Pituitary LH Response to LHRH in Rats After Treatment with PMS-hCG or Ovariectomy Followed with Estrogen and Progesterone[1] (37729)

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We have recently reported that the induction of cortical spreading depression in female rats is followed by the release of either pituitary LH or prolactin, depending on the hormonal status of the animal: LH release (1) in the ovariectomized estrogen and progesterone ($OVX-E_2-P$)-treated subject (2) and prolactin release (3) in the PMS-hCG "pseudopregnant" rat (4). These contrasting specific responses to the same neural input suggested that brain or pituitary thresholds might have been altered differentially by the hormonal environment established by the two treatments. The effects of cortical spreading depression on the multiple unit electrical activity of the hypothalamus were similar in PMS-hCG- and $OVX-E_2-P$-treated rats (5), suggesting that the critical changes might be occurring at the pituitary level. Furthermore, ovarian steroids have a demonstrated capacity to influence pituitary responsiveness to injected median eminence extracts and natural or synthetic luteinizing hormone releasing hormone (LHRH) (6–13). It therefore became desirable to test pituitary responsiveness to LHRH in PMS-hCG- and $OVX-E_2-P$-treated rats. Pituitary concentrations and contents of LH and prolactin in PMS-hCG-treated animals are also reported.

Material and Methods. Adult (250–300 g) Sprague–Dawley (Simonsen) female rats were maintained under controlled illumination (14 hr light, 10 hr darkness). One group was ovariectomized and treated 1–2 months later with a single sc injection of estradiol benzoate (50 μg/0.05 ml) and progesterone (25 mg/0.5 ml). They were used on the third day after treatment. Another group of intact females was treated with sc injections of 300 IU in 0.6 ml of equine gonadotropin (PMS) (NIAMDD-PMSG-1,NIH) and 120 IU in 0.12 ml of human chorionic gonadotropin (hCG) (Pregnyl, Organon, Inc.) 53–56 hr later. They were used 1 week after the PMS was injected.

Continuous ether anesthesia was employed through the experimental period. One femoral artery was cannulated with a heparinized polyethylene tube (PE 50) which was used for both blood sampling and LHRH administration. After surgery was completed, 50–70-min recovery time was allowed before sampling was started. Under continued ether anesthesia, two control blood samples (0.5 ml) were taken 20 min apart before LHRH was administered. Subsequent samples were drawn at 10, 20, 40, and 80 min. After centrifugation, plasma was stored frozen prior to RIA procedures, and blood cells were resuspended in physiological saline for reinjection following withdrawal of the next sample. Synthetic LHRH was kept in a stock solution in 0.1 *N* HCl. Immediately before injection, a dilution of 200 ng/ml in normal saline was prepared, and the pH adjusted to 6.5–7.0 with phosphate buffer. Luteinizing hormone releasing hormone was injected at doses of 40 ng in 0.2 ml or 100 ng in 0.5 ml/animal.

Another group of PMS-hCG-treated rats were rapidly (10–15 secs) killed by decapitation between 8:15 and 8:30 AM or 6:15 and 6:30 PM (1815 and 1830). The anterior

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

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

pituitaries were rapidly removed, weighed, and homogenized in culture tubes containing sea sand (Merck) and cold phosphate buffer at pH 7.0. The homogenate was diluted to 0.5 or 1.0 mg/ml, centrifuged, and the supernate stored frozen for radioimmunoassay (RIA).

Plasma and pituitary luteinizing hormone and prolactin were measured in duplicate by RIA using NIAMDD-Rat LH-RP 1 and NIAMDD-Rat Prolactin-RP-1 as standards. Means were compared applying Student's *t* test.

Results. Luteinizing hormone responses in the two groups are shown graphically in Fig. 1. Control levels of plasma LH in the OVX-E_2-P rats were fairly stable and consistently higher (110 ± 20 ng/ml) than those in animals receiving PMS-hCG (< 30 ng/ml). Following injection of LHRH in the OVX-E_2-P group, maximum values of plasma LH were attained by 10 min with either the 40- or 100-ng dosage. This was followed by a somewhat more gradual decline, with the 40-ng group reaching control levels of plasma LH by 80 min after treatment. The 100-ng LHRH group peaked at roughly twice the maximum value reached by the 40-ng subjects and had not dropped to the control level at the last bleeding 80 min after injection.

