BIOLOGY OF REPRODUCTION 24, 820–830 (1981)

Effects of Estradiol and Progesterone on Plasma Gonadotropins, Prolactin, and LHRH in Specific Brain Areas of Ovariectomized Rats[1]

PHYLLIS M. WISE,[2] PATRICIA CAMP-GROSSMAN
and CHARLES A. BARRACLOUGH

Department of Physiology,
School of Medicine,
University of Maryland,
Baltimore, Maryland 21201

ABSTRACT

We examined the effects of physiological concentrations of estradiol (E_2) and progesterone (P) on plasma LH, FSH, and prolactin (PRL) and on LHRH concentrations in several microdissected brain regions in ovariectomized (OVX) rats. One week after ovariectomy (Day 0), rats received Silastic capsules containing only sesame oil or 37.5, 75.0 or 150 μg E_2/ml of oil s.c. The E_2 capsules produced plasma estrogen concentrations of 7.0, 9.6, or 15.4 pg/ml, respectively, on Day 2 whereas oil-treated controls had 5.4 pg/ml of E_2 in plasma. All three E_2-treated groups of rats had LH surges of comparable magnitude during the afternoon of Day 2. Two Silastic capsules of P (50 mg/ml) were implanted at 0900 h on Day 2 into E_2-treated rats. In animals in which E_2 levels were 7.0 or 9.6 pg/ml, P only moderately amplified the LH surges. However, when plasma E_2 concentrations reached 15 pg/ml, P treatment evoked a massive release of LH and advanced the time of release by 1 h. In contrast, the stress of inserting oil capsules into ether-anesthetized E_2-treated rats at 0900 h on Day 2 had no effect on afternoon LH surges in these animals. FSH surges occurred only in rats receiving both E_2 and P. PRL surges were induced using the highest concentrations of E_2 and were advanced in time by P. When the highest dose of E_2 was used, LHRH concentrations in the median eminence (ME) increased prior to and decreased during the E_2 P-induced surge. These changes were not paralleled by any changes in the other anterior brain regions we examined (suprachiasmatic preoptic nucleus, suprachiasmatic nucleus, medial preoptic nucleus, anterior hypothalamic nucleus, and retrochiasmatic area). No changes in LHRH concentrations were observed under any other steroid treatment regimen. Higher plasma E_2 concentrations induced by the 150 μg/ml E_2 capsule were correlated with higher LHRH concentraions in the median eminence concomitant with a parallel trend in some of the anterior brain areas: MPN ($P<0.06$), SPN ($P<0.06$), and RCA ($P<0.03$).

INTRODUCTION

Estrogen induces gonadotropin and prolactin (PRL) surges in ovariectomized (OVX) rats when administered under a variety of experimental paradigms (Blake, 1977; Caligaris et al., 1971a; DePaolo and Barraclough, 1979a; Goodman, 1978a; Henderson et al., 1977b; Legan et al., 1975; Neill, 1980). When estradiol (E_2)-primed OVX rats are treated with progesterone (P), LH surges are temporally advanced and peak concentrations are greatly amplified (Aiyer and Fink, 1974; Caligaris et al., 1971b; DePaolo and Barraclough, 1979a; Kalra et al., 1973; Mann and Barraclough, 1973). While the positive feedback sites of action of E_2 on gonadotropin secretion are thought to reside predominantly within the preoptico-anterior hypothalamic area in rats (Blake, 1977; Clemens et al., 1976; Goodman, 1978b; Kalra and McCann, 1975; Kawakami et al., 1978), E_2 also directly increases pituitary responsiveness to LHRH (Aiyer and Fink, 1974; Arimura and Schally, 1971; Cooper et al., 1974; Drouin et al., 1976; Vilchez-Martinez et al., 1974). In contrast, in vivo studies indicate that P enhances the positive feedback effects of E_2 on gonadotropins primarily by its action within the brain (Aiyer et al., 1976; DePaolo and Barraclough, 1979a). The positive feedback effects of E_2 on PRL secretion are thought to

Accepted January 13, 1981.
Received August 19, 1980.
[1] Supported by USPHS Grant HD 02138.
[2] Reprint requests: Dr. P.M. Wise, Dept. of Physiology, University of Maryland School of Medicine, 660 W. Redwood St., Baltimore, MD 21201.

be at both the hypothalamic (Bishop et al., 1972; Caligaris and Taleisnik, 1974; Nagasawa et al., 1969; Ratner and Meites, 1964; Weisel et al., 1978) and pituitary level (Ben-David et al., 1964; Chen et al., 1976; Lu et al., 1971; Nicoll and Meites, 1962; Raymond et al., 1978).

Estrogen-inducible P cytosol receptors exist within neurons of the medial basal hypothalamus and preoptic area which have physicochemical properties similar to those in the uterus. Further, these receptors increase in a dose-dependent fashion with increasing doses of E_2 (Kato et al., 1978; Moguilewsky and Renaud, 1979). We reasoned that if this P receptor ultimately is involved in the augmented secretion of LH, we should observe increased pituitary gonadotropin release if we progressively elevate plasma E_2 levels and maintain P at constant physiological concentrations in plasma. We have examined this possibility in the present study.

P may augment E_2-induced LH surges by affecting the concentrations of LHRH present within the preoptico-suprachiasmatic- tuberoinfundibular system (PSTS). We previously observed that changes in LHRH concentration occur in microdissected areas of PSTS during proestrus which can be correlated with increases in plasma E_2 and with the proestrous gonadotropin surge (Wise et al., 1981). For these reasons we also examined the temporal effects of E_2 or E_2P on LHRH concentrations in these same microdissected regions of PSTS prior to and during the time of steroid-induced gonadotropin surges.

