PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 374–379 (1980)

# Pituitary Refractoriness to Luteinizing Hormone Releasing Hormone: Its Importance in Ending the Luteinizing Hormone Surge in the Cyclic Rat (40881)

CHARLES A. BLAKE AND LAURA L. GARNER

*Department of Anatomy, University of Nebraska Medical Center, Omaha, Nebraska 68105*

*Abstract.* A study was conducted to assess the importance of pituitary refractoriness to luteinizing hormone releasing hormone (LHRH) in ending the preovulatory surge of luteinizing hormone (LH) and in modulating the associated surge of follicle-stimulating hormone (FSH) in rat plasma. Phenobarbital-blocked proestrous rats were infused iv with LHRH at a constant rate of 50 ng/hr to restore the rising and plateau phases of the spontaneous surges of LH and FSH in plasma. Refractoriness to LHRH was demonstrated for LH but not FSH release when LHRH was infused beyond 2 hr. The plasma LH declined from high levels from 2 to 4.5 hr of infusion while the plasma FSH remained at an elevated plateau. The decline in plasma LH was not as rapid as that observed when the infusion was ended. Injection of a large dose (1 μg) of LHRH at 2 or 3.5 hr after the start of infusion caused substantial increases in plasma LH and FSH but only the LH response after the later injection was less than that observed in other rats given the injection earlier. The results suggest that pituitary refractoriness to LHRH plays a minor role in expediting the decline in the plasma LH during the latter portion of the LH surge and that it has no appreciable effect on the pattern of the plasma FSH at this time.

In the female rat, a preovulatory surge of luteinizing hormone (LH) in serum occurs during the afternoon and evening of proestrus. Associated with this increase in serum LH is a rise in serum follicle-stimulating hormone (FSH) concentrations. This rise in serum FSH has been described as the first phase of FSH release to differentiate it from the second or selective phase of FSH release which starts during late proestrus and continues into estrus at a time when LH is low in serum (1–3).

It is now generally accepted that hypothalamic LH releasing hormone (LHRH) plays a major role in stimulating the adenohypophysis to result in the LH surge and first phase of FSH release (4–10). There is evidence to suggest that the end of the LH surge is the result of at least two events, a decrease in hypothalamic LHRH release and pituitary refractoriness to LHRH (7).

In the present study, we have assessed the relative importance of the pituitary refractory mechanism in ending the LH surge and examined if pituitary refractoriness to LHRH may play a significant role in determining the pattern of the plasma FSH during the early evening of proestrus.

*Materials and methods.* Virgin, female Sprague–Dawley rats (Simonsen Laboratory, Gilroy, Calif.) were kept in a room with automatically controlled temperature (24–26°C) and lighting (light on 0500 to 1900 hr daily). Following a 3-week period of acclimation to their quarters, rats were monitored daily by vaginal lavage to determine the stage of the estrous cycle. Only rats which displayed two or more consecutive 4-day estrous cycles were used at proestrus. The body weights of the rats were 223 to 275 g at this time.

All rats were injected ip with sodium phenobarbital (75 mg/kg body wt) between 1200 and 1300 hr proestrus to block the proestrous increases in plasma LH and FSH (3, 7, 8). Two polyvinyl cannulae were then implanted into the right atrium of the heart as previously described (7) prior to 1400 hr. One cannula was used for constant rate LHRH (Beckman, lot No. CO851) infusion (50 ng/hr; 0.47 ml/hr). This procedure restores and synchronizes the majority of the proestrous LH surge and associated rise in plasma FSH (7, 8). The other cannula was used for blood collections and the pulse injection of 1.0 μg LHRH in 0.2 ml saline.



0037-9727/80/070374-06$01.00/0
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Rats were divided into one of the following six groups of four to six rats each:

1. Saline infusion for 4.5 hr from 1500 to 1930 hr. Blood was collected at 1500, 1630, 1700, 1710, 1730, and 1930 hr.
2. LHRH infusion for 3 hr from 1500 to 1800 hr. Blood was collected at 1500, 1630, 1700, 1710, 1730, and 1800 hr.
3. LHRH infusion for 3 hr from 1500 to 1800 hr and a pulse LHRH injection at 1700 hr. Blood was collected at 1500, 1630, 1700, 1710, 1730, and 1800 hr.
4. LHRH infusion for 4.5 hr from 1500 to 1930 hr. Blood was collected at 1500, 1800, 1830, 1840, 1900, and 1930 hr.
5. LHRH infusion for 4.5 hr from 1500 to 1930 hr. Blood was collected at 1500, 1800, 1830, 1840, 1900, and 1930 hr.
6. LHRH infusion for 4.5 hr from 1500 to 1930 hr and a pulse LHRH injection at 1830 hr. Blood was collected at 1500, 1800, 1830, 1840, 1900, and 1930 hr.

