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Interactions of Estradiol and Progesterone on Pituitary Gonadotropin Secretion: Possible Sites and Mechanisms of Action

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ABSTRACT

The effects of estradiol (E_2) alone or with progesterone (P) on spontaneous or LHRH-induced releases of LH/FSH were examined 14–19 days after ovariectomy of rats. At 14 days, a Silastic E_2 capsule was implanted s.c. (Day 0) and 72 h later (Day 3) 2 groups of rats received either sesame oil or progesterone (2 mg) s.c. Hourly blood samples were collected sequentially from 1200–1800 h on Day 3 and again at 0900 h and 1600 h on Days 4 and 5 (96 and 120 h post E_2). Control groups included proestrous and untreated ovariectomized rats. Based on the data obtained we concluded that: a) Daily LH but not necessarily FSH surges occur on Days 2, 3, 4 and 5 but not on the day following E_2 treatment (Day 1). b) The responsiveness of the pituitary gland to 250 ng LHRH is equal in both E_2-treated (Day 3) and proestrous rats. However, the magnitude of the spontaneous LH surge in E_2-treated rats (Day 3), was 60% less than in normal proestrous animals. Thus, E_2 seems to reduce the amount of endogenous LHRH released on Day 3. c) Progesterone, given at 0900 h to E_2-treated rats (Day 3), resulted in afternoon LH and FSH surges that were earlier in their onset and of greater magnitude than those in E_2-treated or normal proestrous rats. However, no plasma LH increases occurred in E_2P-treated rats on Days 4 and 5 unlike in rats receiving only E_2; d) If spontaneous LH surges were blocked with Nembutal on Day 3, the magnitude of the LH surge on Day 4 in E_2-treated rats was 2-fold greater than that observed on Day 4 in rats which spontaneously released LH on Day 3. E_2P-treated rats which received Nembutal on Day 3 also had spontaneous LH surges on Day 4 which were less in concentration than those of E_2-treated animals on Day 4 or those obtained at 1600 h on Day 3 in the E_2P-treated group. e) Pituitary gland responsiveness to LHRH in E_2P-treated rats is equal to that of E_2-treated rats on Day 3. The responsiveness of the pituitary gland on Day 4 in E_2P-treated rats is significantly less than that in E_2-treated rats on Day 4; however, it is equal to the responsiveness found on Day 3 in E_2P-treated rats. Therefore, the stimulatory effect of P appears to be due to an increased release of endogenous LHRH. The failure of LH/FSH surges to occur on Day 4 in E_2P-treated rats may not be due to the loss of 24 h periodicity in the intrinsic neural signal or to a severe decline in the releasable LH pituitary pool. Rather it could be due to the failure of the hypothalamus to discharge sufficient LHRH to evoke LH/FSH surges presumably because of inadequate releasable LHRH reserves.

INTRODUCTION

The importance of the ovarian sex steroids in facilitating preovulatory releases of pituitary LH and FSH is well recognized. Originally, Everett (1948) observed that estrogen, when administered to 5-day cyclic rats on diestrus Day 2, advanced ovulation by 24 h. In ovariectomized (OVX) rats, preovulatory LH-like surges occur when single (Legan et al., 1975; Blake, 1977) or multiple (Caligaris et al., 1971a; Swerdloff et al., 1972) injections of estrogen are administered. Similarly, OVX rats that have received Silastic capsules containing estradiol-17β (E_2) exhibit daily afternoon LH surges (Henderson et al., 1977) for as long as 10 days (Legan and Karsch, 1975). Although these Silastic implants produce plasma E_2 levels which are within normal physiological ranges for proestrous rats (75–90 pg/ml), as little as 12 pg/ml of plasma E_2 will exert a positive feedback action on LH release in gonadectomized female rats (Goodman, 1978).

Progesterone also will advance or postpone

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the preovulatory discharge of LH by 24 h in cyclic female rats depending upon the hour and day of the cycle that it is given (Everett and Sawyer, 1949; Zeilmaker, 1966). Further, a biphasic action of P on LH release has been observed in E_2-primed OVX rats. In these preparations, greater plasma concentrations of LH are obtained in E_2P-treated animals than in rats which receive only estrogen. However, following LH surges in such preparations, further daily preovulatory LH-like releases are absent in contrast to animals receiving only estrogen (Caligaris et al., 1971b; Blake, 1977; Freeman et al., 1976). For example, P facilitates the release of LH (Brown-Grant and Naftolin, 1972) and FSH (Brown-Grant and Greig, 1975) when it is given on proestrous morning but it inhibits the delayed estrous LH surge if given on either proestrous morning (Blake, 1977) or afternoon in pentobarbital-treated rats (Redmond, 1968; Freeman et al., 1977). Mann and Barraclough (1973) have suggested that the diurnal release of P that occurs during the early morning hours of proestrus may be important in the timing and the full expression of the spontaneous preovulatory LH release on proestrous afternoon.

In this investigation, we have undertaken a longitudinal study on the effects of prolonged periods of elevated plasma E_2 in OVX rats on temporal patterns and plasma concentrations of LH and FSH. The synergistic and inhibitory actions of P in this preparation on the spontaneous secretion of gonadotropins and on pituitary gland responsiveness to LHRH were also evaluated. The results of these studies have been presented previously in abstract form (DePaolo and Barraclough, 1978).

MATERIALS AND METHODS

Adult female Sprague-Dawley rats (ARS/Madison, WI) weighing 220–260 g were maintained in a temperature (22–24°C) and light (lights on 0400–1800 h) controlled environment prior to use. Food and water were supplied *ad libitum*. Vaginal lavages were taken each day and only rats displaying at least 2 consecutive 4 day cycles were selected for use as proestrous controls. Other rats exhibiting 2 or more 5 day cycles were bilaterally ovariectomized (OVX) and were placed in various experimental groups at 14 days postcastration.