MATERIALS AND METHODS

Adult female Sprague-Dawley rats (Zivic Miller, Allison Park, PA) weighing between 200–250 g were maintained in a controlled environment (22–24°C; lights on 0400–1600 h) prior to use. Food and water were supplied ad libitum. Estrous cyclicity was monitored by daily vaginal lavages, and only those females having 4–5 day cycles were used. All rats were bilaterally ovariectomized (OVX), and 1 week later (0900 h, Day 0) they received s.c. implants of Silastic capsules containing either oil (Group 1) or E_2 (Groups 2–8). E_2 capsules were 30 mm in length (1.57 mm i.d., 3.8 mm o.d., Dow Corning) and contained 20 mm lengths of various concentrations of estradiol-17β dissolved in sesame oil (Table 1). Progesterone Silastic capsules were 30 mm long and contained 20 mm lengths of P (50 mg/ml) suspended in sesame oil. Two P capsules were inserted s.c. into certain groups of E_2-treated OVX rats (Groups 3, 5, 7) on Day 2 at 0900 h (Table 1). Groups of oil- (Group 1) and E_2-treated rats (Groups 2, 4, 6) received capsules which contained only oil on Day 2 at 0900 h (Table 1). A final group (Group 8) of rats received E_2 (150 μg/ml) on Day 0 and did not receive any capsules on Day 2.

Steroid hormones were mixed overnight in oil with a magnetic stirrer before being placed into capsules. The capsules were constructed by the method of Legan et al. (1975) and were stored in oil solutions containing the same steroid concentrations as those within the capsule. These capsules were incubated in castrated female rats (s.c.) for 12–18 h prior to being implanted into experimental animals. This incubation period reduces the transitory plasma hormone peak that normally occurs if nonincubated capsules or capsules preincubated in water or methanol are placed into OVX animals (Henderson et al., 1977a).

Experiment 1. Serum Estradiol and Progesterone Concentrations in Animals Treated with 150 μg/ml [E_2(150)] on Day 0 With or Without Progesterone on Day 2

To determine the effect of implanting estradiol and progesterone capsules on concentrations of circulating serum steroid, animals treated with E_2 (150) at 0900 h on Day 0 and with oil or P at 0900 h on Day 2 were decapitated at 0800, 1000, or 1600 h on Day 0; 1300 or 1600 h on Day 1; and 1000, 1300, or 1600 h on Day 2. Trunk blood was collected, centrifuged, and the serum separated and stored frozen (−20°C) until it was assayed for hormone concentrations.

Experiment 2. Plasma Gonadotropin and Prolactin Profiles on Day 2 in Sequentially Bled Rats

Those animals in which sequential blood samples were taken to measure plasma gonadotropins and prolactin received jugular cannulae on Day 0. Animals were bled (0.6 ml) at 0900 h and at hourly intervals between 1200–1700 h on Day 2. The 0900 h blood samples (Groups 1–7) were taken while the animals were anesthetized with ether to insert the progesterone or oil capsules. The 0900 h blood sample taken from Group 8 and all other blood collections were made from unrestrained, unanesthetized rats. All rats were decapitated at 1700 h on Day 2, and trunk blood was collected and assayed for steroid hormone concentrations.

Experiment 3. LHRH Concentrations in Discrete Brain Regions of Steroid-Treated Rats

Animals used to determine PSTS-LHRH concentrations received the same oil or steroid treatment regimens as Groups 1, 2, 3, 6, and 7. These rats were decapitated at 1000, 1200, or 1500 h on Day 2, and the brains were quickly removed. The following brain areas were microdissected, extracted in 0.1 N HCl, and stored as described previously (Selmanoff et al., 1980): median eminence (ME), retrochiasmatic area (RCA), suprachiasmatic nucleus (SCN), suprachiasmatic preoptic nucleus (SPN), medial preoptic nucleus (MPN), and anterior hypothalamic nucleus (AHN). Protein concentrations were measured in the pellet using the Bradford dye binding method (Bradford, 1976). Trunk blood was measured for LH, FSH, E_2, P (Wise et al., 1979), and PRL (Niswender et al., 1969)

concentrations by radioimmunoassay methods. NIA-MDD RP-1 were used as standards for LH, FSH, and PRL.

LH, FSH, and PRL data were subjected to Cochran's test (Winer, 1971) for homogeneity of variance and found to be heterogeneous. Therefore, subsequent statistical analyses were performed on the $\log_{10}$ of the data. Data were analyzed by three-way analysis of variance: hormone (presence vs absence of progesterone) × dose (estradiol 37.5 μg/ml vs 75 μg/ml vs 150 μg/ml) × time (0900–1700 h for plasma hormones, 1000 vs 1200 vs 1500 h for LHRH). Differences between two individual means were tested using Newman-Keuls multiple range analysis.

RESULTS

Experiment 1. Effects of Implanting E_2 (150) Capsules on Day 0 and P or Oil Capsules on Day 2 on Subsequent Serum E_2 and P Concentrations

Serum E_2 before 0800 h on Day 0 and at various times after capsule implantation (1000 h Day 0–1600 h, Day 2) are shown in Table 1. When E_2 (150) capsules were implanted s.c. they produced a transient rise in serum E_2 which stabilized within 24 h. These serum E_2 levels remained constant during the hours examined on Day 2 (1000, 1300, 1600 h). Progesterone concentrations prior to inserting a P capsule were constant (Table 1). The stress of implanting oil capsules in rats briefly anesthetized with ether produced a transient rise in serum P concentrations 1 h later; however, these concentrations never were as high as those produced when P-filled capsules were inserted s.c. Serum P concentrations in these groups (Groups 3, 5, 7) remained constant from 1000–1600 h on Day 2.

Experiment 2. Effects of Steroid Treatment on Plasma E_2, P, LH, FSH, and PRL Concentrations

To determine whether the stress of capsule implantation has any effect on E_2-induced LH surges, four animals received E_2 implants on Day 0 and were not further treated on Day 2 (Group 8, Table 2). E_2-induced LH surges were not significantly different at any time compared with animals receiving oil implants (Group 6) at 0900 h on Day 2 ($F = 0.096$, $P<0.75$). Thus the ether stress produced in these studies had no effect on the timing or amplitude of the E_2-induced surge.

