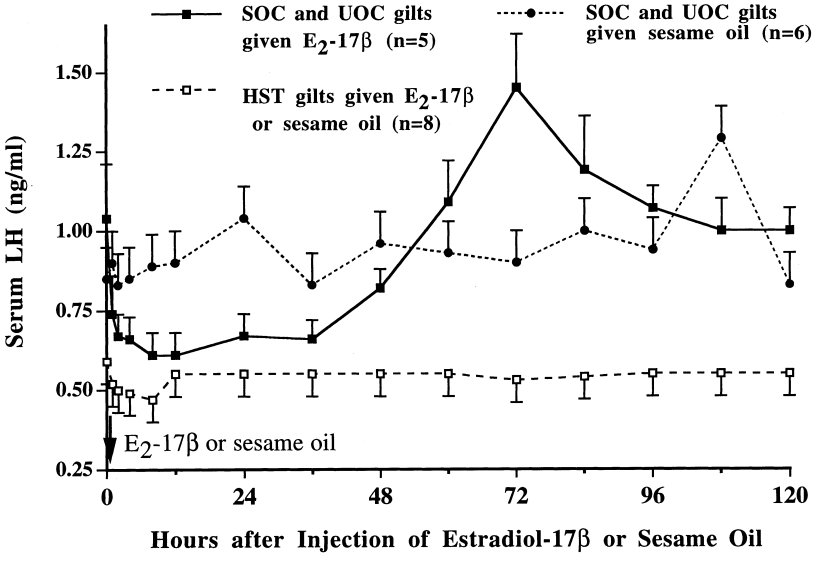

Fig. 3. Biphasic effect of a single i.m. injection of estradiol-17β on sequential profiles of LH during 120 h in HST (○) and SOC as well as UOC gilts (●) as compared with those in sesame oil injected controls (△).

Table 2
Effect of hypophyseal stalk transection on pituitary, thyroid and adrenal glands in ovariectomized gilts

		HST		SOC and UOC[a]	
Gland	Units	*N*	Weight	*N*	Weight
Pituitary	(mg)	8	228 ± 8.2*	10	332 ± 13.4
Thyroid	(g)	7	11.7 ± 1.0	10	13.2 ± 1.1
Adrenal	(g)	7	4.3 ± 0.3	8	5.7 ± 0.5

[a] Control equals pooled values for sham-operated and unoperated control gilts because no differences were detected in ANOVA between these groups ($P > 0.05$).

* $P < 0.05$.

weights remained similar ($P > 0.05$) in HST and controls. Gross examination of pituitary glands from HST gilts revealed tissue necrosis in the anterior one-third of these glands, immediately ventral to the severed end of the stalk; the remainder of the gland appeared normal. Coronal histological sections of the middle one-third of the gland from HST gilts revealed viable secretory parenchyma comparable to coronal sections of pituitary from controls (Fig. 4). Differential staining of adenohypophysis from HST gilts revealed viable appearing acidophils, basophils and chromophobes similar to SOC and UOC. Although adrenal gland weights were similar in HST and control gilts, coronal sections of adrenal revealed cortical cells with less cytoplasm compared with controls (Table 2, Fig. 5). Thyroid gland weights in HST were similar to controls (Table 2), but thyroid histology from HST gilts revealed cuboidal epithelial cells and accumulation of acidophilic colloid in large follicles compared with normal columnar epithelial cells and smaller follicles with basophilic colloid in controls (Fig. 6).

3.3. Effect of pulsatile LHRH infusion and E_2-17β treatment on LH secretion

In the third study, LH secretion in response to 2 μg of LHRH i.v. was greater ($P < 0.01$) in ovariectomized, control gilts than in gilts that had received hourly LHRH for 48 h (Fig. 7A). The overall means were 2.9 versus 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 i.m. injections of E_2-17β did not differ ($P > 0.10$, Fig. 9A). Clearly, the negative response to the first E_2-17β injection was followed by an increase in serum LH concentrations after the second E_2-17β injection. After 7 days of hourly LHRH infusions and the E_2-17β treatment, LH concentrations were similar in the two groups, but the response to an i.v. 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 (Fig. 7B).