The volume of the 1500-hr blood samples was 0.6 ml. The volume of the remaining samples was 0.5 ml. Thus, a total of 2.6 ml was withdrawn from all rats prior to withdrawing the last blood sample at which time the experiment was ended. The total blood volume removed from individual rats ranged from 11 to 14%. However, each rat received an injection of 0.0 to 0.4 ml saline after each bleeding. The amount injected was calculated to add to the volume of saline vehicle infused to approximate replacement of the volume of blood withdrawn.

Blood samples were centrifuged at 4°C for 10 min to separate plasma from cells. Plasma was stored frozen at −20°C until subsequent radioimmunoassay of LH and FSH by the method of Niswender *et al.* (11) as described previously for LH (12) and FSH (10). LH was measured in a single determination on 100 μl plasma for the first blood sample from each rat. The remaining samples were assayed in a single determination on 3 and 20 μl and in duplicate on 10 μl. The 10-μl duplicate determinations were averaged. The amount of plasma which caused nearest 40% displacement of the iodinated LH from the anti-ovine LH sera was used to calculate the LH value. FSH was measured in a single determination on 150 μl plasma. LH and FSH are expressed as micrograms per milliliter and nanograms per milliliter plasma, respectively, in terms of the NIAMDD reference preparations. The blood samples withdrawn from the saline-infused rats (Group 1) were assayed for LH and FSH in a single assay for each hormone. The remainder of the blood samples were assayed in a single assay for each hormone. A serum pool was assayed for LH (10 μl) and FSH (150 μl) in quadruplicate in each assay. The intraassay coefficients of variation were less than 6 and 4% for LH and FSH, respectively, while the interassay coefficients of variation were 10 and 5%, respectively. The sensitivities of the LH assays were $\leq$1 ng while those for FSH were $\leq$20 ng. Statistical comparisons of data were performed by one-way analysis of variance for separate groups to determine changes over time. Comparisons between groups were made by two-way analysis of variance (treatment and time periods). Subsequent Newman−Keuls tests were performed. Student's $t$ test was used to test for significant differences in plasma LH and FSH increments after pulse injections of LHRH. We consider any $P$ value less than 0.05 to indicate statistical significance. Significance was tested at the 0.05 and 0.01 levels.

*Results.* Plasma LH and FSH concentrations did not rise during a 4.5-hr infusion of saline in five rats (not illustrated). The plasma LH remained below 0.045 μg/ml from 1500 to 1930 hr while the plasma FSH never exceeded 210 ng/ml during this period.

The mean plasma LH concentrations for the remainder of the rats are shown in Fig. 1. As mean plasma LH values for the different groups of rats treated identically initially (LHRH infusion at 50 ng/hr) and bled at the same times prior to and during LHRH infusion were not different from

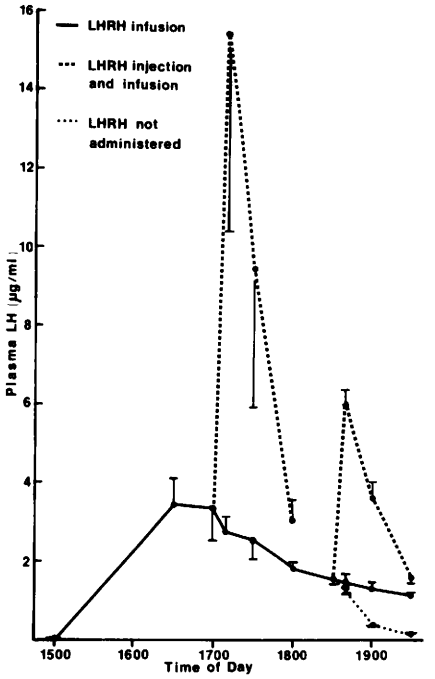


FIG. 1. Effects of continuous constant-rate iv LHRH infusion (50 ng/hr) and a bolus iv injection of 1 μg LHRH at either 1700 or 1830 hr on mean ± SE plasma LH concentration in phenobarbital-blocked proestrous rats. Each point represents the mean of 4 to 25 values. Plasma LH decreased from 1700 to 1930 hr during LHRH infusion and a pulse LHRH injection at 1700 hr with continued infusion was more effective than that at 1830 hr with continued infusion in elevating the plasma LH.