Plasma E_2 concentrations in oil-treated OVX rats at 1700 h on Day 2 were 5.4 ± 0.6 pg/ml while P levels in these rats were 1.0 ± 0.1 ng/ml (Group 1, Table 3). When Silastic capsules (Group 2) which contained 37.5 μg/ml E_2 were inserted, they significantly ($P<0.05$) elevated plasma E_2 concentrations in OVX rats to 7.1 ± 0.6 pg/ml but did not affect plasma P levels in OVX controls by 1700 h on Day 2 (Table 2). These plasma E_2 concentrations were sufficient to reduce plasma LH levels in OVX rats significantly by 0900 h (223 ± 17 ng/ml) and 1200 h (215 ± 8 ng/ml) on Day 2, and they also evoked LH surges during the afternoon of Day 2 (F

TABLE 1. Serum steroid concentrations in OVX and steroid-treated rats at various times on Days 0–2.

Day	Time (h)	Animals/ group	Serum steroid concentrations	
			E_2 (pg/ml)	P (ng/ml)
0	0800[a]	6	5.1 ± 0.5	0.9 ± 0.3
0	1000[b]	5	68.9 ± 9.3	2.2 ± 0.4
0	1600[b]	4	37.7 ± 6.6	1.6 ± 0.2
1	1300[b]	5	18.5 ± 3.2	1.3 ± 0.2
1	1600[b]	5	18.7 ± 2.2	2.0 ± 0.5
2	1000[bc]	6	22.8 ± 1.1	6.1 ± 1.7
2	1000[bd]	5	16.2 ± 3.8	13.9 ± 0.9
2	1300[bd]	6	14.5 ± 1.8	11.8 ± 1.7
2	1600[bd]	6	15.8 ± 1.2	11.7 ± 1.6

[a] Animals were bilaterally ovariectomized. One week later (Day 0) a Silastic capsule containing E_2 (150 μg/ml) was inserted at 0900 h.

[b] E_2 capsule inserted at 0900 h on Day 0.

[c] Oil capsule inserted at 0900 h on Day 2 under ether anesthesia.

[d] P capsules (2 × 50 mg/ml) were inserted at 0900 h Day 2.

TABLE 2. Plasma LH (ng/ml) in OVX, E_2-treated rats: Effect of ether anesthesia at 0900 h on Day 2 (mean ± SEM).

	Time (h)						
	0900	1200	1300	1400	1500	1600	1700
Group 6[a] [E_2 (150) + ether] (n = 10)	188 ± 15	147 ± 10	172 ± 19	477 ± 59	1140 ± 213	1044 ± 136	628 ± 73
Group 8[b] [E_2 (150) − ether] (n = 4)	218 ± 18	160 ± 16	189 ± 50	517 ± 121	1017 ± 133	919 ± 215	568 ± 153

[a]Animals in Group 6 were treated with E_2 at 0900 h on Day 0 and treated with two oil capsules at 0900 h on Day 2 under ether anesthesia.

[b]Group 8 rats received the same treatment as Group 6 rats on Day 0, but they did not receive any further treatment on Day 2.

ratio for time = 209.55, P<0.0001, Table 4, Fig. 1). However, the peak plasma LH levels obtained on Day 2 at 1500 h (1151 ± 216 ng/ml) are only approximately one-third those normally observed in cyclic rats on proestrous afternoon (∿3500 ng/ml; Barraclough et al., 1979). Silastic capsule implants of P into E_2-primed rats (Group 3) at 0900 h on Day 2 significantly (P<0.05) increased plasma P concentrations in OVX rats to 13.9 ± 1.2 ng/ml (Table 3). These elevated P plasma levels (F ratio for hormone = 296.02, P<0.0001; F ratio for hormone × time = 13.44, P<0.0001) stimulated LH surges in Group 3 rats whose onset was advanced by 60 min and whose peak concentrations were significantly greater (∿62%) than those obtained in rats receiving only E_2 capsules (P<0.01) (Fig. 1).

When Silastic capsules containing E_2 concentrations of 75 μg/ml were implanted into OVX rats (Group 4), they significantly increased plasma E_2 levels (9.6 ± 0.5 pg/ml) over those obtained in Group 2 rats (Table 3). Plasma LH levels at 0900 h (211 ± 5 ng/ml) and 1200 h (183 ± 17 ng/ml) were significantly reduced when compared with those of OVX controls (P<0.05), and this E_2 plasma concentration induced LH surges whose peak concentrations were no different from Group 2 values (F ratio for dose = 11.08, P<0.0001, Fig. 1, Table 4). P capsules, when inserted into rats with these E_2 plasma levels (Group 5), augmented the LH surge to a degree similar to that obtained in Group 3 rats (F ratio for dose × hormone × time = 3.28; P<0.0002, Fig. 1).

When capsules containing E_2 concentrations of 150 μg/ml were implanted into OVX rats (Group 6), plasma E_2 levels at 1700 h on Day 2 were 15.3 ± 0.9 pg/ml and plasma LH concentrations were reduced to 188 ± 14 ng/ml and 147 ± 10 ng/ml at 0900 and 1200 h, respectively. Although these plasma E_2 concentra-

TABLE 3. Plasma steroid concentrations in OVX, steroid-treated rats.

	Day 0	Day 2		Plasma steroid concentrations (mean ± SEM)	
	Capsule concentrations		Animals/		
Group	E_2 (μg/ml)	P (mg/ml × 2)	group	E_2 (pg/ml)	P (ng/ml)
1	Oil	Oil	8	5.4 ± 0.6	1.0 ± 0.1
2	37.5	Oil	9	7.1 ± 0.6	1.5 ± 0.3
3	37.5	50	10	7.0 ± 0.4	13.9 ± 1.2
4	75.0	Oil	11	9.6 ± 0.5	1.5 ± 0.3
5	75.0	50	11	9.5 ± 0.5	14.2 ± 1.2
6	150.0	Oil	9	15.3 ± 0.9	1.2 ± 0.2
7	150.0	50	9	15.4 ± 0.7	15.2 ± 1.4
8	150.0	No treatment	4	15.2 ± 0.6	1.3 ± 0.3

TABLE 4. Plasma LH, FSH and PRL concentrations in OVX rats (mean ± SEM).