3.4. Effect of pulsatile LHRH infusion and E_2-17β treatment on LH secretion after hypophyseal stalk transection

One HST gilt died in the fourth study, and in another the nylon disc realigned allowing for partial reconnection between the pituitary and hypothalamus. Data for this incomplete HST gilt were excluded from statistical evaluation of the data but are presented in the

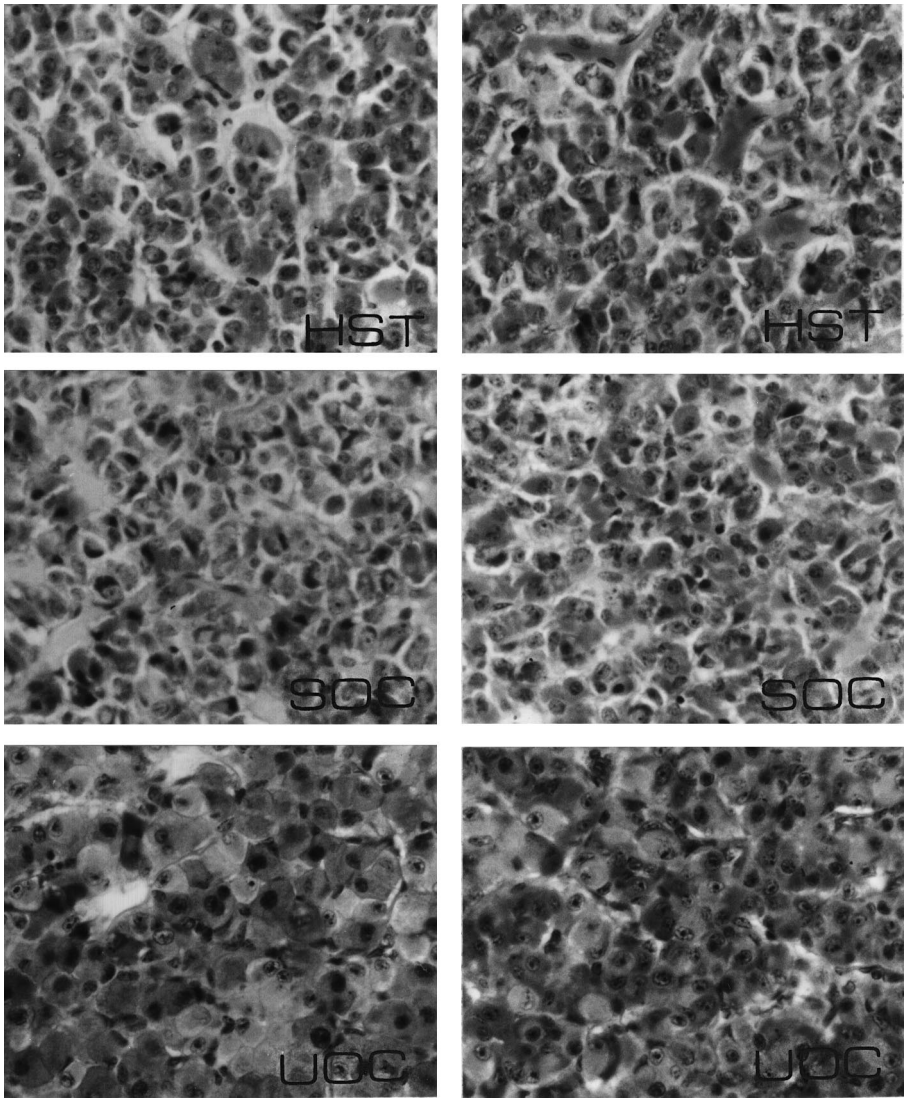

Fig. 4. Photomicrographs of adenohypophysis at sacrifice on day 9 in (a) hypophyseal stalk-transected (HST); (b) sham-operated (SOC) and (c) unoperated control (UOC) gilts. These histological cross sections are from the middle one-third of the anteromedial basophilic part of the adenohypophysis. The sections were stained differentially with performic acid-Alcian blue-periodic acid-(PAS)-Schiff-orange G. Pituitary cell types were identified; blue Alcian blue-positive PAS-negative basophils (associated with δ cells, thyrotrophs), violet to purple Alcian blue-positive PAS-positive basophils and rose Alcian blue-negative PAS-positive basophils (associated with β and γ cells, gonadotrophs), yellow to orange G-positive acidophils (associated with adrenocorticophs and somatotrophs), red to brown acidophils (carminophils associated with lactotrophs) and chromophobes. Light cells, some of which may be chromophobes, stain faint pink with PAS. An abundance of basophils (gonadotrophs and thyrotrophs) with a large cytoplasmic component was evident in anteromedial regions of the adenohypophysis in HST gilts and comparable to those in SOC and UOC. Normal appearing acidophils were dispersed in anteromedial regions of the adenohypophysis in stalk-transected and control gilts. Light-staining chromophobes were found in this region of the gland in approximately equal distributions in HST, SOC and UOC. Histological sections indicated survival of adenohypophyseal cells 9 days after HST, with a population of basophils near that found in SOC and UOC ($\times$400).