Sodium pentobarbital (Nembutal 31 mg/kg BW, i.p.) was given at 1140 h and LHRH (Beckman, lot no. D0420) was diluted with physiological saline (0.15 M NaCl) and was administered in a dose of 250 ng i.v. at 1300 h when appropriate. All capsules were implanted at 0900 h into OVX rats. The critical period in our laboratory, as defined by Everett et al. (1949) is from 1300–1500 h.

Plasma samples (0.6 cc) were obtained from jugular cannulae in all rats and physiological saline equal to the blood volume removed was reinjected i.v. after each collection period as previously described (DePaolo et al., 1979). The animals in which plasma samples were obtained over several days were returned to the light controlled room each day after blood removal.

Silastic capsules 15 mm in length (1.57 mm i.d., 3.18 mm o.d., Dow Corning) containing 5 mm of crystalline E_2 (Sigma) were constructed according to the method of Legan et al. (1975). Capsules were incubated in distilled water (22°C) for 60 min prior to implantation to minimize the transitory plasma estradiol peak that occurs following insertion of nonincubated capsules. Three days after implanting empty or E_2 capsules (Day 3), either 200 μl of sesame oil or 2 mg of P (in oil) were injected s.c. at 0900 h. The experimental paradigm used for these experimental groups is illustrated in Fig. 1. Descriptions of the manipulations performed in the various experimental groups is provided with the results of the study.

Radioimmunoassays

Estradiol and progesterone. Plasma estradiol-17β concentrations were measured by radioimmunoassay using a specific antisera raised in sheep against E_2 (Wright et al., 1973) and a modification of the procedure of Wright et al. (1973) as previously described (DePaolo et al., 1979).

Plasma P concentrations were measured using a modification of the procedure of Thorneycroft and Stone (1972) which has been previously described (DePaolo et al., 1979) and an antibody (GDN #337) supplied by Dr. Gordon Niswender. The specificity of this antiserum has been reported previously (Smith et al., 1975).

LH and FSH. Luteinizing hormone concentrations in plasma samples were measured by the ovine-ovine radioimmunoassay procedure described by Niswender et al. (1968). Values are presented in terms of a rat pituitary standard prepared against the B160 standard supplied to us by Dr. V. L. Gay. This preparation has been shown to have a LH biological potency of 0.17 X NIH-LH-S1 as measured in the ovarian ascorbic acid depletion assay.

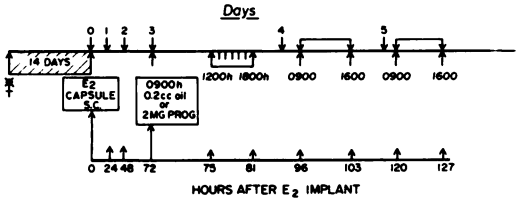

FIG. 1. Experimental paradigm employed in these studies. Rats were ovariectomized and 14 days later a Silastic E_2 capsule was implanted (s.c.) on Day 0. On Day 3 (72 h post-E_2) groups of rats received either sesame oil or P in oil (2 mg s.c.) at 0900 h. Rats were bled sequentially on Day 3 from 1200–1800 h and on Days 4 and 5 at 0900 h and 1600 h.

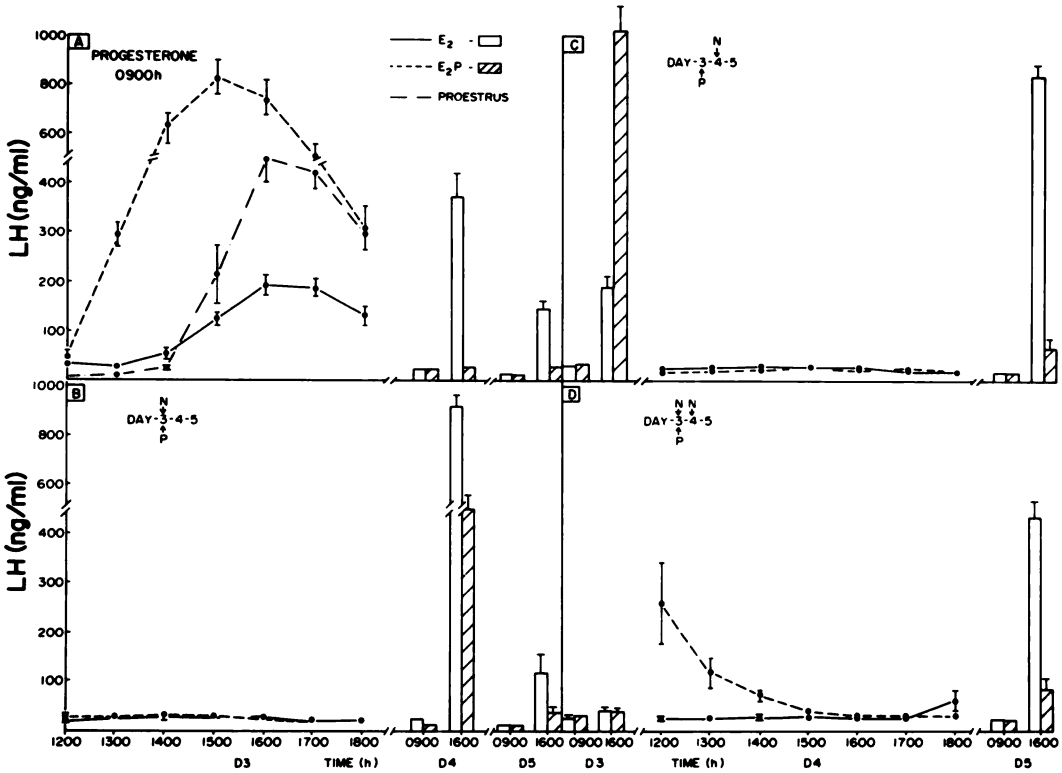

FIG. 2A. Temporal patterns and concentrations of LH which occurred in normal proestrous rats as compared with castrated E_2 or E_2P treated animals 3, 4 and 5 days after inserting a Silastic E_2 capsule. Spontaneous LH surges did not occur on Days 4 or 5 in E_2P treated rats.