	Time (h)						
	0900[a]	1200[b]	1300[b]	1400[b]	1500[b]	1600[b]	1700[c]
LH (ng/ml)	290 ± 25*	335 ± 31*	280 ± 34	311 ± 18	305 ± 16	335 ± 55	315 ± 42
FSH (ng/ml)	859 ± 179	1382 ± 60*	1177 ± 34	1127 ± 88	1097 ± 98	1385 ± 106	1175 ± 107
PRL (ng/ml)	35 ± 4†	31 ± 5†	22 ± 1†	45 ± 4†	43 ± 10†	29 ± 6†	38 ± 6†

[a]Blood removed via jugular cannulae under light ether anesthesia (Day 2). Oil capsule inserted s.c.

[b]Blood removed via jugular cannulae from unanesthetized, unrestrained rats (Day 2).

[c]Trunk blood collected after decapitation (Day 2).

*Significantly greater ($P<0.05$) than all groups of steroid-treated rats.

†Significantly less than E_2P(75)-, E_2(150)-, and E_2P(150)-treated rats.

tions were significantly greater ($P<0.05$) than those obtained in Groups 2 or 4 (Table 3), the steroid-induced LH surges obtained were comparable in temporal patterns and concentrations to those obtained in these groups (Fig. 1). When P capsules were implanted into rats having plasma E_2 levels of 15.4 ± 0.7 pg/ml (Group 7), markedly higher peak concentrations of LH were obtained (5580 ± 896 ng/ml) and the surge began 1 h earlier (Fig. 1). These LH concentrations are approximately fivefold greater than those observed in E_2-treated Groups 2, 4, and 6 and two- to threefold greater than in E_2P-treated Groups 3 and 5.

Following OVX, plasma FSH concentrations (Table 4) increased significantly over those basal gonadotropin concentrations obtained in cyclic rats (data not shown). E_2 suppressed elevated plasma FSH concentrations in OVX rats only at 1200 h on Day 2. The effect of estradiol did not depend upon the concentration of hormone (F ratio for dose = 2.868, $P<0.06$). It appears that FSH surges did not occur in E_2-treated rats (F ratio for dose × time = 1.66, $P<0.08$). Significant increases ($P<0.05$) in plasma FSH were observed only in E_2P-treated animals in Groups 3 and 7 but not in Group 5 starting at 1400 h (F ratio for hormone × time = 2.615, $P<0.02$; Table 5).

E_2-treatment (F ratio for dose = 10.165, $P<0.0001$) resulted in a significant increase in baseline plasma prolactin concentrations at 0900 h on Day 2 in Groups 5, 6, and 7 only ($P<0.05$, Table 6). A prolactin surge occurred during the afternoon in Groups 2–7 (F ratio for time = 12,673, $P<0.0001$). P treatment of E_2-primed rats affected the surge (F ratio for hormone × time = 2.394, $P<0.03$): it advanced the PRL surge only when E_2 was administered at higher concentrations (Groups 5 and 7 significantly elevated at 1200 h; Groups 3, 4, and 6 significantly elevated at 1400 h) (Table 6).

Experiment 3. Effect of Steroid Treatment on LHRH Concentrations in Specific Brain Areas

Three-way analysis of variance of ME-LHRH concentrations revealed a significant interaction between the presence or absence of P and time (F ratio for hormone vs time = 7.154, $P<0.0002$). When plasma E_2 was elevated to ∿15 pg/ml and P capsules were implanted at 0900 h on Day 2 (Group 7), ME-LHRH concentrations were higher at 1200 h ($P<0.05$) and lower at 1500 h ($P<0.05$) than in animals treated with E_2 alone (Group 6, Fig. 2). Furthermore, one-way analysis of variance revealed a significant effect of time in E_2P(150)-treated rats: ME-LHRH concentrations increased significantly between 1000 and 1200 h ($P<0.05$) and decreased significantly between 1200 and 1500 h ($P<0.05$). Under other steroid treatment protocols (Groups 2, 3, and 6), when steroid-induced gonadotropin surges were of less magnitude, no change in ME-LHRH was observed prior to or concomitant with the surge. No LHRH concentration changes occurred during the day in SPN, SCN, RCA, MPN, or AHN (Table 5) in treatment Groups 2, 3, 6, and 7.