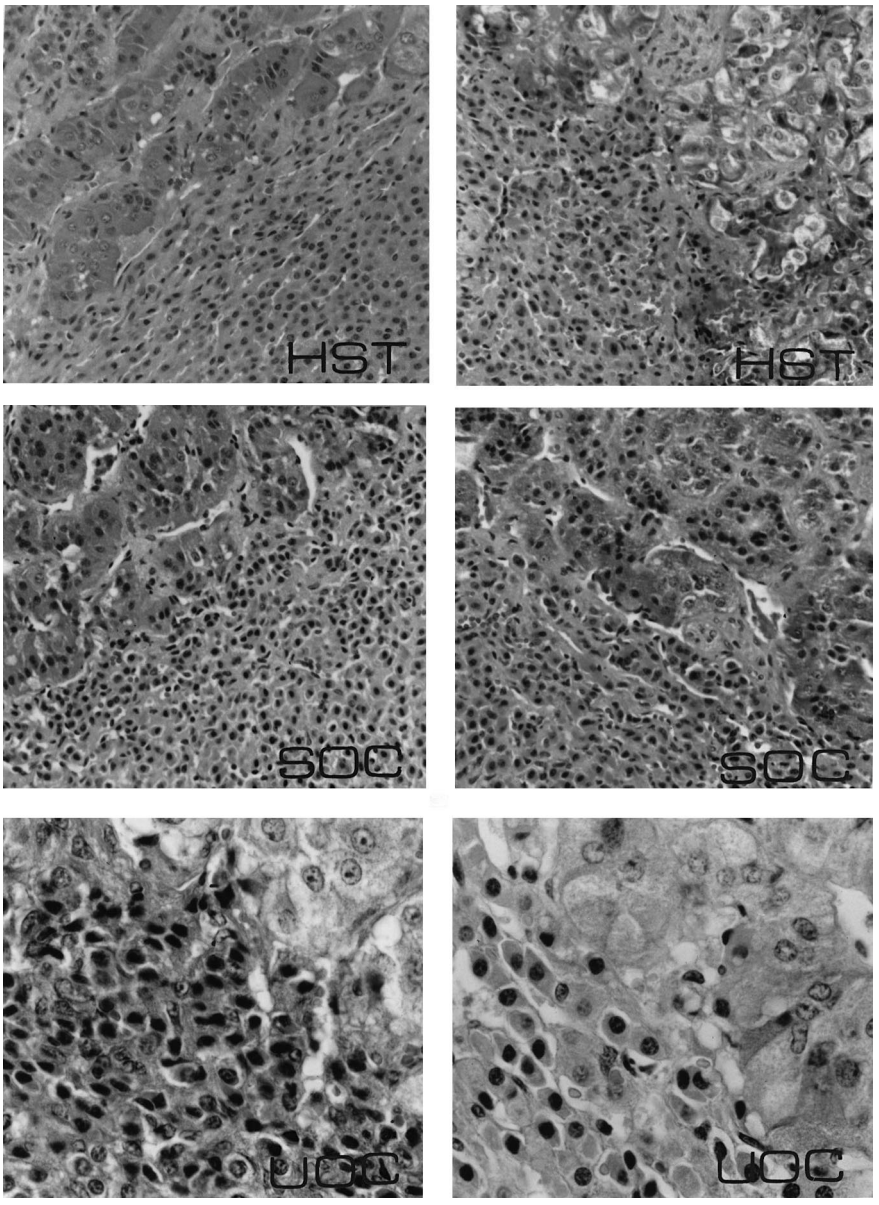

Fig. 5. Photomicrographs of coronal sections of adrenal cortical and medullary zones in HST, SOC and UOC gilts. After HST widths of adrenal cortex and medulla remained similar to controls, but cells in the cortex were less numerous and contained less cytoplasm (×360).