B. The effects of Nembutal (N), administered at 1140 h to E_2 or E_2P treated rats. The blockade of spontaneous LH surges on Day 3 resulted in higher concentrations of LH being released at 1600 h on Day 4 in E_2-treated rats (compared with Fig. 2A, Day 4). Also, spontaneous LH surges occurred in E_2P treated rats on Day 4 but not on Day 5.

C. Effects of Nembutal administered on Day 4 at 1140 h, on spontaneous LH surges. The E_2 treated rats released significantly higher concentrations of LH on Day 5 than did rats shown in Fig. 2A, Day 5.

D. Effects of Nembutal, given on Days 3 and 4, on spontaneous LH surges on Days 3, 4 and 5. In E_2P treated rats, spontaneous LH surges apparently had begun prior to Nembutal treatment. Abbreviations in this and subsequent figures: E_2, estradiol-17β; P, progesterone; N, Nembutal. Vertical lines in this and subsequent figures represent SEM.

Concentrations of plasma FSH were measured by radioimmunoassay as described previously (Chappel and Barraclough, 1976). The values are expressed in terms of NIAMDD rat RP-1 (2.1 X NIH-FSH-S1). Statistical evaluations between and among groups were performed using Duncan's multiple range test.

RESULTS

LH in Proestrous, Estrous and Castrated Rats

Changes in plasma LH concentrations throughout the afternoon of proestrus (n = 6) and at 0900 h and 1600 h estrus are illustrated in Fig. 2A and Table 1. The administration of Nembutal at 1140 h (n = 5) effectively blocked the proestrous preovulatory LH surge; however, an LH surge occurred 24 h later (Table 1). Treatment of Nembutal-blocked proestrous rats (n = 7) with LHRH (250 ng i.v.) at 1300 h resulted in a significant rise in plasma LH by 5 min ($P<0.05$) and in peak concentrations at 60 min postinjection (Table 2).

Plasma LH levels were significantly elevated 17 days following ovariectomy of cyclic rats from basal diestrous levels of 8 ± 1ng/ml to 246 ± 56 ng/ml. Placement of empty Silastic capsules 14 days after ovariectomy did not significantly alter plasma LH concentrations in castrate rats 3 days later (Day 3).

TABLE 1. Plasma LH and FSH concentrations in proestrous (Pr) and estrous rats: effects of Nembutal (N).

H of day	LH (ng/ml) Normal proestrus	LH (ng/ml) Proestrus and Nembutal	LH (ng/ml) P< Pr vs Pr + N	FSH (ng/ml) Normal proestrus	FSH (ng/ml) Proestrus and Nembutal	FSH (ng/ml) P< Pr vs Pr + N
1200	8 ± 1[a]	4 ± 1	ns	175 ± 8	148 ± 30	ns
1300	11 ± 2	7 ± 1	ns	202 ± 7	151 ± 33	0.05
1400	27 ± 5	6 ± 1	0.01	272 ± 40	183 ± 11	0.01
1500	216 ± 59[c]	4 ± 1	0.01	362 ± 59[d]	208 ± 25	0.01
1600	469 ± 49[c]	5 ± 1	0.01	551 ± 55[c]	238 ± 20[d]	0.01
1700	444 ± 31[c]	4 ± 1	0.01	629 ± 42[c]	229 ± 10[d]	0.01
1800	300 ± 40[c]	4 ± 1	0.01	582 ± 31[c]	233 ± 21[d]	0.01
			Estrus			
0900	7 ± 1	3	ns	295 ± 21[c]	140 ± 4	0.01
1600	10 ± 1	286 ± 67[bc]	0.01	250 ± 25[c]	392 ± 22[bc]	0.01

[a]Mean ± SEM.

[b]$P<0.01$ compared to 0900 h estrus.

[c]$P<0.01$ compared with 1200 h proestrus.

[d]$P<0.05$ compared with 1200 h proestrus.

TABLE 2. Plasma LH and FSH concentrations in Nembutal blocked proestrous rats and in estrous rats after LHRH treatment. Mean ± SEM.

	Proestrus							Estrus	
	Min post-LHRH injection							H of day	
Group	0	5	15	30	60	90	120	0900	1600
LH (ng/ml)	9 ± 1	34 ± 4[b]	102 ± 12[a]	116 ± 17[a]	143 ± 22[a]	64 ± 9[a]	41 ± 13[b]	6 ± 1	48 ± 21
FSH (ng/ml)	168 ± 11	183 ± 11	207 ± 13	214 ± 21[b]	241 ± 19[a]	295 ± 44[a]	288 ± 24[a]	352 ± 25[a]	277 ± 31[a]

[a] $P<0.01$ compared with 0 time proestrus.
[b] $P<0.05$ compared with 0 time proestrus.

Plasma Steroid Concentrations after E_2 and P Treatment

Groups of steroid-treated castrate rats (n = 3–5) were decapitated at 0900 h, 1000 h or 1600 h on Day 0, at 0900 h or 1600 h on Day 3, or at 1600 h on Day 4 or Day 5 and plasma was assayed for E_2 and P concentrations.

The plasma concentration changes of E_2 and P with time, after implanting or injecting these steroids into OVX rats, are shown in Table 3. One hour after the insertion of an E_2 capsule into castrated rats (1000 h), plasma E_2 levels were greater than 1 ng/ml. Thereafter, E_2 levels in plasma declined precipitously within the next 5 h to reach values which were approximately 3 times greater than those observed on the morning of proestrus in our rat colony (82 pg/ml peak concentrations). Five days after capsule insertion, plasma E_2 levels were comparable to peak proestrous E_2 values.

The injection of P (2 mg, s.i.) elevated plasma concentrations of this steroid to 100 ng/ml which is in marked excess of the peak P levels obtained on proestrous afternoon in our colony (40 ng/ml). Thereafter, P in plasma declined to reach preinjection levels by 1600 h on Day 5.

