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### Permalink

<https://escholarship.org/uc/item/53q3j02p>

### Journal

Neuroendocrinology, 23(3)

### ISSN

0028-3835

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### Publication Date

1977

### DOI

10.1159/000122663

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## **Evidence for the Dependence of Serum Luteinizing Hormone Surge on a Transient, Enhanced Secretion of Gonadotropin-Releasing Hormone from the Hypothalamus**

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**Key Words.** Gonadotropin-releasing hormone · Hypothalamus · LH surge · Nembutal blockade · Ovariectomized estradiol-treated rats

**Abstract.** Data that a substantial, transient release of gonadotropin-releasing hormone (GnRH) from the hypothalamus is a prerequisite for the serum luteinizing hormone (LH) surge are presented. Ovariectomized rats, in which daily afternoon LH peaks can be induced by estradiol benzoate (EB), were used as the experimental model. These rats present a homogenous, synchronized population having low hypothalamic stores of GnRH, thus facilitating detection of small physiological fluctuations in the levels of hypothalamic GnRH. Blockade, by Nembutal administration, of the serum LH surge on 2 consecutive afternoons results in elevated GnRH levels in the hypothalamus (1.79 ng in blocked rats vs 0.94 ng in controls). Abolition of LH secretion by administration of antiserum to GnRH, unlike the Nembutal blockade, does not affect GnRH levels. These results indicate that the afternoon LH surge is dependent on a transitory, enhanced release of GnRH from the hypothalamus, reflected by a depletion of GnRH stores.

In order to demonstrate the physiological role of gonadotropin-releasing hormone (GnRH) in the regulation of luteinizing hormone (LH) release, correlations have been sought between the secretion of these hormones during various physiological states [SORRENTINO and SUNDBERG, 1975]. Reliable measurements of GnRH in serum are currently unavailable [DE LA CRUZ and ARIMURA, 1975], due to secretion of minute amounts and the rapid clearance of the hormone [CLEMENS *et al.*, 1975]. We have, therefore, looked for a

<sup>1</sup> In partial fulfillment of the requirements for the Ph.D. degree of the Graduate School of the Weizmann Institute of Science.

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Received: October 4th, 1976; revised MS accepted: February 4th, 1977.

model in which gonadotropin release may be attributed to a concurrent disappearance of GnRH from the hypothalamus.

Massive gonadotropin secretion occurs during the afternoon of proestrus in cyclic female rats and also after disruption of the negative feedback by ovariectomy. In the latter instance, the high serum levels of LH are accompanied by an 80% reduction in hypothalamic GnRH content [SHIN and HOWITT, 1974]. Estradiol benzoate (EB) administration to ovariectomized rats lowers serum LH levels markedly, and daily afternoon 'surges' resembling the proestrous LH peak occur for the following 3 days [LEGAN *et al.*, 1975]. Both the proestrous and the EB-induced LH surge can be postponed for 24 h by the administration of pentobarbital (Nembutal) at 13.00 h [HOFFMAN and SCHWARTZ, 1965]

The ovariectomized, EB-treated rat was used as an experimental model for establishing a relationship between hypothalamic GnRH content and the LH surge.

### *Materials and Methods*

Wistar-derived female rats of the departmental colony were ovariectomized at 8–10 weeks of age and 2–3 weeks later were given s.c. injections of 50  $\mu\text{g}$  of EB (Ikapharm, Ramat-Gan, Israel) in peanut oil. Rats were sacrificed by decapitation and trunk blood was collected for LH measurement. GnRH was extracted as previously described [KOCH and BARAM, 1976]: the medial basal hypothalamus, consisting of the median eminence and the arcuate and lower ventromedial nuclei (average weight, 8 mg), was excised within 2 min. Tissues, immersed in 1 ml of 0.01 M phosphate-buffered saline (pH 6.9) were boiled for 3 min, homogenized, reboiled and spun for 10 min at  $12,000 \times g$ . The supernatant was analyzed for GnRH by radioimmunoassay [KOCH *et al.*, 1973]. Serum LH was determined by the double antibody radioimmunoassay technique [DAANE and PARLOW, 1971], using kits kindly provided by the National Institute of Arthritis and Metabolic Diseases Rat Pituitary Program. Results are expressed in terms of the RP-1 reference preparation.