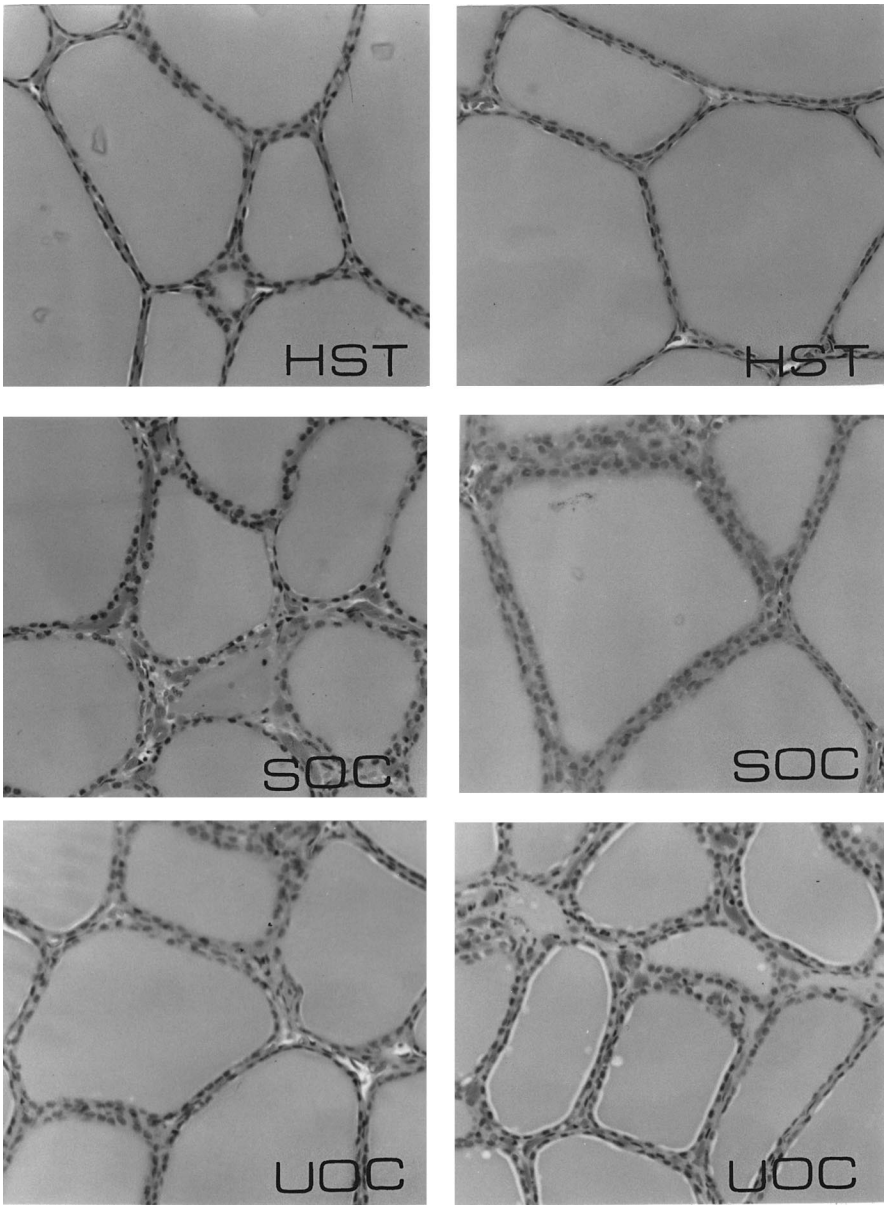

Fig. 6. Photomicrographs of thyroid glands from HST, SOC and UOC gilts. In HST, abundant large follicles were filled with acidophilic colloid and lined with cuboidal epithelial cells with little cytoplasm. In SOC and UOC, vacuoles are evident at periphery of follicles, colloid stained (Mallory triple stain) predominantly basophilic, and columnar epithelial cells with abundant cytoplasm lined the follicle wall (×360).

