Serum Estradiol and Progesterone Concentrations in Ovariectomized Estrogen- and Progesterone-Primed and PMS-hCG-Treated Rats[1] (37728)

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In an attempt to explain why cortical spreading depression differentially triggered pituitary luteinizing hormone (LH) release in ovariectomized estrogen- and progesterone-primed rats (OVX-E_2-P) (1) and prolactin output in equine gonadotropin- and human chorionic gonadotropin (PMS-hCG)-treated animals (2), studies were made of the responsiveness of these preparations to exogenous luteinizing hormone releasing hormone (LHRH) (3) and of the concentrations and contents of LH and prolactin in their pituitaries (3, 4). Synthetic LHRH induced a significant rise in plasma LH in OVX-E_2-P-primed rats at doses which were completely ineffective in PMS-hCG-treated animals (3). Furthermore, pituitary and plasma LH concentrations were lower while plasma prolactin concentrations were higher in PMS-hCG-treated rats (3).

Both estrogen and progesterone have been shown to influence the "spontaneous" as well as the LHRH-induced release of LH (5–7). These steroid hormones also affect prolactin secretion (8, 9). The observed differences in LH and prolactin dynamics in OVX-E_2-P-primed and PMS-hCG-treated rats suggested the desirability of studying peripheral steroid concentrations as a possible causative factor.

Material and Methods. Adult (250–300 g) Sprague–Dawley (Simonsen) female rats were housed under controlled illumination (14 hr light, 10 hr dark). Daily vaginal smears were taken for 2–3 weeks to ascertain cycle regularity before any treatment was applied.

Four groups of animals were studied: (a) *PMS-hCG.* Equine gonadotropin (PMS) (NIAMD-PMSG-1,NIH) was injected sc at a dose of 300 IU in 0.6 ml. Human chorionic gonadotropin (hCG) (Pregnyl, Organon, Inc.) was administered sc 53–56 hr later (120 IU in 0.12 ml). Animals were used 7 days after the injection of PMS. (b) *OVX-E_2-P.* One or two months after ovariectomy, single sc injections of estradiol benzoate (50 μg in 0.05 ml) and progesterone (25 mg in 0.5 ml) were given, and animals were used 3 days later. (c) *OVX.* Ovariectomized controls were used 1–2 months after surgery. (d) *Cycling rats.* These were sampled on diestrus day 2 (D-2) or estrus (E).

Trunk blood was collected after decapitation between 11 AM and 3 PM in the PMS-hCG, OVX-E_2-P, and OVX groups and between 4 and 5 PM in the cycling rats. The blood was allowed to clot at 4–6° for 1–2 hr. After centrifugation, the serum was separated and stored frozen until assayed for steroids.

Progesterone and estradiol were quantitated by the radioimmunoassay method of Abraham and associates (10), using antisera of known specificity prepared and purified according to the procedures recommended by Thorneycroft *et al.* (11).

Student's t test was used to compare the differences between means.

Results and Discussion. Both estradiol and progesterone levels in the PMS-hCG and OVX-E_2-P groups were significantly ($p < 0.001$) higher than in the D-2 or E group (Fig. 1). While estradiol was higher ($p < 0.001$) in OVX-E_2-P than in PMS-hCG ani-

[1] Supported by grants from NIH (NS 01162 and HD-01177) and the Ford Foundation.

[2] Fellow of the Foundations' Fund for Research in Pyschiatry (Grant 72-525).

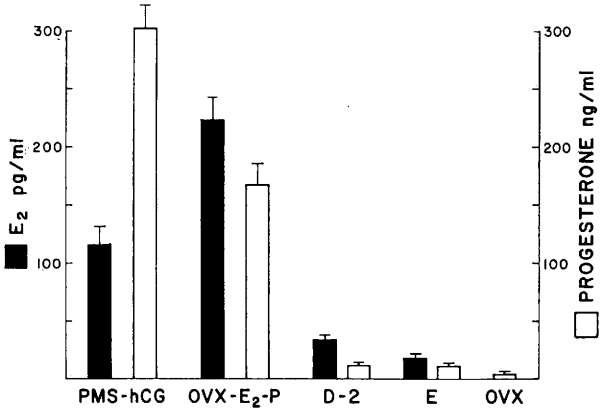

FIG. 1. Serum concentrations (mean ± SE) of estradiol (E_2) and progesterone (P) in cycling rats during estrus (E) and second day of diestrus (D-2), in ovariectomized rats (OVX), in ovariectomized rats primed with estradiol and progesterone (OVX-E_2-P), and in intact PMS-hCG-treated rats. The number of animals per group in the nine columns from left to right in the figure were 10, 19, 8, 14, 5, 5, 5, 5, 5, respectively.