each other, these data have been combined. Infusion of LHRH at 50 ng/hr in the phenobarbital-blocked rat caused plasma LH concentration to rise by 1.5 hr to a level within the high range of values observed during the spontaneous LH surge (Fig. 1 vs Ref. (14)). The plasma LH remained elevated at an apparent plateau with an additional 0.5 hr infusion and fell gradually during the following 2.5 hr of infusion from 1700 to 1930 hr ($P < 0.01$). This decline represents pituitary refractoriness for LH release in response to LHRH. Despite the maintenance of constant elevated levels of LHRH in the blood by this infusion technique (15), the plasma LH falls after reaching peak or elevated plateau levels. The decline in plasma LH represents a decrease rather than a cessation of pituitary LH release in response to LHRH. Termination of LHRH infusion caused LH to fall more rapidly in the plasma (Fig. 1).

A bolus iv injection of a large dose (1 μg) of LHRH at either 2.0 or 3.5 hr after the start of infusion caused a large increase in plasma LH (Fig. 1). The increases in LH values above the plasma LH concentrations observed during continued LHRH infusion represent the additional effect of the 1 μg LHRH administered by iv injection as the infusion pump was not turned off after the iv injection. The mean increment in plasma LH (mean of LH increments for individual rats) at 10 min after injection was greater ($P < 0.01$) in rats injected at 2 hr than in those injected at 3.5 hr after the start of infusion (11.2 ± 3.2 vs 4.3 ± 0.3 μg/ml).

The mean plasma FSH concentrations for the same rats are shown in Fig. 2. As mean plasma FSH values for the different groups of rats treated identically initially (LHRH infusion at 50 ng/hr) and bled at the same times prior to and during LHRH infusion were not different from each other, these data have been combined. Infusion of LHRH at 50 ng/hr caused the FSH to rise in the plasma approximately three-fold by 1700 hr proestrus to levels which are similar to those observed during the spontaneous

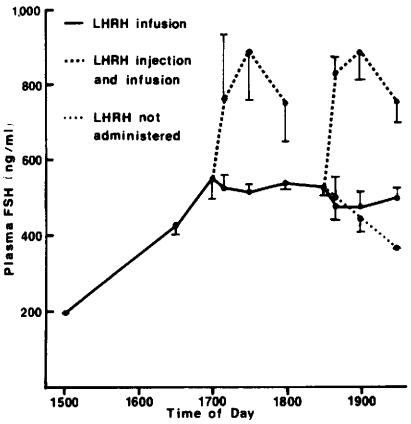


FIG. 2. Mean ± SE plasma FSH concentrations are plotted for the same rats in which plasma LH was measured and plotted in Fig. 1. Plasma FSH did not decrease from 1700 to 1930 hr during LHRH infusion and a pulse LHRH injection at 1700 hr with continued infusion was not more effective than that at 1830 hr with continued infusion in elevating the plasma FSH.

FSH rise during proestrus (3, 8). In contrast to that observed for plasma LH, the plasma FSH did not fall by 1930 hr during continued LHRH infusion. The maintenance of elevated FSH in the plasma represents continued pituitary FSH release in response to LHRH. Cessation of LHRH infusion caused plasma FSH to fall significantly by 60 min after the end of infusion ($P < 0.01$).

A bolus iv injection of 1 $\mu$g LHRH at either 2.0 or 3.5 hr after the start of the continuous LHRH infusion caused a significant increase ($P < 0.05$) in the plasma FSH at 10 min after injection (Fig. 2). The mean increments in plasma FSH at both 10 and 30 min after injection were not different in rats injected with LHRH at 2.0 hr than in those injected at 3.5 hr after the start of infusion (296 ± 61 vs 308 ± 41 ng/ml at 10 min and 428 ± 80 vs 363 ± 70 at 30 min).