*Experimental design: Experiment A.* Ovariectomized rats were given s.c. injections of EB (50  $\mu\text{g}$ ) on the morning of day I. On day III at 13.00 h, a group of rats ('once-blocked') received an i.p. injection of pentobarbital (Nembutal, Abbott, France; 35 mg/kg). Another group ('twice-blocked'), received additional Nembutal doses on the following day at 12.00 and 14.00 h (20 mg/kg), while a 3rd group served as controls. All rats were sacrificed on day IV at 16.00 h, when the serum LH-surge is maximal.

*Experiment B.* The first experiment was repeated, except for the timing. Nembutal was injected at 09.00 on day III and at 08.00 and 10.00 h on day IV; animals were killed at noon on day IV.

*Experiment C.* Antiserum to GnRH (anti-GnRH) [KOCH *et al.*, 1973] or normal rabbit serum was administered (0.5 ml, i.p.) to ovariectomized, EB-treated rats at 11.30 h on day III. The rats were bled by heart puncture at 16.00 h on day III and were sacrificed at 16.00 h on day IV.

### *Results*

Ovariectomy causes a dramatic depletion of hypothalamic GnRH stores, which are partially repleted by EB (table I). The steroid lowers the high post-castration levels of serum LH (table I) and induces daily afternoon surges of LH, which are accompanied by a decrease in hypothalamic GnRH content (table II). These surges, and the concurrent GnRH depletion, may be abolished by the administration of Nembutal at 12.00–14.00 h, the critical period for the LH surge; the LH surge on the following day is exceptionally large (1,681 vs 913 ng/ml; table II). A morning injection, at 09.00 h, does not influence the amount of GnRH in the medial basal hypothalamus (table II). When the LH surge is blocked on 2 consecutive days by the administration of Nembutal, the amounts of hypothalamic GnRH found are twice as high as those of untreated controls (table II).

The EB-induced surge of LH in ovariectomized rats is GnRH-dependent, as it can be prevented by the administration of anti-GnRH (table III). The amounts of GnRH in hypothalami of anti-GnRH-treated rats are not significantly different from those of control animals (table III).

### *Discussion*

Seeking evidence for the dependence of the LH surge on a transient depletion of GnRH from the hypothalamus, we have used the ovariectomized, EB-treated rat, an animal model which offers several advantages: (1) hypothalamic GnRH stores are reduced, and inter-animal variations are minimal, so that even small fluctuations in GnRH levels are accentuated; (2) EB treatment of an ovariectomized rat lowers the high post-castration serum LH levels and induces daily afternoon surges resembling those of proestrus, which are likewise amenable to manipulation by Nembutal. We have abolished the LH surge for 2 consecutive days by daily administration of Nembutal. Such 'twice-blocked' rats were found to have twice the control amount (1.79 vs 0.94 ng/rat) of hypothalamic GnRH (table II). The accumulation of hypothalamic GnRH is not due to nonspecific or toxic effects of Nembutal, since it occurs only if the anaesthetic is given at the critical time for the LH surge (table II); this suggests that the release of GnRH, resulting in depletion of hypothalamic stores, causes the LH-surge. Admittedly, the hypothalamic depot of GnRH is determined by a dynamic equilibrium between input and output, i.e. the synthesis and the degradation or secretion of the peptide,

**Table I.** Effects of ovariectomy and EB replacement on hypothalamic GnRH content and serum LH levels<sup>1</sup>

Treatment	GnRH (ng/hypothalamus)	Serum LH (ng/ml)
Intact (diestrous)	2.75 ± 0.3 <sup>2</sup>	50 ± 3.1
Ovariectomized	0.58 ± 0.1*	1,129 ± 156*
Ovariectomized + EB, 50 µg/rat	0.91 ± 0.1**	235 ± 18**

<sup>1</sup> Rats were treated with EB 2 weeks after castration and were sacrificed on the morning of the 4th day after treatment.

