PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 859–862 (1974)

Influence of Estradiol Benzoate on Pituitary Responsiveness to LH-RH at Different Stages of the Estrous Cycle in Rats[1] (38206)

J. A. VILCHEZ-MARTINEZ,[2] A. ARIMURA, AND A. V. SCHALLY

Veterans Administration Hospital, and Tulane University School of Medicine, New Orleans, Louisiana 70146

It has been shown that the pituitary responsiveness to LH-RH differs at various stages of the estrous cycle (1, 2). Moreover, we have demonstrated that estradiol benzoate (EB) has a biphasic effect on the pituitary response to LH-RH in normal diestrous rats. First, there is an inhibition which occurs 2–6 hr after the administration of steroid (2), and later, an augmentation after 14–48 hr (2–4). It was also demonstrated that pretreatment with progesterone (P) suppressed the LH release induced by small doses of LH-RH, but did not affect the response to high doses of LH-RH (4). However, the combined administration of estrogen and progesterone strongly suppressed the pituitary response to LH-RH (4). It is possible that endogenous steroids, especially endogenous progestins interact with exogenous estrogen in the response of the pituitary to LH-RH. Since plasma progestin levels vary at various stages of the estrous cycle, the effect of EB on the pituitary responsiveness might vary depending on the stage of administration. Therefore, we studied the effect of the administration of EB on the LH-release induced by LH-RH at different stages of the estrous cycle and the temporal pattern of the effect of EB administered on the morning of estrus.

Materials and Methods. Adult female rats of the SD strain (Charles River Co.) weighing approximately 200 g were used throughout the experiment. They were housed in animal quarters with controlled temperature and light (14 hr light and 10 hr darkness), and had free access to Purina Laboratory Chow and water. The animals were kept under these conditions for 14 days before daily examination of vaginal smears were started. Only rats which showed at least 2 successive, regular 4 day cycles were used.

Experiment I: At 9 am of each stage of the estrous cycle, the animals were divided into 2 groups. One group was injected with 10 μg EB in 0.5 ml sesame oil sc, while the other was injected with 0.5 ml sesame oil only. Twenty-four hr later, they were injected iv under light ether anesthesia with 0.2 ml acidified saline (0.01 *M* acetic acid/0.15 *M* NaCl) or 0.4 μg synthetic LH-RH (Hoechst lot R-4, kindly supplied by Prof. G. Vogel and Dr. R. Geiger, Farbwerke Hoechst, Frankfurt).

Experiment II: The rats were treated with either 0.5 ml sesame oil or 5 μg EB in 0.5 ml sesame oil at 9 am on the day of estrus. Four, 9, and 14 hr later, they were injected iv under ether anesthesia with 0.2 ml acidified saline or 0.4 μg synthetic LH-RH.

In all experiments, each animal was bled from the jugular vein before and 20 min after the injection of LH-RH or saline. The blood samples were kept at 4° overnight, centrifuged, and sera was separated and kept frozen until assayed for LH. LH values were determined in duplicate by the double-

[1] This study was partly supported by USPHS Research Grant AM-07467 and AM-09094, The Population Council, New York, N. Y., and VA.

[2] Fellow of the University of Zulia, Maracaibo, Venezuela.

TABLE I. Effect of EB on the Basal Levels of Serum LH at 9 am during Different Stages of Estrus Cycle in Rats.

Treatment[a]	DI	DII	Proestrus	Estrus
Oil	1.51 ± 0.17	0.45 ± 0.13[b]	0.86 ± 0.13[b]	0.84 ± 0.18[b]
EB	1.02 ± 0.12[c]	0.30 ± 0.13	0.52 ± 0.22	0.83 ± 0.18

The values are expressed as ng/ml of NIH-LH S_{17}. Ten to 11 animals were used per group.
[a] 10 μg of EB or oil was administered 24 hr earlier.
Duncan's new multiple range test.
[b] Significantly different from the respective basal LH value at DI ($P < 0.05$).
[c] Significantly lower than the value of respective oil-treated control group.

antibody radioimmunoassay method for rat LH as described by Niswender *et al.* (5). NIH-LH-S_{17} was used as the reference preparation. The difference between serum LH concentration before and after injection of acidified saline or LH-RH was calculated for each animal and used as the response parameter of the pituitary responsiveness. Duncan's new multiple range test was used to compare the mean response among the different groups (6).