LH in Steroid Treated Experimental Groups

Estradiol-17β capsules were inserted at 0900 h on Day 0 and jugular cannulae were inserted 24 h later (Day 1). This group of rats (n = 12) were injected 48 h later with sesame oil (Day 3) and were bled sequentially at hourly intervals from 1200–1800 h on Day 3 and again at 0900 h and 1600 h on Days 4 and 5 (96 and 120 h post-E_2 implantation). A separate group of castrated rats (n = 5) were bled sequentially at 1300 h, 1500 h and 1700 h on Days 1 and 2 after E_2 capsule placement.

When a Silastic capsule containing E_2 was implanted into OVX rats, at 0900 h on Day 0, significant afternoon elevations of LH did not commence until 54 h later. Plasma LH levels on Day 1 were 28 ± 2 ng/ml at 1300 h, 33 ± 4 ng/ml at 1500 h and 30 ± 5 ng/ml at 1700 h. On Day 2, the plasma LH values were 39 ± 7 ng/ml, 154 ± 23 ng/ml, 348 ± 54 ng/ml at 1300 h, 1500 h and 1700 h, respectively. The effects of E_2 on plasma LH concentrations throughout the afternoon of Day 3 and on morning and afternoon LH levels 4 and 5 days post E_2 implantation are shown in Fig. 2A. Three days

TABLE 3. Plasma E_2 and P concentrations in castrate steroid treated rats. Mean ± SEM.

Time (H and days)	E_2[a] (pg/ml)	E_2 in E_2P treated rats[b] (pg/ml)	P in oil treated E_2 castrates (ng/ml)	P in P treated E_2 castrates[d] (ng/ml)
0900 Day 0	15 ± 2	15 ± 1	...	...
1000 Day 0	>1000	>1000	...	...
1600 Day 0	236 ± 31	236 ± 25	...	...
0900 Day 3	220 ± 12	241 ± 20	1.5 ± 0.2	1.5 ± 0.1
1600 Day 3	216 ± 25	238 ± 41	1.8 ± 0.3	>100
1600 Day 4	135 ± 11	150 ± 10	1.6 ± 0.2	14.9 ± 1.8
1600 Day 5	119 ± 8	119 ± 10	2.5 ± 0.3	3.7 ± 0.4

[a]Plasma E_2, capsule implanted at 0900 h Day 0.

[b]Plasma E_2, capsule implanted at 0900 h Day 0, P injected at 0900 h Day 3.

[c]Plasma P in E_2 treated rats injected with sesame oil Day 3.

[d]Plasma P in E_2 treated rats injected with P on Day 3.

of E_2 treatment significantly reduced the high plasma LH concentrations in castrate rats to levels which were about 4-fold higher than those obtained in intact diestrous rats (33 ± 4 vs 8 ± 1 ng/ml). On the afternoon of Day 3 post-E_2-treatment, plasma LH rose significantly above basal levels at 1400 h ($P<0.05$). However, despite such animals having higher basal LH levels at 1200 h of Day 3, the magnitude of the LH surge at 1600 h was ∿60% less in the castrate E_2-treated rats than in normal proestrous animals (194 ± 21 vs 468 ± 49 ng/ml). The diminished release of LH in E_2 treated rats was apparent in each animal when compared with individual mean values in normal proestrous rats. In contrast, LH concentrations in E_2 treated rats at 1600 h on Day 4 were comparable to proestrous controls whereas plasma LH values at 1600 h on Day 5 were similar to those obtained at 1600 h on Day 3 (Fig. 2A).

Administration of P at 0900 h on Day 3 to E_2 treated rats (n = 11) did not reduce LH levels in castrate rats below those observed after E_2 treatment alone. However, P treatment resulted in afternoon LH surges on Day 3 that were earlier in their onset and of greater magnitude than the LH surges observed in control proestrous or in E_2 treated castrate rats (Fig. 2A). This facilitation of E_2-dependent LH surges by P was shortlived because no plasma increases in this gonadotropin occurred in E_2P treated rats on either Days 4 or 5 as contrasted to animals which received only E_2.

To determine whether this facilitating action of P on pituitary LH release is due to an increased release of endogenous LHRH or to an increased responsiveness of the pituitary gland to LHRH, the following experiment was performed. Both E_2 treated and E_2P treated rats were given Nembutal at 1140 h and LHRH (250 ng i.v.) at 1300 h on Day 3. Nembutal effectively blocked the LH surges on the afternoon of Day 3 in both E_2 treated (n = 9) and E_2P treated (n = 9) rats (Fig. 2B). In such animals, the magnitude of the LH surge at 1600 h on Day 4 in E_2 treated rats was ∿2-fold greater than that which would have occurred in E_2 treated animals which had not received Nembutal on Day 3 (compare Fig. 2B with 2A). The E_2P treated rats also exhibited LH surges on Day 4 but of significantly less magnitude ($P<0.01$) than either the E_2 treated group on Day 4 or the E_2P treated group at 1600 h on Day 3. Further, on Day 5, an LH surge was absent in the E_2P treated group but it still occurred in rats which received only E_2 (Fig. 2B).

Plasma LH was significantly elevated in Nembutal-blocked E_2 (n = 11) and E_2P treated rats (n = 11) within 5 min following the injection of LHRH ($P<0.01$) (Fig. 3). Peak plasma LH concentrations occurred at 30 min post-injection (as compared with 60 min in proestrous controls) and declined thereafter. Further, plasma LH values at 30 min were significantly greater ($P<0.01$) in E_2 treated vs E_2P treated rats (Fig. 3). As well, greater plasma LH concentrations were observed in E_2 or E_2P treated castrates after LHRH (600 ± 47 and 450 ± 41 ng/ml, respectively) than in Nembutal blocked proestrous rats (143 ± 22 ng/ml) (Table 2). Interestingly, E_2P treated as well as

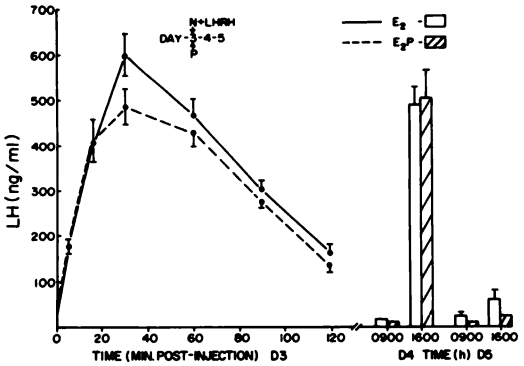

FIG. 3. Induction of LH release by LHRH (250 ng) in Nembutal blocked E_2 and E_2P treated rats on Day 3. Pituitary responses in both groups were similar on Day 3; 1600 h LH concentrations on Day 4 in these groups were identical.