When plasma E_2 concentrations reached ∿15 pg/ml, ME-LHRH concentrations were

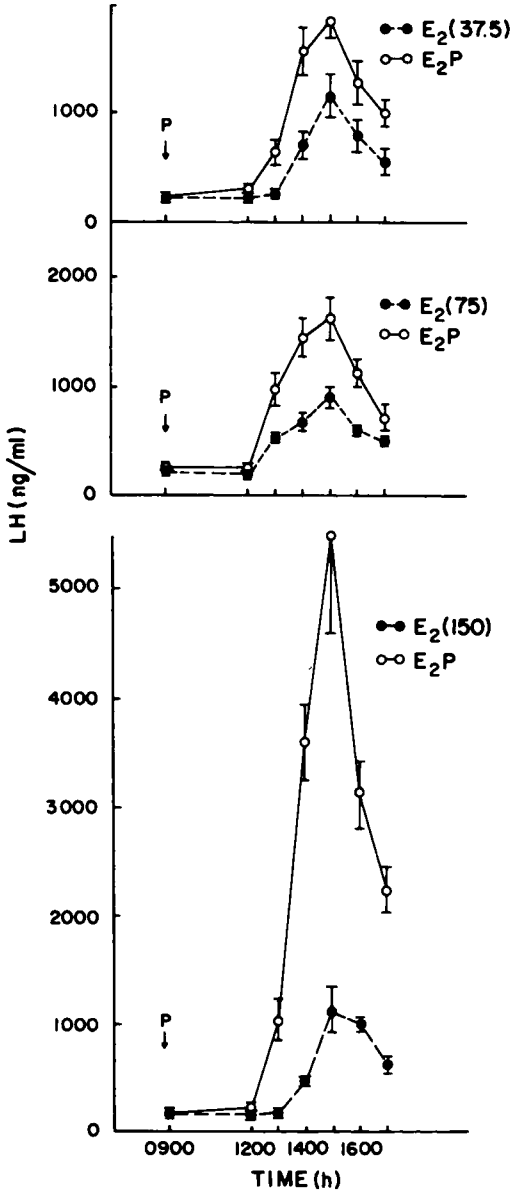

FIG. 1. Plasma E_2 was progressively elevated by use of Silastic capsules. When capsules containing 37.5, 75.0, or 150 μg/ml E_2 were implanted (Day 0), spontaneous LH surges of equal magnitude were obtained even though plasma E_2 levels increased from 5.4 (OVX) to 7.1 (37.5) to 9.6 (75.0) to 15.3 (150) pg/ml. P slightly amplified the LH surge in rats with E_2 levels of 7.1–9.6 pg/ml. In rats with E_2 levels of 15.3 pg/ml, P produced a massive discharge of LH. P advanced the time of the LH surge by 1 h in all groups.

significantly higher (Groups 6 and 7) than when plasma E_2 was lower (Groups 2 and 3; F ratio for dose = 51.983, $P<0.0001$). The higher doses of E_2 had a significant effect on LHRH concentrations in the RCA ($P<0.01$) and showed the same trend in the MPN ($P<0.06$) and SPN ($P<0.06$) (Table 7). No effect was observed in the SCN or AHN.

DISCUSSION

These experimental results demonstrate that when low *physiological* plasma concentrations of E_2 are produced in OVX rats, LH surges occur. However, greater LH peak concentrations are not achieved when plasma E_2 levels are elevated from 7–15 pg/ml, although such progressive increases in plasma E_2 have profound effects on the ability of P to enhance and advance the gonadotropin surge. We examined the responsiveness of E_2-treated rats to P 48 h later since previous studies demonstrated that maximal cytosol receptor concentrations are observed at this time (Moguilewsky and Renaud, 1979). Thus, whereas plasma E_2 concentrations of 7–10 pg/ml allow P to stimulate slightly larger gonadotropin surges (60–80%), when E_2 concentrations are increased to ∿15 pg/ml for 48 h, the P-induced surge is fivefold greater than in E_2-treated controls. The differential priming ability of E_2 may be related to the ability of such plasma concentrations to induce differential amounts of hypothalamic P receptors. Interestingly, intermediate plasma concentrations of estradiol did not cause P to stimulate an LH surge of intermediate amplitude. It is possible that a threshold number of P receptors is needed for the greatly enhanced gonadotropin response, and this threshold is achieved when ∿15 pg of E_2 are present in plasma. Indeed, the ability of P to induce lordotic behavior in OVX E_2-treated rats is correlated temporally with the appearance of a critical number of E_2-induced P receptors in specific areas of the brain (Moguilewsky and Renaud, 1979). Statistically significant FSH surges occurred only in E_2P-treated rats and similar findings have been reported previously (DePaolo and Barraclough, 1979). Furthermore, peak FSH plasma concentrations on Day 2 did not depend upon the initial priming dose of E_2.

LHRH concentrations in specific brain areas and changes in these concentrations prior to and concomitant with steroid-induced surges

TABLE 5. Plasma FSH concentrations (ng/ml) in steroid-treated OVX rats (mean ± SEM).

Steroid (μg/ml)	Time (h)						
	0900	1200	1300	1400	1500	1600	1700
E_2(37.5)	1097 ± 55	1008 ± 44	1012 ± 94	1092 ± 89	1188 ± 108	1121 ± 108	1306 ± 75
E_2P(37.5)	913 ± 106	1065 ± 71	1168 ± 85	1322 ± 163*	1681 ± 192	1483 ± 194*	2003 ± 229*
E_2(75)	933 ± 108	921 ± 83	1210 ± 278	1062 ± 56	1082 ± 82	1000 ± 103	1213 ± 100
E_2P(75)	939 ± 69	960 ± 108	1210 ± 141	1420 ± 174*	1373 ± 214*	1419 ± 208*	1506 ± 270*
E_2(150)	798 ± 54	849 ± 53	872 ± 57	878 ± 76	992 ± 86	1110 ± 89	1588 ± 284
E_2P(150)	735 ± 52	850 ± 54	1003 ± 217	1291 ± 208*	1629 ± 253*	1690 ± 259*	2123 ± 225*

*Significantly different from 0900 h FSH concentrations in equivalently steroid-treated animals.

differ depending upon the steroid treatment regimen. Only when plasma LH reached peak concentrations that were comparable to those obtained on proestrus (Group 7) were changes in ME-LHRH measurable. In this experimental group, progesterone stimulated a significant rise in ME-LHRH between 1000–1200 h and the LH surge was accompanied by a significant drop in LHRH between 1200–1500 h. Perhaps the amount of LHRH released into portal plasma from the ME is not immediately replaced by more LHRH synthesized in and transported from anterior brain areas. Simpkins et al. (1980) reported progesterone-induced fluctuations in regions within the mediobasal hypothalamus. The changes in ME-LHRH concentrations were not accompanied by similar changes in any of the LHRH containing anterior brain areas we examined. This is in contrast to our previous observations in proestrous rats where simultaneous parallel LHRH changes occur in SCN, RCA, and MPN prior to and during the proestrous LH surge (Wise et al., 1981). Thus the effect of P on LHRH may be limited to ME. Alternatively, it is possible that 1) the effect of P on anterior brain areas is too small to be detected using RIA methodology, or 2) axonal transport of LHRH to the ME from the anterior hypothalamic areas occurs simultaneously with rapid new synthesis during the 3 h interval between P treatment and autopsy. In this scheme, net changes in anterior hypothalamic LHRH would not be detected in our studies. When steroids induced LH surges of lesser magnitude (Groups 2, 3, 6), no changes in LHRH were detectable in any of the brain areas examined despite the fact that other investigators report elevated LHRH concentrations in portal plasma during steroid-induced surges (Sarkar and Fink, 1979). Presumably, the amounts of LHRH released are minimal and they may be accompanied by the synthesis of equivalent concentrations of LHRH to replenish the released hormone thus making it impossible to measure any net LHRH changes. Indeed, Pilotte et al. (1980) found that small elevations in portal plasma dopamine concentrations were not accompanied by comparable changes in ME dopamine turnover rates (an index of release). This observation suggests that

TABLE 6. Plasma prolactin concentrations (ng/ml) in steroid-treated OVX rats (mean ± SEM).