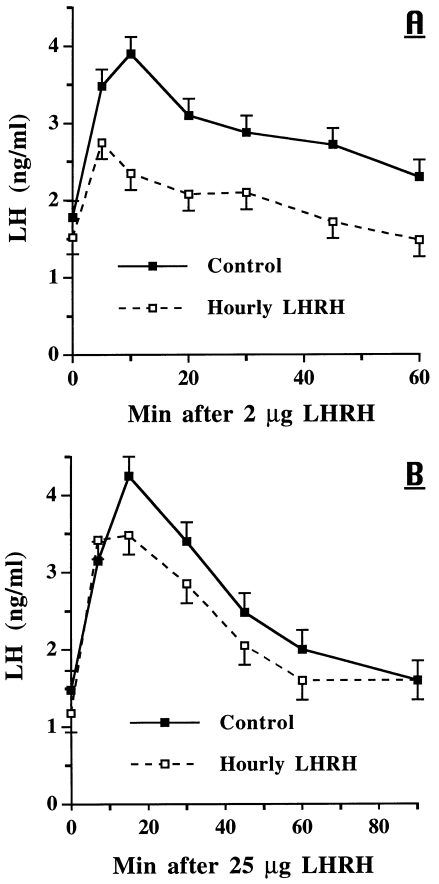

Fig. 7. Response to LHRH i.v. in ovariectomized gilts. Treatment groups were controls ($N = 4$) infused hourly with diluent and gilts ($N = 4$) infused hourly with 2 μg LHRH. (**A**) The response to 2 μg LHRH i.v. 48 h after hourly infusions began; group: $P < 0.01$; time: $P < 0.01$; group × time: $P < 0.08$. (**B**) Response in these same gilts to 25 μg LHRH i.v. on day 7 after initiation of hourly infusions combined with E_2 (20 μg/kg BW) i.m. on days 2 and 4; group: $P < 0.01$; time: $P < 0.01$; group × time: $P > 0.10$.

figures to document the marked differences in LH secretion relative to complete HST. Concentrations of LH were less ($P < 0.01$) in the four HST gilts that had received 48 h of hourly LHRH than in UOC (Fig. 8A). 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 (Fig. 9B), but after the second injection of E_2-17β, LH concentrations increased in UOC's and were greater than in LHRH-infused gilts from 72–96 h after initial E_2-17β ($P < 0.05$). The time by group interaction approached significance ($P < 0.06$). When these gilts were given a 25 μg bolus of LHRH at the end of the study, the response was greater ($P < 0.05$; Fig. 8B) in UOC's, and the time by group interaction was significant ($P < 0.01$). For the one gilt that was classified as an incomplete HST, her response to LHRH on days 0 and 7 more

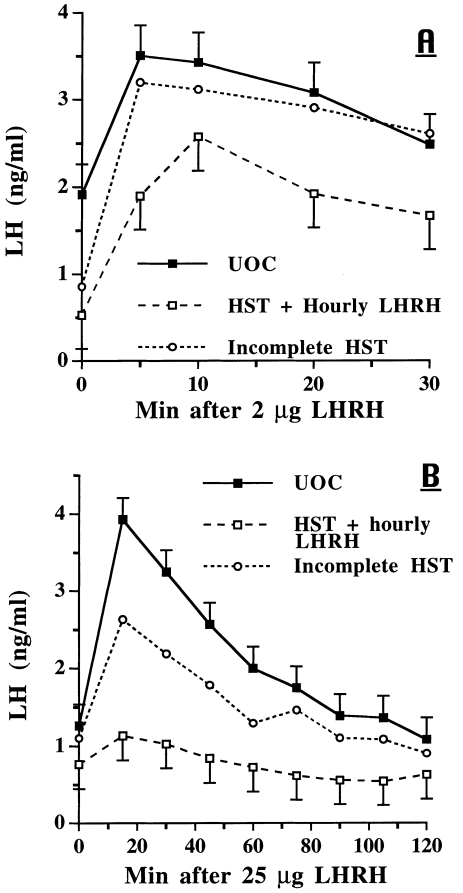

Fig. 8. Response to LHRH i.v. in ovariectomized gilts. Treatment groups were UOC ($N = 5$) and HST gilts ($N = 4$) infused hourly with 2 μg LHRH. (**A**) The response to 2 μg LHRH i.v. 48 h after hourly infusions began; group: $P < 0.03$; time: $P < 0.01$; group × time: $P > 0.10$. (**B**) Response in the same gilts to 25 μg LHRH i.v. on day 7 after initiation of hourly infusions combined with E_2 (20 μg/kg BW) i.m. on days 2 and 4; group: $P < 0.05$; time: $P < 0.01$; group × time: $P < 0.01$. Data from one gilt classified as an incomplete HST, due to misalignment of the nylon disc, are presented for comparison only.