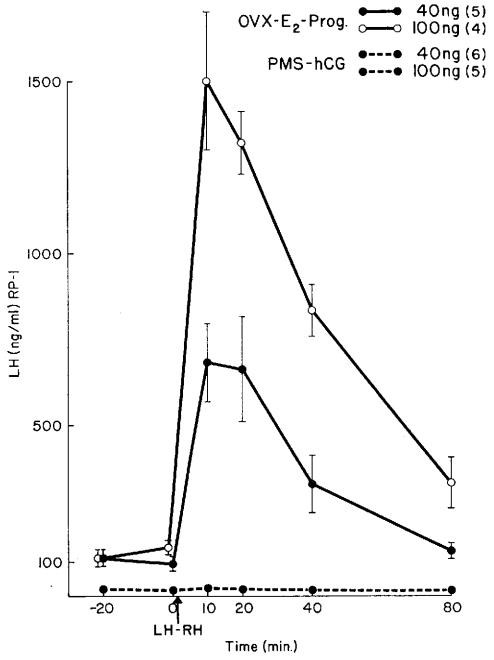

FIG. 1. Effects of intravascular injection of LHRH on plasma LH in PMS-hCG- and ovariectomized estrogen- and progesterone-treated rats.

At marked variance with the responses observed in the OVX-E_2-P group, plasma LH values in PMS-hCG animals failed to show any rise following injection of either LHRH dosage (Fig. 1). In fact, the LH values remained consistently lower than 30 ng/ml, practically at the limit of sensitivity of the assay (≅ 20 ng/ml), throughout the whole 80-min period after treatment with LHRH.

Pituitary luteinizing hormone and prolactin concentrations (μg/mg) and contents (ng/gland) are shown in Table I. Values are quoted relative to the time of day at which autopsy was performed. Prolactin content showed a tendency, approaching statistical significance, to be higher in animals sacrificed in the late afternoon while LH content showed a trend in the opposite direction. The most striking items are the relatively very low values for concentration and content of pituitary LH, which are to be discussed below.

Discussion. Control plasma luteinizing hormone levels in PMS-hCG animals were consistently lower than in OVX-E_2-P rats and were close to or actually below the sensitivity of the assay. These levels are at variance with those reported previously by us (1) using ovine LH in the radioimmunoassay. The possibility exists of a cross-reaction in the RIA system between the injected hCG and ovine LH. Anyhow, when rat LH was used in the RIA, plasma LH levels remained consistently low and were not affected by cortical spreading depression (unpublished results), a finding in full accord with our previous report. In fact, the present results show that even after intra-arterial injection of LHRH at doses up to 100 ng/rat, plasma LH concentrations did not rise above control values. On the contrary, in OVX-E_2-P animals, 40 ng LHRH induced a 6–7-fold increase and 100 ng a 15-fold increase in plasma LH, both with peaks at 10 min. With the higher

TABLE I. Pituitary LH and Prolactin Concentrations and Total Contents in PMS-hCG Treated Rats.

Time	N	Pituitary weight (mg)	Prolactin		LH	
			μg/mg	μg/pit.	μg/mg	μg/pit.
AM (0815–0830)	4	18.45 ± 1.73[a]	1.07 ± 0.04	19.01 ± 1.29	4.73 ± 0.65	88.60 ± 13.15
PM (1815–1830)	4	19.50 ± 1.21	1.21 ± 0.07	23.37 ± 0.64[b]	3.27 ± 0.55	65.57 ± 14.39
Total	8	18.97 ± 1.00	1.14 ± 0.08	21.2 ± 1.06	4.01 ± 0.45	75.44 ± 10.27

[a] Mean ± SE.
[b] $p = 0.025$.

dose, the LH values were still elevated above control concentrations at 80 min after LHRH injection, a finding in accord with results reported by Arimura *et al.* (14), who injected LHRH iv to rats under urethan anesthesia.