Steroid (μg/ml)	Time (h)						
	0900	1200	1300	1400	1500	1600	1700
E_2(37.5)	79 ± 16	114 ± 39	232 ± 59	354 ± 68*	371 ± 83*	353 ± 73*	200 ± 47
E_2P(37.5)	71 ± 16	251 ± 96	305 ± 95	403 ± 105*	426 ± 86*	315 ± 56*	414 ± 75*
E_2(75)	71 ± 9	71 ± 20	299 ± 73*	309 ± 44*	227 ± 61*	220 ± 45	172 ± 33
E_2P(75)	129 ± 37	329 ± 69*	506 ± 80*	469 ± 64*	330 ± 39*	254 ± 44	288 ± 35
E_2(150)	225 ± 29	107 ± 26	285 ± 84	417 ± 84*	585 ± 109*	694 ± 100*	444 ± 84*
E_2P(150)	137 ± 18	408 ± 91*	746 ± 134*	612 ± 60*	640 ± 48*	528 ± 48*	476 ± 45*

*Significantly different from 0900 h PRL concentrations in equivalently steroid-treated animals.

TABLE 7. LHRH concentrations (pg/μg protein; mean ± SEM) in specific brain areas of steroid-treated OVX rats.

Treatment[a]	Time (h) 1000	1200	1500	Three-way analysis of variance F value	P<
Suprachiasmatic preoptic nucleus (SPN)					
Group 2	0.25 ± .09	0.23 ± .05	0.24 ± .07		
Group 3	0.25 ± .10	0.17 ± .04	0.27 ± .06		
Group 6	0.40 ± .17	0.41 ± .13	0.28 ± .07		
Group 7	0.37 ± .07	0.27 ± .05	0.30 ± .09		
Effect of dose of E_2				3.439	0.06
Effect of presence of P				0.296	0.59
Effect of time				0.311	0.74
Suprachiasmatic nucleus (SCN)					
Group 2	0.19 ± .05	0.18 ± .04	0.22 ± .06		
Group 3	0.19 ± .04	0.18 ± .03	0.20 ± .04		
Group 6	0.16 ± .04	0.24 ± .04	0.23 ± .07		
Group 7	0.21 ± .02	0.30 ± .10	0.22 ± .07		
Effect of dose of E_2				1.222	0.27
Effect of presence of P				0.258	0.62
Effect of time				0.485	0.62
Retrochiasmatic area (RCA)					
Group 2	0.26 ± .08	0.18 ± .10	0.24 ± .04		
Group 3	0.22 ± .05	0.21 ± .02	0.25 ± .05		
Group 6	0.38 ± .09	0.34 ± .08	0.40 ± .09		
Group 7	0.32 ± .08	0.29 ± .06	0.49 ± .14		
Effect of dose of E_2				9.577	0.01
Effect of presence of P				0.004	1.00
Effect of time				1.285	0.28
Medial preoptic nucleus (MPN)					
Group 2	0.10 ± .03	0.14 ± .03	0.11 ± .02		
Group 3	0.10 ± .02	0.11 ± .01	0.10 ± .03		
Group 6	0.13 ± .03	0.15 ± .03	0.17 ± .04		
Group 7	0.16 ± .03	0.12 ± .02	0.12 ± .02		
Effect of dose of E_2				3.537	.06
Effect of presence of P				1.504	.22
Effect of time				.085	.91
Anterior hypothalamic nucleus (AHN)					
Group 2	0.05 ± .00	0.06 ± .02	0.04 ± .01		
Group 3	0.06 ± .02	0.12 ± .03	0.06 ± .02		
Group 6	0.04 ± .01	0.09 ± .01	0.05 ± .01		
Group 7	0.09 ± .03	0.05 ± .01	0.06 ± .01		
Effect of dose of E_2				0.120	0.73
Effect of presence of P				1.468	0.26
Effect of time				3.046	0.06

[a] Animals were steroid-treated as described in the Materials and Methods.

portal plasma hormone changes are a more sensitive index than are changes in brain concentrations in terms of dopamine activity. A parallel situation may exist in terms of brain changes in LHRH and portal plasma hormone changes. Kalra et al. (1978) also have reported changes in medial basal hypothalamic (MBH) LHRH concentrations during P-induced gonadotropin surges. However, the time sequence of LHRH changes relative to the LH surge was different from that observed by us. Further, in their study LHRH was measured in a more

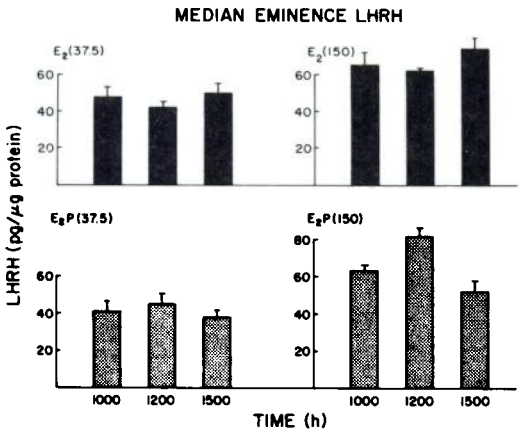

FIG. 2. ME-LHRH concentrations in OVX rats primed with 37.5 or 150 μg/ml E_2 on Day 0 with or without P (50 mg/ml) treatment on Day 2. LHRH concentrations were significantly elevated in rats treated with higher E_2 concentrations. E_2P (150) animals exhibited a significant increase between 1000–1200 h and a significant decrease between 1200–1500 h on Day 2. No other steroid-treated group showed any diurnal pattern concomitant with the LH surge.

grossly dissected MBH brain area and it is not possible to differentiate whether the patterns of change observed by them were limited to the ME or involved other hypothalamic nuclei.

