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Inhibitory and Facilitatory 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 (38026)

K. J. COOPER,[1] C. P. FAWCETT AND S. M. MCCANN[2]

Department of Physiology, The University of Texas Southwestern Medical School at Dallas, Dallas, Texas 75235

Several recent reports have indicated that the pituitary gland of the female rat exhibits an enhanced response to LH-RF/FSH-RF stimulation during late diestrus day 2 and proestrus which corresponds closely with the period of heightened estrogen secretion (1–4). However, estrogen treatment in the ovariectomized rat has been shown to both inhibit and augment pituitary sensitivity to releasing factor therapy. Low doses of estradiol 17β (100 ng) depressed pituitary responsiveness to synthetic LH-RF 3 hr after iv injection (5) while pharmacological levels of estradiol benzoate (50 μg) increased LH-RF/FSH-RF-evoked secretion of LH *in vivo* and LH and FSH release *in vitro* 72 hr after estrogen treatment (6). The object of the study reported here was to determine whether low doses of estradiol 17β could enhance pituitary sensitivity to a releasing factor preparation known to possess both LH and FSH-releasing properties subsequent to the initial inhibitory action, and to determine the time course for such a response.

Material and Methods. All animals were adult female Sprague-Dawley rats weighing 220–250 g and maintained under a lighting regime of 14 hr light/10 hr dark (lights on at 5 AM) at a temperature of 21 ± 2°C. Vaginal smear cytology was observed daily and only those animals exhibiting regular four day estrous cycles were allocated to the experiment. All animals were ovariectomized and retained for a period of three weeks before use. The effects of an LH-RF/FSH-RF-evoked stimulation of LH and FSH release were examined both *in vivo* and *in vitro* at various times following injection of saline or estradiol 17β. The estrogen solution was prepared by dissolving estradiol 17β in a very small volume of ethanol and diluting with physiological saline to a concentration of 200 ng in 200 μl for each injection. The estrogen was administered iv at intervals of 3, 6 or 9 hr before treatment with the releasing factor extract of ovine hypothalami which had been purified by ultrafiltration and gel filtration (1, 2).

For the *in vivo* experiments, the releasing factor preparation was used at a single dose of 100 μl diluted to 200 μl with physiological saline. This dose was chosen to lie near the middle of the previously determined linear log dose-response curve obtained with the extract in diestrous animals (2). The diluted stimulant was introduced over a period of 60 sec into the jugular vein of saline control or estrogen-treated, ether-anesthetized animals (8–10 animals/group) immediately after obtaining a control pre-injection blood sample (1 ml). Subsequent blood samples were removed similarly 10, 20 and 30 min later. Control rats were injected with LH-RF/FSH-RF at 3 (3 rats), 6 (3 rats) and 9 hr

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(4 rats) after saline injection. Since the LH and FSH increments were similar at all times, the results were pooled to form a single saline control group.

For the *in vitro* study, the pituitary glands of estrogen or saline-treated rats were removed, divided into halves and randomly distributed among the incubation flasks to reach a total of four hemipituitaries per flask (5–6 flasks/group). They were preincubated for 30 min in medium 199 under an atmosphere of 95% O_2 and 5% CO_2 at a pH of 7.2 and a temperature of 37°C which was immediately followed by a 3 hr incubation with the releasing factor preparation or medium alone. The hypothalamic extract was used at a single dose of 1 μl added to the medium of each flask and the pH readjusted to 7.2 if necessary. This dose was near the middle of the linear log dose-response curve obtained with the preparation with diestrous animals. The *in vivo* and *in vitro* LH and FSH values obtained from injection or incubation of the same doses of releasing factor material in diestrus day 1 animals are also included for comparative purposes.

The plasma and incubation medium LH and FSH concentrations were estimated by radioimmunoassay using the method of Niswender *et al.* (7) for LH, expressed as ng/ml NIH-LH-S1, and using the appropriate kits supplied by NIAMD for FSH, expressed as ng/ml in terms of the RP-1 reference preparation supplied with the kits.[3] Statistical evaluation of the differences between means was by the paired *t* test for the *in vivo* experiments, and by analysis of variance for the *in vitro* data.

Results. Effect of pre-treatment with estrogen on pituitary responsiveness to an LH-RF/FSH-RF preparation in vivo. The pre-injection LH and FSH levels in saline-pretreated, ovariectomized controls were markedly elevated above the levels for diestrous animals (Tables I and II). Administration of 200 ng of estradiol 17β significantly depressed endogenous LH release for up to 9 hr ($P < 0.01$), although the plasma LH concentration did not fall to the level found in diestrous controls. Plasma FSH titres remained unaffected by estrogen treatment.