E_2 treated rats which received LHRH on Day 3 had spontaneous LH surges on Day 4. Further, the magnitude of the LH surge on Day 4 was similar in E_2 and E_2P treated rats (Fig. 3). Plasma LH values in E_2 treated rats at 1600 h on Day 5 did not differ significantly from the 0900 h values.

If spontaneous gonadotropin surges in the steroid treated rats were allowed to occur on Day 3 but were blocked by Nembutal on Day 4, plasma LH concentrations at 1600 h on Day 5 in E_2 treated rats (n = 5) were significantly greater ($P<0.01$) in magnitude than normally would have occurred on this day (compare Day 5 in Fig. 2A with 2C). Also, plasma LH concentrations in E_2P treated rats (n = 6) at 1600 h on Day 5 were significantly higher than the values at 0900 h on this day.

The injection of LHRH in rats which had spontaneous gonadotropin surges blocked by Nembutal on Day 4 (Fig. 2C) resulted in significant elevations of LH in both E_2 treated (n = 6) and E_2P treated (n = 6) rats (Fig. 4A). Not only were greater peak concentrations of LH obtained after an LHRH pulse injection in E_2 as compared to E_2P treated animals on Day 4, but the peak plasma LH values obtained in E_2 treated rats were also of greater magnitude than those observed after LHRH treatment of Nembutal blocked E_2 treated rats on Day 3 (compare Fig. 4A with Fig. 3). Responses of E_2P treated rats to LHRH were similar on both Days 3 and 4 (compare Fig. 3 with Fig. 4A). There were no significant differences in plasma LH concentrations at 1600 h on Day 5 in E_2 treated rats regardless of whether LHRH had

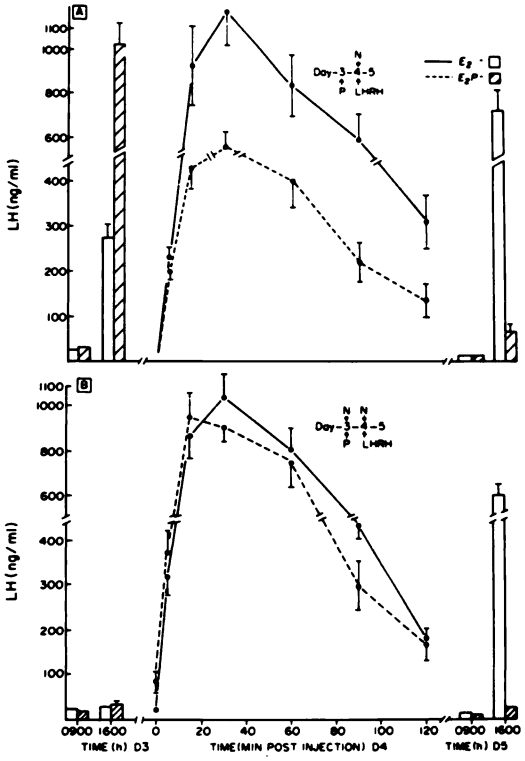

FIG. 4A. Effects of LHRH on LH secretion in E_2 and E_2P treated rats which received Nembutal at 1140 h on Day 4. These animals had spontaneous LH surges on Day 3. Greater concentrations of LH were released in E_2 as compared with E_2P treated animals.

B. Effects of LHRH on LH secretion in E_2 and E_2P treated rats which received Nembutal on both Days 3 and 4. These animals did not have spontaneous LH surges on Day 3. The LH response in both groups was similar.

or had not been injected on Day 4.

If preovulatory LH surges were blocked on Days 3 and 4 by Nembutal, the spontaneous release of this gonadotropin was significantly greater at 1600 h on Day 5 in E_2 treated rats (n = 6). However, we were unsuccessful in completely preventing the spontaneous release of LH in both E_2 and E_2P treated rats (n = 5) on Day 4 (Fig. 2D). In the E_2 treated group, plasma LH levels were significantly higher ($P<0.01$) at 1800 h as compared with 1700 h values. The elevated plasma LH values observed at 1200 h in E_2P treated rats on Day 4 suggest that the spontaneous surge had begun prior to the administration of Nembutal. The injection of LHRH on Day 4 to E_2 treated (n = 7) or E_2P treated (n = 6) rats which received Nembutal on both Days 3 and 4 resulted in LH

surges of equal magnitude and duration (Fig. 4B).

FSH in Proestrous and Castrated Rats

The plasma concentration changes in FSH throughout the afternoon of proestrus are presented in Table 1. The administration of Nembutal to proestrous rats at 1140 h failed to suppress completely the afternoon rise in FSH because this gonadotropin was significantly ($P<0.05$) elevated above basal concentrations at 1600 h. In this same group of rats, a delayed rise in plasma FSH occurred on estrus. After the administration of LHRH at 1300 h to Nembutal treated proestrous rats (Table 2), the first significant rise ($P<0.05$) in FSH occurred at 30 min postinjection with peak concentrations being reached at 90 min. At 120 min, plasma FSH concentrations were significantly higher ($P<0.01$) than those measured at 1500 h in Nembutal treated rats.

Seventeen days after ovariectomy, plasma FSH levels were ∿10-fold higher than those observed in intact diestrous rats (2148 ± 235 vs 201 ± 7 ng/ml).