Results. In the oil-treated control rats, the highest serum LH level was found on the day of diestrus I (DI), declined on the day of diestrus II (DII), rose slightly on the day of proestrus (PE), and remained at this elevated level on the day of estrus (Table I). The same trend was noted in the EB-treated rats. EB did not modify the basal levels of LH at any stage of the cycle, except for DI when the basal serum levels were significantly lower than in the respective oil control rats.

In the oil-treated rats, 0.4 μg synthetic LH-RH raised serum LH levels significantly, but the injection of acidified saline had no effect. However, the magnitude of the increase after administration of LH-RH varied according to the stages of the estrous cycle (Fig. 1). The greatest response was obtained at PE and estrus, and the lowest response at DII. On the other hand, a 10 μg 24 hr pretreatment with EB always increased the pituitary response to LH-RH, regardless of the stage of cycle. But the pattern of changes in the pituitary responsiveness during the cycle was not affected, the greatest response still occurring at PE and estrus and the lowest response at DI.

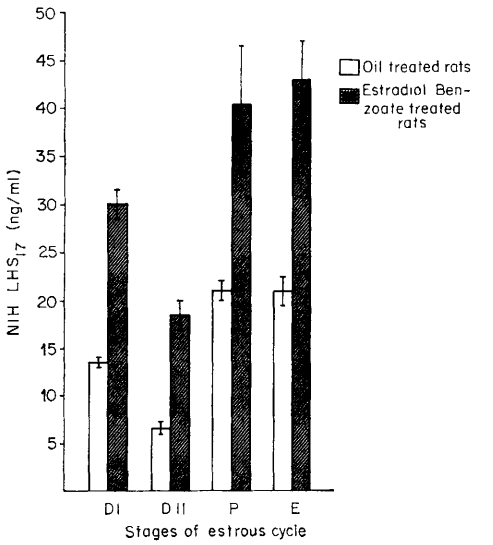

FIG. 1. Net increment in serum LH levels after iv injection of 0.4 μg LH-RH in different stages of the estrous cycle of normal rats injected 24 hr previously with sesame oil or with 10 μg of EB. The oil and EB were injected at 9 am. Five to 6 rats were used per group.

In the estrus rats, serum LH level remained unchanged at 4 and 9 hr after the injection of oil, but was lower at 14 hr ($P < 0.05$). Pretreatment with 5 μg of EB lowered the basal serum level of LH 9 hr after the injection (Table II). The injection of LH-RH significantly increased serum LH levels in all groups. However, the magnitude of increase varied at different times (Fig. 2). In the oil-injected control rats, the greatest response was found at 4 hr, and the lowest at 14 hr. In the EB treated rats, the greatest response was observed at 4 hr after injection of EB. The response in

TABLE II. Effect of EB on the Basal Level of Serum LH in Estrous Rats.

Treatment[a]	Time after treatment		
	4 hr	9 hr	14 hr
Oil	0.76 ± 0.08[b]	0.81 ± 0.14[b]	0.40 ± 0.07
EB	0.54 ± 0.06	0.32 ± 0.05[c]	0.54 ± 0.17

The values are expressed as ng/ml NIH-LH S_{17}. Ten to 11 animals per group were used.

[a] 5 μg EB or oil were injected at 9 a.m.

Duncan's new multiple range test.

[b] Significantly higher than the basal LH value at 14 hr ($P < 0.05$).

[c] Significantly lower than the value of respective oil-treated control group.

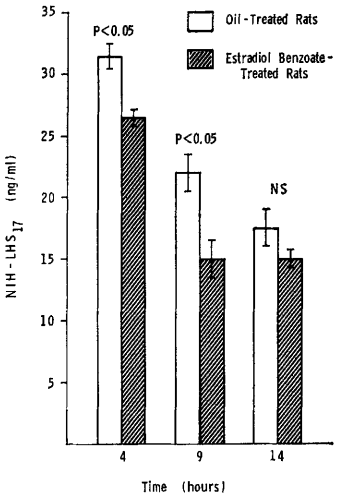

FIG. 2. Net increment in serum LH levels after iv injection of 0.4 μg LH-RH in estrous rats at various time intervals after pretreatment with oil or 5 μg of EB sc. Oil or EB was injected at 9 am. Five to 6 rats were used per group.