Injection of the LH-RF/FSH-RF preparation caused a significant rise in plasma LH within 10 min in saline and estrogen-

[3] Antiovine LH was provided through the courtesy of Dr. G. Niswender (Colorado State University) and Dr. L. Reichert (Emory University) generously supplied the purified ovine LH for radioiodination. Kits for the determination of FSH were obtained through the NIAMD-NIH Pituitary Hormone Program.

TABLE I. Effect of a Single Injection of an LH-RF/FSH-RF Preparation on Plasma LH in Diestrous Rats, and in Saline or Estrogen-Treated, Ovariectomized Rats.

Group	No. of rats	Plasma LH (ng/ml)		
		Initial	Increment after injection at: 10 min	30 min
Diestrus (day 1)	8	1.6 ± 0.4[a]	6.6 ± 0.7**	3.7 ± 0.6
Ovariectomized				
Saline control	10	15.6 ± 2.4	8.7 ± 1.7*,****	4.0 ± 1.3
3 hr after estrogen	10	5.6 ± 1.1	2.3 ± 0.4*,***	1.2 ± 0.3
6 hr after estrogen	10	6.8 ± 0.9	7.3 ± 1.4**	4.4 ± 1.0
9 hr after estrogen	10	7.2 ± 1.2	15.3 ± 1.9**,****	8.5 ± 1.7

[a] Mean ± SEM.

* $P < 0.05$ for LH-RF/FSH-RF evoked release.

** $P < 0.01$ for LH-RF/FSH-RF evoked release.

*** $P < 0.01$ compared to all other groups at 10 min.

**** $P < 0.025$ for saline control vs estrogen treated at 9 hr.

TABLE II. Effect of a Single Injection of an LH-RF/FSH-RF Preparation on FSH Release in Diestrous Rats, and in Saline or Estrogen-Treated Ovariectomized Rats.

Group	No. of rats	Plasma FSH (ng/ml)		
		Initial	Increment after injection at: 10 min	30 min
Diestrus (day 1)	8	192 ± 21[a]	21 ± 14	32 ± 12
Ovariectomized				
Saline control	10	1843 ± 319	47 ± 37	42 ± 35
3 hr after estrogen	10	1509 ± 294	58 ± 26	5 ± 8
6 hr after estrogen	10	1924 ± 253	71 ± 21	60 ± 25
9 hr after estrogen	10	1896 ± 247	52 ± 22	161 ± 36*

[a] Mean ± SEM.

* $P < 0.05$ for LH-RF/FSH-RF evoked release at 30 min compared to 10 min.

injected animals which decreased during the next 20 min. Pituitary LH response to releasing factor stimulation, which was significantly inhibited 3 hr after estrogen therapy, returned to the saline-treated control level by 6 hr and was significantly augmented by 9 hr. LH-RF/FSH-RF stimulation also significantly raised plasma FSH concentrations within 30 min after injection in rats injected with estrogen 9 hr earlier.

Effect of pre-treatment with estrogen on pituitary responsiveness to an LH-RF/FSH-RF preparation in vitro. There was a significant suppression of unstimulated LH and FSH release as a result of estrogen treatment when the glands were removed at 3 hr after the injection which returned to the saline control level by 6–9 hr (Tables III and IV). However, the lowered unstimulated value at 3 hr was still significantly above that observed for diestrus day 1 rats. Addition of the releasing factor preparation elevated the secretion of both LH and FSH in all treated groups, the gonadotropin contents reaching a significantly higher level for both FSH and LH 6–9 hr after estrogen injection than in any of the other groups.

Discussion. The ability of intravenous estradiol to inhibit LH release within 3 hr of its injection in ovariectomized rats is confirmed by the present study *in vivo* for LH and *in vitro* for the unstimulated glands for both LH and FSH. Furthermore, the continuing inhibition of endogenous release with this low dose of estrogen, which persisted for at least 9 hr in the present experi-

TABLE III. Effect of an LH-RF/FSH-RF Preparation on LH Release from Anterior Pituitaries Incubated *in vitro* from Diestrous, and from Saline or Estrogen-Treated, Ovariectomized Rats.

Group	LH Output (ng/ml/3 hr)	
	Unstimulated (5)	Stimulated (6)[a]
Diestrus	105 ± 21	227 ± 29*
Ovariectomized		
Saline-injected control	1664 ± 148	2429 ± 144*
3 hr after estrogen	1132 ± 94**	2151 ± 178*
6–9 hrs after estrogen	1875 ± 167	3903 ± 238*,***

[a] Number of flasks/group in parenthesis.

* $P < 0.01$ for LH-RF/FSH-RF induced LH release for each treatment group.