FSH in Steroid Treated Experimental Groups

Three days after the insertion of an E_2 capsule, plasma FSH levels were suppressed significantly below the values obtained at this time in nontreated castrates (Fig. 5). However, in contrast to an LH rise which occurred on the third afternoon after E_2 treatment, plasma FSH in these animals remained unchanged. Plasma FSH concentrations were significantly elevated at 1600 h on Day 4 when compared with the 0900 h values obtained on this day. No significant changes in plasma FSH occurred on Day 5.

When progesterone was administered to a group of E_2 treated rats at 0900 h on Day 3 (Fig. 5), basal concentrations of plasma FSH were not significantly altered below those values obtained in rats which received only E_2. However, in contrast to rats which received only E_2, E_2P treated rats had an FSH surge during the afternoon of Day 3. The first significant rise ($P<0.01$) in plasma FSH occurred at 1400 h and peak concentrations were reached by 1600 h. The temporal patterns of FSH in plasma in the E_2P treated rats closely resembled those observed on proestrous afternoon,

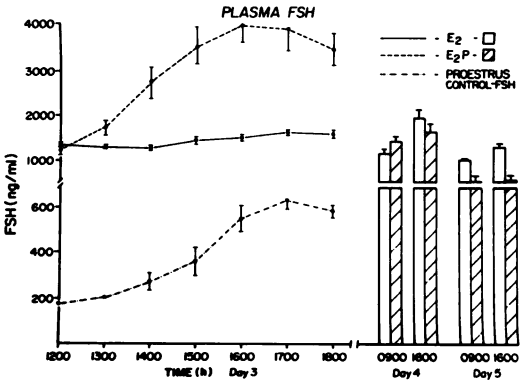

FIG. 5. Temporal patterns and concentrations of plasma FSH which occurred in normal proestrous rats as compared to castrated E_2 or E_2P treated rats on Days 3, 4 and 5. Spontaneous FSH surges occurred on Day 4 but not on Days 3 or 5 in rats receiving only estrogen. Plasma FSH concentrations in E_2P treated rats were not significantly different at 1600 h when compared with concentrations at 0900 h on Days 4 or 5.

although the onset of the surge occurred 1 h earlier in the steroid treated rats. Further, the peak FSH plasma concentrations obtained at 1600 h on Day 3 were significantly higher ($P<0.01$) than the FSH levels obtained in castrated rats (3976 ± 358 vs 2148 ± 235 ng/ml). No significant increases in plasma FSH occurred on either Days 4 or 5.

Nembutal, when administered on Day 3, blocked the preovulatory FSH surge which normally would have occurred that afternoon in E_2P treated rats (Table 4). However, Nembutal was unable to maintain plasma FSH at basal concentrations. Rather, significant increases ($P<0.05$) in FSH were observed by 1500 h in E_2P treated rats and by 1700 h in E_2 treated rats. Further, significant increases ($P<0.01$) in plasma FSH levels, as in LH levels, were observed at 1600 on Day 4 in both E_2 and E_2P treated groups.

The effects of LHRH on FSH release in E_2 or E_2P treated castrate rats given Nembutal at 1140 h on Day 3 are shown in Table 5. In both steroid treated groups, LHRH induced a significant rise ($P<0.01$) in plasma FSH within 15 min after injection. This gonadotropin was still elevated at 120 min post-LHRH injection but had declined to basal levels by 0900 h of Day 4. A second significant rise in plasma FSH occurred on Day 4 at 1600 h in E_2 and E_2P treated rats. Statistical comparisons at various times post-LHRH treatment between the

TABLE 4. FSH plasma levels in E_2 or E_2P treated castrate rats given Nembutal (N) on Day 3. Mean ± SEM.

	Day 3							Day 4		Day 5	
	H of day							H		H	
Group	1200	1300	1400	1500	1600	1700	1800	0900	1600	0900	1600
E_2 + N	855 ± 25	801 ± 26	864 ± 90	841 ± 33	954 ± 55	1053 ± 88[a]	1046 ± 91[a]	890 ± 45	2192 ± 176[b]	1005 ± 101	1229 ± 90
E_2P + N	913 ± 64	1071 ± 89	974 ± 127	1136 ± 88[a]	1133 ± 54[a]	1197 ± 62[a]	1299 ± 58[a]	1079 ± 63	2055 ± 191[b]	1010 ± 101	1031 ± 70
E_2 + N vs E_2P + N	ns	0.05	ns	0.05	ns	ns	0.05	ns	ns	ns	0.05

[a] $P<0.05$ compared with 1200 h Day 3.

[b] $P<0.01$ compared with 0900 Day 4.

TABLE 5. Plasma FSH levels in E_2 and E_2P treated castrates given Nembutal (N) and LHRH on Day 3. Mean ± SEM.

	FSH (ng/ml plasma)										
	Day 3							Day 4		Day 5	
	Min Post-LHRH injection							H of day			
Group	0	5	15	30	60	90	120	0900	1600	0900	1600
E_2 + N and LHRH Day 3	853 ± 46	970 ± 49	1223 ± 62[a]	1394 ± 68*	1516 ± 100[a]	1460 ± 90[a]	1331 ± 80[a]	961 ± 52	1737 ± 129[b]	911 ± 40	1115 ± 59
E_2P + N and LHRH Day 3	965 ± 41	1044 ± 44	1325 ± 56[a]	1578 ± 108*	1610 ± 98[a]	1549 ± 76[a]	1503 ± 56[a]	1152 ± 40	2157 ± 148[b]	1080 ± 90	1043 ± 76
E_2 + N vs E_2P + N	ns	ns	ns	ns	ns	ns	ns	ns	0.05	ns	ns

[a] $P<0.01$ compared with 0 time Day 3.

[b] $P<0.01$ compared with 0900 h Day 4.

various groups are shown in Table 5.

Administration of Nembutal on Day 4 or on Days 3 and 4 blocked the FSH rises which would have normally occurred in E_2 or E_2P treated rats. In other groups, injections of LHRH elicited comparable increases in plasma FSH concentrations in both E_2 and E_2P treated rats.