closely resemble UOC than HST gilts (Fig. 7). The response of this gilt to hourly LHRH plus the first E_2-17β injection produced a much shorter interval for the negative effect of E_2-17β, but after the second E_2-17β injection, the LH response more closely mimicked that observed in the UOC's (Fig. 9B).

4. Discussion

The main findings from these studies 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

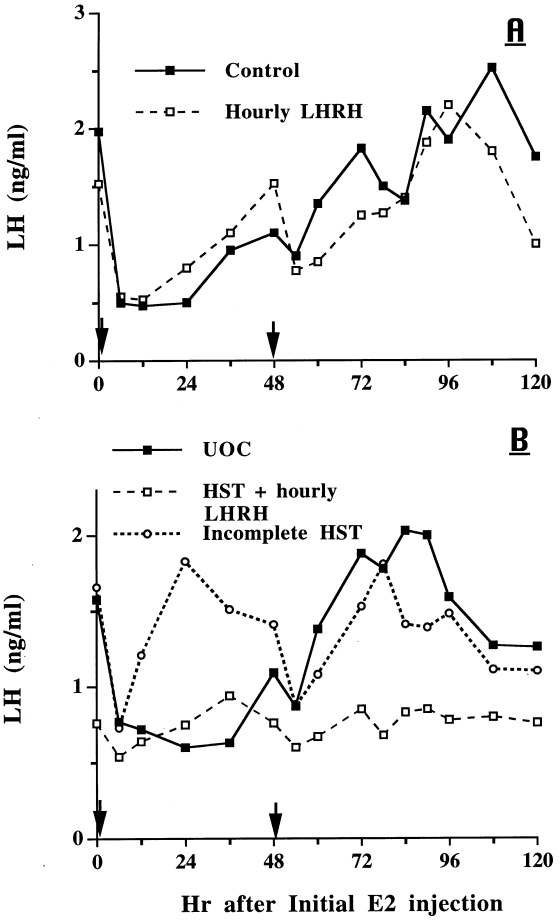

Fig. 9. Response to E_2-17β (20 μg/kg BW; arrows designate time of i.m. injection) in ovariectomized gilts. (**A**) Treatment groups were controls infused hourly with diluent and gilts infused hourly with 2 μg LHRH; group: $P > 0.10$; time: $P < 0.01$; group × time: $P > 0.10$. (**B**) Treatment groups were HST gilts that received hourly infusion of 2 μg LHRH and UOC; group: $P < 0.01$; time: $P < 0.01$; group × time: $P < 0.06$. Data from one gilt classified as an incomplete HST, due to misalignment of the nylon disc, are presented for comparison only.

(3) pulsatile LH secretion occurs in ovariectomized gilts in response to hypothalamic stimulation. These studies established that a single i.m. 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. This dosage of E_2-17β produced circulating concentration of E_2-17β six-fold greater than those reported by Kesner et al. (1987) in gilts treated with estradiol benzoate. Additionally, two injections of this dosage of E_2-17β 48 h apart produced a similar negative followed by positive response in LH secretion. However, in HST gilts, estrogen treatment elicited a detectable 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 and

conclusions of Kesner et al. (1987, 1989) and Stickan et al. (1999) that estrogen manifests its positive feedback on LH secretion primarily through the central nervous system, and estrogen at excessively elevated concentrations is no more effective than concentrations observed during the estrous cycle.