** $P < 0.01$ for unstimulated release vs all other ovariectomized groups.

*** $P < 0.01$ for stimulated release vs all other ovariectomized groups.

TABLE IV. Effect of an LH-RF/FSH-RF Preparation on FSH Release from Anterior Pituitaries Incubated *in vitro* from Diestrous, and from Saline or Estrogen-Treated Ovariectomized Rats.[a]

Group	FSH output (ng/ml/3 hr)	
	Unstimulated (5) [a]	Stimulated (6) [a]
Diestrus	2507 ± 224	3962 ± 268*
Ovariectomized		8815 ± 457*
Saline-injected control	6831 ± 434	8178 ± 519*
3 hr after estrogen	4975 ± 361**	14746 ± 978*,***
6–9 hrs after estrogen	8015 ± 513	

[a] Number of flasks/group in parenthesis.
* $P < 0.01$ for LH-RF/FSH-RF induced FSH release for each treatment group.
** $P < 0.01$ for unstimulated release vs all other ovariectomized groups.
*** $P < 0.01$ for stimulated release vs all other ovariectomized groups.

ment, is also similar to that reported by Negro-Vilar *et al.* (5) who used either 100 or 1000 ng of estradiol 17β and observed that plasma LH was lowered for at least 6 hr. The initial decline in plasma LH was accompanied by reduced sensitivity to the LH-RF/FSH-RF *in vivo* at 3 hr; however, there was no suppression of the stimulation by the hypothalamic preparation with pituitaries of animals sacrificed at 3 hr after estrogen and incubated for 3 hr in the presence of the LH-RF/FSH-RF preparation. This may reflect the relatively short duration of the inhibition which was also not apparent at 6 hr both *in vivo* and *in vitro*. Plasma LH levels remained low in the estrogen-treated animals at both 6 and 9 hr in spite of the return to normal sensitivity at 6 hr and the enhanced responsiveness at 9 hr to LH-RF/FSH-RF. This dichotomy between the lowered endogenous release of LH by the glands and the augmented responsiveness to releasing factors suggests that the inhibitory effect of estrogen persisted at the hypothalamic level (8) longer than at the pituitary.

At 9 hr after injection of estrogen, pituitary responsiveness to the hypothalamic preparation was enhanced *in vivo* for LH and possibly also for FSH at 30 min after injection, and was clearly enhanced for both hormones *in vitro*. The relatively small effects of the extract on FSH release *in vivo* are in agreement with other work from this laboratory with single injections of the synthetic decapeptide (5). Apparently, for the FSH-secreting cell to respond, it requires a relatively long exposure to hypothalamic principles which can be obtained either by infusion of the decapeptide (9), by subcutaneous injection (10), or by incubation with glands *in vitro*.

The causes of the alterations in hypophyseal sensitivity to releasing stimulation induced by estradiol are not known. The augmented responsiveness at 9 hr might be the result of the continuing estrogen-induced inhibition of endogenous hormone secretion thereby resulting in accumulation of a readily releasable pool of gonadotropins in the gland. Since it was earlier suggested that the steroid influences might alter peptide or protein synthesis in the gland to produce specific inhibitory or stimulatory proteins which could inhibit or stimulate release respectively (11), the possibility remains that estrogen may induce a more fundamental alteration in the releasing process.

Whatever the mechanism by which estrogen exerts an influence on the pituitary, it seems likely that the greater sensitivity to LH-RF/FSH-RF injections during proestrus reported by Cooper *et al.* (1, 2), Aiyer *et al.* (3) and Martin *et al.* (4) is the result of the rapid rise in estrogen secretion between diestrus day 2 and the morning of proestrus in the normal cyclic rat. These results further emphasize the importance of fluctuations in steroid secretion during the estrous cycle as a mechanism for regulating the degree to which the pitui-

tary gland is able to respond to the secretion of hypothalamic neurohormones, and thereby determine the timing of events in the reproductive cycle.

Summary. A single intravenous injection of 200 ng of estradiol significantly depressed both LH release *in vivo* for up to 9 hr and unstimulated LH and FSH release *in vitro* at 3 hr. The releasing factor preparation evoked significantly less LH release *in vivo* at 3 hr after estradiol than in saline-injected controls; however, by 6–9 hr after estradiol, the response to LH-RF/FSH-RF stimulation was noticeably enhanced for both LH and FSH release *in vitro* and *in vivo* when compared to the response in the ovariectomized controls, even though plasma LH was still suppressed.

It is concluded that subsequent to an initial suppression of gonadotroph sensitivity to LH-RF/FSH-RF stimulation, there is an augmented hypophyseal responsiveness to the releasing factor following estrogen treatment.

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