mals, the reverse was the case for progesterone ($p < 0.001$). Also, estradiol levels were higher ($p < 0.01$) in D-2 than in E rats. No significant differences in progesterone values could be observed between D-2 and E animals. The results reported by Barraclough *et al.* (12) on progesterone levels measured by gas–liquid chromatography were somewhat higher than ours irrespective of the day of the cycle. It should be noted that while we collected the blood following rapid decapitation, these investigators took it as a terminal sample after ovarian blood had been collected under pentobarbital anesthesia. They observed differences between changes in ovarian secretion rates and peripheral levels and attributed them to an adrenal source of progesterone. Therefore, the stress factor might explain the discrepancy between their results and ours. Progesterone levels in ovariectomized animals averaged 1.9 ng/ml, which represents approximately 17% of the values found in D-2 or E animals. We assume that this amount is of adrenal origin.

In the groups studied, the ratios of estradiol (pg/ml) to progesterone (ng/ml) were as follows: D-2, 2.80; E, 1.63; OVX-E_2-P, 1.30; and PMS-hCG, 0.38. Differences between the OVX-E_2-P and PMS-hCG animals were due not only to higher progesterone levels but also to lower estradiol values in the PMS-hCG group. This difference could be dependent on a long-term sustained steroid secretion by the PMS-hCG-stimulated ovaries as compared with conditions in OVX-E_2-P-primed animals in which the relatively short-term exogenous steroid levels are mainly a function of the metabolic clearance rate.

These results, considered with the past hormonal history of the two conditions, give a basis for explanation of the differences in LH dynamics (3). Estradiol has been reported to increase the responsiveness to LHRH in intact rats, while progesterone seems to have the opposite effect (5–7). When both steroids are given to intact animals, a further decrease in responsiveness to LHRH results (7). This latter condition probably resembles to some extent the state in PMS-hCG animals, inasmuch as the increased (exogenous) steroid concentration is superimposed on an already equilibrated pituitary–gonad secretory system. The prolonged action of high estrogen levels in the PMS-hCG rats appears to have inhibited LH synthesis and depleted the pituitary content of LH while fostering prolactin secretion. The high progesterone levels do not inhibit prolactin secretion but may elevate the threshold to exogenous LHRH.

It would appear that the relatively short-term action of high levels of estrogen and progesterone applied to the OVX rat at the height of LH secretion blocks the "spontane-

ous" release by shutting off the discharge of endogenous LHRH, an inhibitory action exerted at the hypothamic level, while lowering the threshold to exogenous LHRH at the pituitary level. Progesterone does not seem to be high enough to reverse estrogen's facilitatory action at the pituitary, whereas the estrogenic action is perhaps too brief to foster a high level of prolactin secretion.

It has been reported that the effects of "electrochemical stimulation" of the median eminence–arcuate complex or the medial preoptic area on LH and prolactin depend on the steroid environment (13, 14). We would completely agree that steroid levels affect the final gonadotropin output to any particular stimulus, but our results do not support the idea that progesterone dominance is necessarily inhibitory to prolactin release. Although the estrogen:progesterone ratio seems to be one of the significant factors in this respect, the results could be dependent on their absolute values as a function of time. This assumption would find support in the fact that levels of both steroids are high in PMS-hCG and OVX-E_2-P animals, yet prolactin release is fostered in the first condition in which progesterone levels are higher. In rats made pseudopregnant by cervical stimulation, progesterone levels are high during the first days of pseudopregnancy (15), as are the circulating prolactin levels (16). The exact sequence is not sufficiently clear to ascertain whether prolactin levels are high due to, or in spite of, elevated peripheral concentrations of progesterone. At any rate, there seems to be no doubt that under certain conditions, high circulating levels of prolactin and progesterone do coexist.

Summary. Estradiol (E_2) and progesterone (P) were measured by radioimmunoassay in peripheral blood serum on the second day of diestrus (D-2) and estrus (E) in normal cycling rats, in ovariectomized (OVX) animals, and in PMS-hCG- and OVX-E_2-P-treated rats. PMS-hCG and OVX-E_2-P animals had significantly higher estradiol and progesterone levels than cycling rats on D-2 or E. Progesterone was significantly higher in the PMS-hCG group while estradiol was higher in OVX-E_2-P-treated rats. These results may provide an explanation of previous findings relative to LH dynamics in OVX-E_2-P- and PMS-hCG-treated animals.

We thank Mrs. Ruth Penardi, Miss Katherine Bangs, and Mrs. Frances Smith for technical and secretarial help and Mr. Bobby McAlister for drawing the figure.

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Received July 30, 1973. P.S.E.B.M., 1973, Vol. 144.