DISCUSSION

The treatment of longterm ovariectomized rats with estrogen results in daily afternoon surges of LH which may begin within 24 h (Legan et al., 1975) or 48 h (Henderson et al., 1977; Blake, 1977) and reoccur every 24 h for as long as 6 days after steroid exposure (Blake, 1977). The present studies confirm and extend some of these observations. However, unlike a previous report (Legan and Karsch, 1975), our results showed that implants of Silastic capsules do not produce steady state plasma E_2 concentrations. Rather, plasma E_2 declines with time from 236 ± 31 pg/ml within 7 h after capsule insertion to 119 ± 8 pg/ml by Day 5. In spite of these changing plasma E_2 concentrations and the fact that they are considerably higher than normal proestrous values (82 pg/ml), daily LH surges occur. This suggests that the preopticohypophysiotropic system is responsive to a wide variety of plasma E_2 concentrations. Alternatively, once the available neuronal and pituitary estrogen receptors are saturated, further estrogen in circulation is without physiological effect.

In the present studies we observed that the magnitude of the LH surge in E_2 treated rats on Day 3 was significantly less than that which occurred normally on the afternoon of proestrus. Further, the resultant peak concentrations in plasma LH after LHRH were considerably higher in Nembutal treated E_2 and E_2P treated vs proestrous rats (compare Table 2 with Fig. 3). These responses suggest either that greater pituitary responsiveness to LHRH (i.e., competence to release LH) occurs in E_2 or E_2P treated vs normal proestrous rats or, more likely, that a larger releasable pituitary LH pool exists in steroid treated castrates than is present in the proestrous pituitary gland. It is well recognized that pituitary concentrations of LH are increased in castrated and in E_2 treated castrated rats (Ramirez and McCann, 1963; Barraclough and Haller, 1970). Seemingly, it is these increased pituitary LH reserves that are released after LHRH administration. Further, in spite of a greater releasable pool of pituitary LH in E_2 treated OVX rats, spontaneous LH surges on Day 3 are less in magnitude than those surges which occur in either normal proestrous or E_2P treated rats. Similar responses have been obtained in acutely castrated rats given E_2 and LHRH (Baldwin et al., 1975). We conclude that less endogenous LHRH is discharged in the E_2 treated rats, perhaps as the consequence of a negative feedback effect of E_2 within the hypothalamus or because of reduced hypothalamic reserves of LHRH.

Alternatively, pituitary responsiveness to LHRH may be less in E_2 treated vs normal proestrous rats. Previous studies in this laboratory have shown that the magnitude of the proestrous preovulatory gonadotropin surge depends on rapidly declining plasma E_2 levels which accompany the rise in plasma LH. If this normal E_2 decline is prevented, then pituitary responsiveness to LHRH is blunted (Turgeon and Barraclough, 1977). Since plasma LH surges do not affect plasma estrogen concentrations in castrated rats bearing Silastic E_2 capsules, pituitary responsiveness to endogenously released LHRH on Day 3 also could be attenuated.

At 1600 h on Day 4 plasma LH concentrations in E_2 treated rats were significantly higher than those obtained on Day 3; if LH release on Day 3 was blocked with Nembutal even greater concentrations of this gonadotropin were released spontaneously into plasma (compare Fig. 2A with Fig. 2B). Further, when plasma LH levels were high on Day 4, they were reduced on Day 5 to the values found on Day 3 at 1600 h. Blockade by Nembutal of LH release on Day 4 resulted in markedly elevated concentrations of this gonadotropin on Day 5.

The increased plasma LH levels that are released spontaneously on Day 4 could be due to: a) increased pituitary LH reserves, b) increased release of endogenous LHRH, c) increased pituitary responsiveness to LHRH (competence to release LH) or, d) any combination of the above. To test some of these possibilities, we blocked the spontaneous LH surge by Nembutal on Day 4 and LHRH was administered. Greater peak plasma LH concentrations were obtained after LHRH treatment on Day 4 than on Day 3. These results imply that an increase in the releasable pool of pituitary LH has occurred over this 24 h interval, which when released could account for the elevated plasma LH concentrations on Day

4. However, other data obtained in these studies suggest that while an increase in the pool size of releasable pituitary LH occurs it may not be of prime importance. If greater depletions of pituitary LH reserve are induced by LHRH on Day 3 than normally occur spontaneously (compare Fig. 3 with Fig. 2A), plasma LH concentrations at 1600 h on Day 4 in both groups are similar. Further, peak plasma LH levels obtained after treatment of Nembutal blocked rats with LHRH on Day 4 are almost identical regardless of whether spontaneous LH surges occurred 24 h earlier (Fig. 4A) or they were blocked with pentobarbital (Fig. 4B) which should increase LH pool sizes.

From these observations it is tempting to conclude that greater concentrations of endogenous LHRH are released on Day 4 than on Day 3. If LHRH release is blocked with pentobarbital on Day 3, then the increased hypothalamic reserve of this hormone is available for release on Day 4. Similarly, blockade of LHRH secretion on Day 4 would make a larger releasable pool of this decapeptide available on Day 5. Thus, when the neural signal for LHRH release is presented each day, plasma LH concentrations of greater or lesser magnitude would occur. In support of this concept are the observations that LHRH content in the medial basal hypothalamus is markedly increased following Nembutal treatment of E_2 treated OVX rats (Baram and Koch, 1977).

An alternative explanation for the differences between the quantity of LH released on Day 3 vs Day 4 in E_2 treated rats may be that pituitary glands on Day 4 are more responsive to LHRH treatment (compare Fig. 3A with Fig. 4A). This responsiveness could be a function of the plasma levels of E_2 which are declining between Days 3 and 4 (Table 3). Henderson et al. (1977) have presented evidence that the magnitude of the afternoon LH surge which occurs in preparations similar to our depends upon circulating E_2 levels.