These results in gilts contrast observations in female rhesus monkeys subjected to HST during the follicular phase of the menstrual cycle (Ferin et al., 1979) or in ovariectomized rhesus monkeys in which the hypophysiotropic area was destroyed and LH secretion re-established by pulsatile infusion of LHRH (Nakai et al., 1978). Although exogenous estrogen augments pituitary responsiveness and induces LH secretion presumably by hypothalamic regulation in rhesus monkeys, the impact of estrogen on adenohypophyseal function likely reflects action of this steroid at both of these loci (Knobil, 1980, 1981). It seems that LHRH has a permissive rather than causative role in controlling LH secretion in rhesus monkeys (Knobil et al., 1980). Estrogen inputs directed at adenohypophyseal loci are sufficient to explain a controlling influence of the steroid on LH secretion in the presence of exogenous LHRH. Exogenous estradiol caused a two-fold increase in adenohypophyseal high-affinity LHRH receptors in ovariectomized cynomolgus macaques (Adams et al., 1981). Although LH release in response to exogenous LHRH is initially suppressed by estradiol, pituitary LHRH receptors correlate positively with estrogen-induced release of LH only during the phase of potentiated response. Thus, estrogenic augmentation (positive feedback) of pituitary responsiveness in monkeys seems to be mediated by an increase in the number of LHRH receptors on the surface of gonadotrophs.

Administration of central nervous system depressants to female pigs and rats (Kesner et al., 1987; Kimura et al., 1995), electro-lesion of the hypophysiotropic area in female rhesus monkeys (Plant et al., 1978) and ewes (Jackson et al., 1978), or interruption of hypophyseal blood circulation in female rhesus monkeys (Ferin et al., 1974), beef calves (Anderson et al., 1981) 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 adenohypophysis (Hotchkiss and Knobil, 1994; Terasawa et al., 1999).

In ovariectomized prepubertal gilts, neural stimuli originating or traversing the neural areas rostral to the median eminence are required for LH secretion (Molina et al., 1986). 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 min 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. Because complete disconnection of the medial basal hypothalamus from the remainder of the central nervous system did not abolish pulsatile and surge secretion of LH in rhesus monkeys, the preoptic area was not involved in the generation of the preovulatory LH surge (Hotchkiss and Knobil, 1994). In ovariectomized rats, pulsatile LH secretion was

maintained after complete deafferentation of the medial basal hypothalamus (Blake and Sawyer, 1974), as in rhesus monkeys.

In ovariectomized ewes, positive feedback action of estrogen is mediated via efferent connections of suprachiasmatic nuclei (SCh) to the medial basal hypothalamus (MBH) (Jackson et al., 1978). Interruption of neural signals from the MBH fails to alter LH secretion. In the rat, hypothalamic control of tonic LH secretion resides within the MBH, and the SCh seems necessary for the preovulatory LH surge (Halász and Pupp, 1965; Halász and Gorski, 1967; Blake, 1977). Estradiol increases the response of the adenohypophysis to exogenous LHRH (Vilchez-Martinez et al., 1974; Aiyer et al., 1974). Estradiol implants in the preoptic area (POA), but not the SCh, elicit LH release (Kalra and McCann, 1975). Estradiol also increases the amount of LHRH released after POA, but not MBH, stimulation (Sherwood et al., 1976). Thus, in the rat, LHRH can act directly on the pituitary to mediate LH secretion, whereas in the rhesus monkey, LHRH has more than a permissive action on LH secretion. In ovariectomized gilts in the present study, the positive feedback action of estrogen required mediation within the brain, and thus, pituitary secretion of LH in response to estrogen requires an increase in LHRH.

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, and E_2-17β may exert its actions on these isolated gonadotrophs. In this study, E_2-17β caused a 60% 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.

That E_2-17β could have affected LH secretion patterns in HST gilts because of surgical trauma and a partly interrupted blood supply to the pituitary is untenable. In gilts subjected to cranial sham operation, surgical trauma was similar to that in HST animals, with the exception of the severance of the hypophyseal stalk and insertion of the nylon disc; yet these sham-operated gilts responded to exogenous E_2-17β in manner similar to that in unoperated controls. Furthermore, estrogen induced a positive increase in LH secretion in the one HST gilt in which the nylon disc allowed partial reconnection of the hypothalamic stalk. Although localized areas of necrosis of the pituitary gland were evident in the stalk-transected gilts, histological examination of coronal sections revealed an abundance of normal basophilic cells in large areas of the adenohypophysis. Previous histological evidence in sexually mature gilts indicated that these basophilic cells remain viable several weeks after HST (Anderson et al., 1967). Cellular viability of the isolated pituitary gland is indicated by a two-fold increase in serum prolactin concentration throughout the postoperative period in HST compared with SOC gilts (Anderson et al., 1982).

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.

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