Treatment of ovariectomized rats with a higher dose of E_2 had a significantly greater effect on ME-LHRH concentrations than treatment with lower E_2 concentrations. Thus LHRH levels were lower in rats treated with E_2 (37.5) (Groups 2, 3) and significantly higher in E_2 (Group 6)- and E_2P (Group 7)-treated rats. Furthermore the effect was also observed in the RCA and the same trend, though not statistically significant, was seen in the SPN and the MPN.

Rats in Groups 5, 6, and 7 had PRL plasma levels which were significantly elevated above concentrations in OVX rats at 0900 h on Day 2. Studies by others in which much higher doses of E_2 were used also found elevated plasma PRL levels, and they attribute this hyperprolactinemia to the actions of this steroid within the hypothalamus (Bishop et al., 1972; Caligaris and Taleisnik, 1974, 1976; Nagasawa et al., 1969; Wiesel et al., 1978) and the pituitary gland (Ben-David et al., 1964; Lu et al., 1971; Raymond et al., 1978). In this study, PRL surges occurred in rats in Groups 3–7 and these observations agree with those of Neill (1980). As well, P advanced the PRL surge but did not affect peak plasma concentrations. Similar observations have been reported by Caligaris and Taleisnik (1974) and Simpkins et al. (1979).

In conclusion, these studies provide some details on an experimental animal model which can be useful in the study of many central neuroendocrine events without concern for the complications produced by pharmacological plasma levels of sex steroids.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the gifts of RIA material (LH, FSH, PRL) made by Dr. A. F. Parlow, the NIAMDD, Dr. T. Nett (anti-LHRH), Dr. G. D. Niswender (anti-ovine LH; anti-P), Dr. G. Collins (anti-E_2) and Dr. L. Reichert (ovine LH). We thank Ms. Patricia Capogrossi, Viktoria Reck-Malleczewen, and Ruth Caldwell for their excellent technical assistance.

REFERENCES

Aiyer, M. S. and Fink, G. (1974). The role of sex steroid hormones in modulating the responsiveness of the anterior pituitary gland to luteinizing hormone releasing factor in the female rat. J. Endocrinol. 62, 553–572.

Aiyer, M. S., Sood, M. D. and Brown-Grant, K. (1976). The pituitary response to exogenous luteinizing hormone releasing factor in steroid-treated gonadectomized rats. J. Endocrinol. 69, 255–262.

Arimura, A. and Schally, A. V. (1971). Augmentation of pituitary responsiveness to LH-releasing hormone (LH-RH) by estrogen. Proc. Soc. Exp. Biol. Med. 136, 290–293.

Barraclough, C. A., Wise, P. M., Turgeon, J., Shander, D., DePaolo, L. and Rance, N. (1979). Recent studies on the regulation of pituitary LH and FSH secretion. Biol. Reprod. 20, 86–97.

Ben-David, M., Dikstein, S., Sulman, F. G. (1964). Effect of different steroids on prolactin secretion in pituitary-hypothalamus organ co-culture. Proc. Soc. Exp. Biol. Med. 117, 511–513.

Bishop, W., Kalra, P. S., Fawcett, C. P., Krulich, L. and McCann, S. M. (1972). The effects of hypothalamic lesions on the release of gonadotropins and prolactin in response to estrogen and progesterone treatment in female rats. Endocrinology 91, 1404–1410.

Blake, C. A. (1977). Effects of estrogen and progesterone on LH release in ovariectomized rats. Endocrinology 101, 1122–1129.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 22, 248–254.

Caligaris, L., Astrada, J. J. and Talesnik, S. (1971a). Release of luteinizing hormone induced by estrogen injection into ovariectomized rats. Endocrionlogy 88, 810–815.

Caligaris, L., Astrada, J. J. and Talesnik, S. (1971b). Biphasic effect of progesterone on the release of

gonadotropin in rats. Endocrinology 89, 331–337.

Caligaris, L. and Taleisnik, S. (1974). Involvement of neurons containing hydroxytryptamine in the mechanism of prolactin release induced by estrogen. J. Endocrinol. 62, 25–33.

Caligaris, L. and Taleisnik, S. (1976). The role of hypothalamic afferents in the release of prolactin induced by ovarian steroids. Neuroendocrinology 21, 139–145.

Chen, C. L., Amenomori, Y., Lu, K. H., Vogt, J. L. and Meites, J. (1970). Serum prolactin levels in rats with pituitary transplants or hypothalamic lesions. Neuroendocrinology 6, 220–227.

Clemens, J. A., Smalstig, E. G. and Sawyer, B. D. (1976). Studies on the role of the preoptic area in the control of reproductive function in the rat. Endocrinology 99, 728–735.

Cooper, K. J., Fawcett, C. P. and McCann, S. M. (1974). Inhibitory and facilitory effects of estradiol-17β on pituitary responsiveness to a luteinizing hormone-follicle stimulating hormone-releasing factor (LH-RF/FSH-RF) preparation in the ovariectomized rat. Proc. Soc. Exp. Biol. Med. 145, 1422–1426.

DePaolo, L. V. and Barraclough, C. A. (1979a). Interactions of estradiol and progesterone on pituitary gonadotropin secretion: Possible sites and mechanisms of action. Biol. Reprod. 20, 1173–1185.

DePaolo, L. V. and Barraclough, C. A. (1979b). Dose dependent effects of progesterone on the facilitation and inhibition of spontaneous gonadotropin surges in estrogen-treated ovariectomized rats. Biol. Reprod. 21, 1015–1023.

Drouin, J., Lagace, L. and Labrie, F. (1976). Estradiol-induced increase of the LH responsiveness to LH releasing hormone (LHRH) in rat anterior pituitary cells in culture. Endocrinology 99, 1477–1481.

Goodman, R. L. (1978a). A quantitative analysis of the physiological role of estradiol and progesterone in the control of tonic and surge secretion of LH in the rat. Endocrinology 102, 142–150.