EB rats at 4 and 9 hrs were significantly smaller than those found in the oil-treated rats at corresponding times. At 14 hr, the magnitude of responses to LH-RH in the oil-treated and the EB treated rats was similarly low, but equal to EB at 9 hr.

Discussion. From the results presented here, it is evident that the LH release after LH-RH administration varied with the stages of the rat estrous cycle. The greatest response was found on the day of proestrus and estrus. The greater response on PE is in agreement with the results observed in our recent studies (2) as well as in others (1, 7). In the hamster, the pituitary response to LH-RH during estrus was significantly smaller than during PE (1). This could be due to the difference of time of experiment relative to estrus, since the pituitary response progressively decreases during the day of estrus as observed in the present study.

Previous reports from our laboratory (2–4) claimed that injection of rats with EB on estrus and DI, augmented the pituitary response to LH-RH after 24–48 hr. In the present experiment, pretreatment with EB brought about a significant increase of the pituitary response to LH-RH in all stages of the estrous cycle.

Recently, we have demonstrated that EB has a biphasic effect on the pituitary response to LH-RH in the diestrous rats (2). In that experiment, 2–6 hr after pretreatment with EB, the injection of synthetic LH-RH failed to raise serum LH. At 9 hr, the magnitude of the pituitary response was the same as that found in the oil-treated rats. At 14 hrs, EB augmented the pituitary response to LH-RH. The similar time study on the effect of EB in estrous rats in the present experiment showed that the pattern of the effect of EB was different than that found in diestrous rats. Four hr after injection of EB, the pituitary response to LH-RH was suppressed, but not abolished. However, the suppression persisted at 9 hr. Fourteen hr after EB, no augmentation of the pituitary response was observed. This difference between DI and estrus day could be due to the difference of endogenous progesterone levels between DI and estrus. The levels of endogenous progesterone are very low in DII, start to rise in the afternoon of PE, remain high during the night of PE and morning of estrus, and then start falling in the afternoon of estrus, reaching low levels in DI (8–12). Therefore, EB injected at 9 am on estrus might interact with circulating P which is elevated at that time. Progesterone given in combination with E is well-known to suppress the pituitary response to LH-RH (4).

In agreement with previous studies, the

basal level of serum LH at 9 am showed some slight fluctuations throughout the cycle (2, 8–13). The highest basal morning values of serum LH level were found in DI and the lowest in DII. Also, the basal serum LH levels showed some variation during the day of estrus. Apparently, there is some diurnal variation of basal level of LH in agreement with previous studies (2, 14).

Summary. The effect of estradiol benzoate (EB) on the pituitary response to LH-releasing hormone (LH-RH) was studied in the different stages of the estrous cycle of rats. An iv injection of 0.4 μg LH-RH always raised serum LH in the oil-treated control group. The magnitude of the increase varied according to the stage of the estrous cycle, being greatest in Proestrus and Estrus, and lowest in Diestrous II (DII). EB injected 24 hr before increased the pituitary response to LH-RH regardless of the stages of the cycle, but did not modify the fluctuation of pituitary responsiveness. The basal levels of LH at 9 am throughout the cycle in the oil-treated control rats were highest in Diestrus I and lowest in Diestrus II. Pretreatment with EB lowered the basal LH levels only on the day of DI. On the day of Estrus, the pituitary response was greatest at 4 hr, and the lowest level at 14 hr in the control rats. Injection of EB suppressed the pituitary response to LH-RH at 4 and 9 hr, and did not alter this response at 14 hr as compared with the response in the oil-injected control rats. The results provide further evidence for the complex interactions of estrogen with other endogenous hormones in the pituitary responsiveness to LH-RH.

The authors are indebted to Mr. L. Garcia, Mrs. M. Esslinger, and Mrs. J. Gauthier for their technical help, to Dr. G. Niswender, Dr. D. Ward, and NIH for the gift of materials used in radioimmunoassays.

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Received Jan. 12, 1974. P.S.E.B.M., 1974, Vol. 146.