These results suggest that the pituitary gland in PMS-hCG-treated animals has either a high threshold to LHRH or a low content of LH, or both. A very low pituitary LH concentration is apparent in our PMS-hCG rats when compared with the OVX and OVX-E_2-P results reported by Blake *et al.* (15); LH concentrations in pituitaries of OVX rats were 20 times and OVX-E_2-P rats 50 times as great as in PMS-hCG animals. The additional observation of low to undetectable concentrations of LH in plasma indicate that LH synthesis is drastically reduced in these rats. According to Novello *et al.* (16), pituitary LH stores are also low in immature PMS-hCG-treated rats, a relationship suggested earlier by McCann *et al.* (17).

Among several possible factors that might be involved in determining the differences in LH dynamics in PMS-hCG and OVX-E_2-P animals are two types of feedback agents: ovarian steroids and the injected gonadotropins. To consider the latter first, the PMS and/or hCG might be acting through a short feedback loop to affect LH dynamics (18). Their presence is suspect because of the aberrant results involving possible cross-reaction with ovine LH in the RIA mentioned above. Hirono *et al.* (19) reported decreased plasma LH values in ovariectomized and cycling rats 1 week after implanting hCG into the median eminence. Whether or not a single injection of hCG would inhibit pituitary LH secretion a week later remains to be proved.

The ovarian steroids secreted in response to the gonadotropins in PMS-hCG-treated rats and supplied in OVX-E_2-P animals undoubtedly influence pituitary LH secretion. It has been reported that estradiol increases pituitary LH concentration and content in ovariectomized rats and that progesterone, in the time and dose relationships employed, does not counteract the estradiol influence (15, 20). Both steroids block the "spontaneous" release of LH in ovariectomized rats (21), resulting in an increase of pituitary LH stores. While OVX-steroid-treated animals are highly sensitive to LHRH as shown here and by others (12, 22), a decreased response develops in intact rats treated simultaneously with the two steroids (13). When given alone to intact female rats, estrogen increases and progesterone decreases the response to median eminence extracts or LHRH (8, 9, 12, 13). The same is true in rabbits (10). Thus, the immediate past hormonal history and estrogen/progesterone ratio appear to be important factors in determining the actual pituitary response. The PMS-hCG condition probably relates more closely with the intact animal treated with steroids than it does with the OVX-E_2-P rat. Direct measurements of circulating estrogen and progesterone in these rats will be made the subject of a separate communication.

With respect to prolactin, it has been shown that daily treatment with low doses of estradiol increases both pituitary and serum concentrations of prolactin in OVX animals and that progesterone counteracts the latter response in doses which do not

affect the pituitary prolactin concentration (23). Chronic treatment with large doses of progesterone alone not only fails to inhibit but actually slightly stimulates prolactin secretion (21, 23). Our values for pituitary concentration and content of prolactin in PMS-hCG rats do not differ significantly from those reported in OVX-E_2-P animals by Blake *et al.* (15). However, plasma prolactin levels are higher in PMS-hCG rats either under continuous ether anesthesia (3) or fully awake (unpublished results). These data suggest an increased pituitary secretion in PMS-hCG-treated rats when compared with OVX-E_2-P-primed animals.

When these results are considered with respect to our previously reported effects of cortical spreading depression on plasma LH and prolactin levels (1, 3), it seems reasonable to conclude that those contrasting results would depend at least partially on differential responsiveness at the pituitary level to the steroid ratios in OVX-E_2-P- and PMS-hCG-treated animals. This, of course, does not rule out the possibility that these same differences in steroid ratios may also affect brain thresholds and the synthesis and release of endogenous LHRH as well.

Summary. Long-term ovariectomized rats primed with estrogen and progesterone responded to exogenous LHRH at dosages of 40 and 100 ng with a sharp rise in plasma LH, peaking at 10–20 min after injection and gradually returning to control levels. In contrast, intact rats treated a week earlier with PMS followed by hCG completely failed to respond to even the higher dosage of LHRH: plasma LH levels remained almost undetectable in these animals. Assays of pituitary extracts revealed a similar concentration and content of prolactin but very depressed values for pituitary LH in these PMS-hCG-treated rats compared with earlier studies in OVX-E_2-P animals.

We thank Dr. A. V. Schally for supplying the synthetic LHRH and Dr. A. F. Parlow and the NIAMDD Hormone Distribution Program for making the RIA kits available. Ms. Frances Smith, Virginia Hoover, and Katherine Bangs and Mr. David Whitmoyer supplied valuable technical assistance.

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Received July 23, 1973. P.S.E.B.M., 1973, Vol. 144.