A second index of endogenous LHRH release is a rise in plasma FSH. In E_2 treated rats, FSH levels remained basal on Day 3 whereas they were significantly elevated at 1600 h on Day 4. Further, releasable pituitary reserves of FSH were similar on Days 3 and 4 as determined by the responses obtained after pulse injections of LHRH (Table 5). The absence of an FSH surge on Day 3 and its presence on Day 4 supports the concept that smaller discharges of endogenous LHRH occur on Day 3 than on Day 4 in E_2 treated rats. Recent studies suggest that pituitary FSH release can occur in response to either relatively high levels of LHRH (Blake, 1976) or to low concentrations of LHRH delivered over a prolonged period (Wise et al., 1978). In the present studies, only when plasma LH was elevated appreciably (an index of high endogenous LHRH secretion) was plasma FSH also elevated.

Further analysis of the 1600 h values for LH and FSH from Days 1–5, after E_2 treatment on Day 0, revealed 24 h rhythmic changes in the plasma concentrations of LH and FSH. Plasma LH levels were unchanged on Day 1 or markedly reduced on Days 3 and 5 when compared with the elevated values obtained on Days 2 and 4. Plasma FSH was elevated significantly only on Days 2 and 4 when plasma LH levels were high. We have presented evidence that the high LH levels obtained on Day 4 most likely are due to an increased release of endogenous LHRH, whereas the low LH values on Day 3 could be related to reduced LHRH discharges. Thus, these rhythmic variations may be the consequence of the quantity of endogenous LHRH released each day as well as to variations in pituitary responsiveness to this decapeptide. Perhaps an interval of 48 h is required to replenish completely the hypothalamic LHRH reserves which could be maximally released on Days 2 and 4.

The administration of P on the morning of Day 3 to E_2 treated rats has a biphasic effect on gonadotropin secretion over the next 48 h. Once P induced gonadotropin surges occur, further 24 h rhythmic releases of LH and FSH are abolished. Freeman et al. (1976) and Banks and Freeman (1978) have made similar observations after P administration to E_2 treated castrates or to Nembutal blocked proestrous rats.

The advancement of the time at which gonadotropin surges occur during the afternoon also has been reported in intact proestrous (Brown-Grant and Greig, 1975; Brown-Grant and Naftolin, 1972; Zeilmaker, 1966) and estrogenized OVX animals (Mann and Barraclough, 1973). Our studies confirm these observations.

As well, the temporal patterns of E_2P induced FSH release are almost identical to normal proestrous FSH surges. Swerdloff et al. (1972) reported that estrogen alone was insuffi-

cient to induce an FSH surge in rats OVX for 15 days and only when P was combined with E_2 was this gonadotropin released. These various data reemphasize the necessity for the release of sufficiently high endogenous LHRH levels to activate FSH gonadotrophs.

To determine if the high plasma FSH and LH levels obtained in E_2P vs E_2 treated rats on the afternoon of Day 3 were the consequence of increased pituitary responsiveness or were due to the release of greater quantities of endogenous LHRH, spontaneous gonadotropin surges were blocked with Nembutal. After the injection of LHRH, peak plasma LH levels were significantly higher in the E_2 vs the E_2P treated OVX animals. Aiyer et al. (1976) have shown that neither pituitary LH content nor concentration is appreciably altered as a consequence of E_2 or E_2P treatment of longterm OVX rats. With this information we conclude that the high plasma LH and FSH concentrations observed on Day 3 (after P treatment) are the result of the release of high levels of endogenous LHRH rather than of changes in either pituitary LH reserves or responsiveness on Day 3.

In contrast, when P is administered to E_2 treated rats on Day 3, it may affect the quantity of LH present in the releasable pituitary pool on Day 4. Because considerably more pituitary LH is released spontaneously on Day 3 in E_2P than in E_2 treated rats, fewer pituitary LH reserves may be present in the former group to be discharged in response to exogenous LHRH administered on Day 4 (Fig. 4A). However, it should be recalled that the amount of LH released in response to a pulse injection of LHRH is similar in E_2P treated rats on Days 3 and 4 which implies that similar releasable pituitary LH reserves exist on both days.

In the present study, spontaneous LH surges occurred in E_2P treated rats on Day 4 if Nembutal was administered on Day 3. In contrast, previous studies by others have shown that when P is given 1 h prior to or 15 min after barbiturate treatment of cyclic proestrous rats, it abolishes the spontaneous release of LH that normally would occur on estrus in barbiturate blocked proestrous rats (Blake, 1977; Freeman et al., 1976). Freeman et al. (1976) suggests that P induces a loss of the "memory" for 24 h periodicity in LH secretion which is "turned on" by estrogen in OVX as well as in cycling rats. The differences in responsiveness to P of rats in our studies and those of others (Blake, 1977; Freeman et al., 1976) may be the result of several factors. a) The preoptico-hypophysiotropic system of longterm OVX rats could respond differently to sex steroids than it does in cyclic proestrous rats. Legan et al. (1975) have shown that daily LH surges persist for 3 days despite removal of E_2 implants in 2-week OVX rats whereas in acutely (24 h) OVX rats continuous exposure to E_2 is necessary to maintain daily LH surges. b) The time that P is administered prior to or after Nembutal (or onset of the critical period) may be important in determining whether a spontaneous LH surge will occur the next day. c) Progesterone, when given to E_2-treated OVX rats, may prevent reaccumulation of an endogenously releasable pool of LHRH once it is discharged (as occurs on Day 3 in E_2P treated OVX rats). Under these circumstances, even though a neural signal is presented each 24 h, insufficient LHRH would be present to induce an LH surge. A major criticism of this hypothesis is the observation that rats treated with P and Nembutal on proestrus do not have spontaneous LH surges on estrus even though releasable LHRH is present within the hypothalamus (Blake, 1977; Freeman et al., 1976). Obviously, without further information on the neuronal molecular events which accompany P treatment of estrogen-primed cyclic or OVX rats, definitive conclusions on the mode of interaction of E_2 with P within the brain to facilitate or block LH release cannot be made.

Nevertheless, the present results provide new information which suggests that the role of E_2 and its interactions with P in regulating gonadotropin secretion is primarily through its effects on LHRH release (and perhaps synthesis) mechanisms. This does not preclude the probability that E_2 also has important effects in modifying pituitary LH reserves.

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