Goodman, R. L. (1978b). The site of positive feedback action of estradiol in the rat. Endocrinology 102, 151–159.

Henderson, S. R., Baker, C. and Fink, G. (1977a). Oestradiol-17β and pituitary responsiveness to luteinizing hormone releasing factor in the rat: A study using rectangular pulses of oestradiol-17β monitored by non-chromatographic radioimmunoassay. J. Endocrinol. 73, 441–453.

Henderson, S. R., Baker, C. and Fink, G. (1977b). Effect of oestradiol-17β exposure on the spontaneous secretion of gonadotropins in chronically gonadectomized rats. J. Endocrinol. 73, 455–462.

Kalra, P. S., Fawcett, C. P., Krulich, L. and McCann, S. M. (1973). The effects of gonadal steroids on plasma gonadotropins and prolactin in the rat. Endocrinology 92, 1256–1268.

Kalra, S. P., Kalra, P. S., Chen, C. L. and Clemens, J. A. (1978). The effect of norepinephrine synthesis inhibitors and a dopamine agonist on hypothalamic LH-RH, serum gonadotropin and prolactin levels in gonadal steroid treated rats. Acta Endocrinol. 89, 1–9.

Kalra, S. P. and McCann, S. M. (1975). The stimulatory effect of gonadotropin release of implants of estradiol or progesterone in certain sites in the central nervous system. Neuroendocrinology 19, 289–302.

Kato, J., Onouchi, T. and Okinaga, S. (1978). Hypothalamic and hypophysial progesterone receptors: Estrogen-priming effect, differential localization, 5α-dihydroprogesterone binding, and nuclear receptors. Steroid Biochem. 9, 419–427.

Kawakami, M., Yoshioka, E., Konda, N., Anita, J. and Visessuran, S. (1978). Data on the sites of stimulatory feedback action of gonadal steroids indispensable for LH release in the rat. Endocrinology 102, 791–798.

Legan, S. J., Coon, G. A. and Karsch, F. J. (1975). Role of estrogen as initiator of daily LH surges in the ovariectomized rat. Endocrinology 96, 50–56.

Lu, K. H., Koch, Y. and Meites, J. (1971). Direct inhibition by ergocornine of pituitary prolactin release. Endocrinology 89, 229–233.

Mann, D. R. and Barraclough, C. A. (1973). Role of estrogen and progesterone in facilitating LH release in 4-day cyclic rats. Endocrinology 93, 694–699.

Moguilewsky, M. and Raynaud, J. P. (1979). The relevance of hypothalamic and hypophyseal progestin receptor regulation in the induction and inhibition of sexual behavior in the female rat. Endocrinology 105, 516–522.

Nagasawa, H., Chen, C. L. and Meites, J. (1969). Effects of estrogen implant in median eminence or serum and pituitary prolactin levels in the rat. Proc. Soc. Exp. Biol. Med. 132, 859–861.

Neill, J. (1980). Neuroendocrine regulation of prolactin secretion. In: Frontiers in Neuroendocrinology. (L. Martini and W. F. Ganong, eds.). Raven Press, New York. pp. 129–155.

Nicoll, C. S. and Meites, J. (1962). Estrogen stimulation of prolactin production by rat adenohypophysis *in vitro*. Endocrinology 70, 272–277.

Niswender, G. D., Chen, C. L., Midgley, A. R., Meites, J. and Ellis, S. (1969). Radioimmunoassay for rat prolactin. Proc. Soc. Exp. Biol. Med. 130, 793–797.

Pilotte, N. S., Gudelsky, G. A. and Porter, J. C. (1980). Relationship of prolactin secretion to dopamine release into hypophysial portal blood and dopamine turnover in the median eminence. Brain Res. 193, 284–288.

Ratner, A. and Meites, J. (1964). Depletion of prolactin-inhibiting activity of rat hypothalamus by estradiol or suckling stimulus. Endocrinology 75, 377–382.

Raymond, L., Beaulieu, M., Labrie, F., Boissier, J. (1978). Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. Science 200, 1173–1175.

Sarkar, D. K. and Fink, G. (1979). Effects of gonadal steroids on output of luteinizing hormone releasing factor into pituitary stalk blood in the female rat. J. Endocrinology 80, 303–313.

Selmanoff, M. K., Wise, P. M. and Barraclough, C. A. (1980). Regional distribution of luteinizing

hormone releasing hormone (LHRH) in rat brain determined by micro-dissection and radioimmunoassay. Brain Res. 192, 421–432.

Simpkins, J. W., Kalra, P. S. and Kalra S. P. (1980). Temporal alterations in luteinizing hormone-releasing hormone concentrations in several discrete brain regions: Effects of estrogen-progesterone and norepinephrine synthesis inhibition. Endocrinology 107, 573–577.

Vilchez-Martinez, J. A., Arimura, A., Debeljuk, L. and Shally, A. V. (1974). Biphasic effect of estradiol-benzoate on the pituitary responsiveness to LH-RH. Endocrinology 94, 1300–1303.

Wiesel, F. A., Fuxe, K., Hokfelt, T. and Agnati, L. F. (1978). Studies on dopamine turnover in ovariectomized or hypophysectomized female rats. Effects of 17β-estradiol benzoate, ethynodioldi-acetate and ovine prolactin. Brain Res. 148, 399–411.

Winer, B. J. (1971). Statistical Principles in Experimental Design. McGraw Hill, New York. p. 208.

Wise, P. M., Rance, N., Barr, G. D. and Barraclough, C. A. (1979). Further evidence that luteinizing hormone releasing hormone also is follicle stimulating hormone releasing hormone. Endocrinology 104, 940–947.

Wise, P. M., Rance, N., Selmanoff, M. K. and Barraclough, C. A. (1981). Changes in radioimmunoassayable LHRH in discrete brain areas of the rat at various times on proestrus, diestrous day 1 and after phenobarbital administration. Endocrinology. In Press.

Zar, J. H. (1974). Biostatistical Analysis. Prentice Hall, Englewood Cliffs. p. 151.