<sup>2</sup> Mean ± SEM for 6–12 rats.

\* p < 0.001 compared to intact.

\*\* p < 0.05 compared to ovariectomized.

**Table II.** Effect of Nembutal blockade of LH surge on hypothalamic content of GnRH

Nembutal treatment	Hypothalamic GnRH, ng/hypothalamus ± SEM	Serum LH, ng/ml ± SEM
Twice-blocked <sup>1</sup>	1.79 ± 0.29	459 ± 48
Once-blocked <sup>1</sup>	1.29 ± 0.09	1,681 ± 197**
Control	0.94 ± 0.06*	913 ± 99**
Twice-blocked, morning <sup>2</sup>	1.04 ± 0.06	276 ± 39
Once-blocked, morning <sup>2</sup>	1.09 ± 0.08	361 ± 89
Control	1.01 ± 0.07	223 ± 24

<sup>1</sup> Nembutal (35 mg/kg) was injected at 13.00 h on day III, and to twice-blocked rats also at 12.00 and 14.00 h on day IV (20 mg/kg each). Rats were killed at 16.00 h on day IV (see experiment A in text).

<sup>2</sup> Nembutal was injected at 09.00 h on day III, and to twice-blocked rats also at 08.00 and 10.00 h on day IV. Animals were sacrificed on day IV, at noon (see experiment B in text).

Comparison with twice-blocked by Student's *t*-test: \*p < 0.02; \*\*p < 0.01; N = 12–17.

**Table III.** Effects of anti-GnRH serum on hypothalamic GnRH content and serum LH levels of ovariectomized, EB-treated rats<sup>1</sup>

Treatment	GnRH in medial basal hypothalamus, ng ± SEM	Serum LH, ng/ml ± SEM
Normal rabbit serum	1.09 ± 0.08	780 ± 143.0
Anti-GnRH	1.02 ± 0.12	85 ± 5.5*

<sup>1</sup> Anti-GnRH serum or normal rabbit serum was injected on the morning of the 3rd day after EB treatment (day III, see experiment C in text). Animals (6/group) were sacrificed at 16.00 h on day IV.

\* Significantly different from normal serum treatment, p < 0.001.

respectively. Thus, gonadectomy results in a gradual decrease of hypothalamic GnRH stores, attaining a new, lower equilibrium, and in elevated serum gonadotropin levels; this may be interpreted as an outcome of the interplay between massive release and augmented synthesis of GnRH. A similar, enhanced GnRH secretion, occurring every afternoon in ovariectomized EB-treated rats, may temporarily disrupt the equilibrium of and cause a decrease in hypothalamic GnRH stores. Further support for this interpretation is lent by the administration of an antiserum against GnRH: the LH-surge is prevented, though hypothalamic GnRH stores are depleted, implying that anti-GnRH, unlike Nembutal, does not interfere with the release of GnRH, but intercepts it on its route to the anterior pituitary.

The resemblance of the EB-induced LH surge to the proestrous surge suggests a similar mechanism for the secretion of LH, i.e. a release of GnRH preceding the gonadotropin peak. The failure to detect any meaningful fluctuations in both serum and hypothalamic GnRH levels on proestrus may be explained by the small amounts secreted: if calculated from the increment in hypothalamic GnRH levels between 'twice blocked' and control rats, the 'GnRH quantum' for one surge of LH is about 0.4 ng.

### *Acknowledgments*

We are grateful to Mr. S. YOSSEF for devoted animal care. This work was supported by a grant of the Ford Foundation and the Population Council Inc., N.Y., to Dr. H. R. LINDNER.

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