*Discussion.* Constant rate iv infusion of LHRH at 50 ng/hr for a period of about 3 hr followed by sequential decreases in the LHRH infusion rate for an additional 3 hr has been shown to simulate the spontaneous LH surge in the phenobarbital-blocked proestrous rat (7, 13). During the 3-hr infusion at 50 ng/hr, the plasma LH rose to an elevated plateau within approximately 1.5 hr and remained there while LHRH was maintained at a constant elevated level in the blood (15). If LHRH infusion at 50 ng/hr was continued beyond approximately 3 hr, the LH declined gradually in the plasma despite the maintenance of elevated levels of LHRH in the blood. Comparison of the rate of decline of LH in plasma in these rats with that in rats in which the infusion was ended as well as with that observed during the declining phase of the spontaneous LH surge clearly suggested that the pattern of LH decline in plasma during the spontaneous surge is the result of at least two events, a decrease in hypothalamic LHRH release and a pituitary refractoriness to LHRH (7).

The results of the present study clearly indicate that the role of pituitary refractoriness to LHRH in ending the LH surge is likely only a modulatory one to decrease pituitary LH release and thus, expedite the fall in the plasma LH. The diminution in the pituitary LH response to LHRH which was demonstrated by the continuous LHRH infusion represents a relative rather than an absolute decrease in the ability of LHRH to release pituitary LH. The pituitary is not releasing LH at its maximal potential after the onset of pituitary refractoriness when rats are infused with LHRH at 50 ng/hr. When a large dose of LHRH was administered to rats during the infusion and after the onset of pituitary refractoriness, a rapid substantial increase in plasma LH occurred. It should be noted, however, that a relative refractoriness to LHRH was also demonstrated with the large dose of LHRH. The magnitude of the LH response was less than that which occurred when the same dose of LHRH was administered prior to the onset of pituitary refractoriness at a time when LH and FSH were elevated to a plateau. It is likely that with spontaneously controlled decreasing amounts of hypothalamic LHRH being released during the latter portion of the spontaneous LH surge, the pattern of the decrease of LH in plasma is modulated to decline somewhat more rapidly due to a diminished ability of the pituitary to release LH in response to LHRH.

Pituitary LH refractoriness to LHRH is demonstrated more strikingly when barbiturate-blocked proestrous rats are infused continuously with LHRH at rates greater than 50 ng/hr or for an extended period of time. Infusion of LHRH at rates greater than 50 ng/hr causes the plasma LH to rise to a higher shortened plateau or to a peak and then to fall rapidly thereafter (7, 16). Infusion of LHRH for 20 hr either at 52 or 156 ng/hr resulted in low plasma LH concentration at the end of infusion. It was assumed (16) that LHRH was no longer effective in releasing LH due to an exhaustion of pituitary LH stores. However, an exhaustion of pituitary LH stores cannot explain pituitary refractoriness to LHRH during the declining phase of the LH surge as evidenced in the present study by the rapid substantial rise in plasma LH observed when 1 $\mu$g LHRH was administered well after the onset of pituitary refractoriness. Correlation of pituitary LH concen-

tration and the plasma LH response to LHRH infusion with changes in the ultrastructural morphology of pituitary LH cells has revealed that the refractory period of LH release during the declining phase of the LH surge is associated with a decreased ability of LH cells to synthesize and package LH into granules in response to LHRH (17). Thus, a decrease in the rate of LH synthesis and packaging into granules could result in less LH available for release in response to constant or decreasing LHRH stimulation and be, at least in part, the mechanism by which the pituitary becomes refractory to LHRH during the declining phase of the LH surge.

Pituitary LH refractoriness to LHRH has also been demonstrated in sheep (18, 19), in the rhesus monkey (20), and in women (21, 22). Injection of 1 mg LHRH at 16 hr after the start of a continuous LHRH infusion in ovariectomized sheep failed to elevate the plasma LH (19). As pituitary LH concentration was not significantly decreased at the end of the 20-hr infusion in these sheep, it was concluded that pituitary refractoriness to LHRH is not due to depletion of pituitary LH stores. Whether or not the refractoriness to LHRH observed with *extended* long-term LHRH infusions which was demonstrated in sheep (18, 19) and in rats (16) is due to impairment in the packaging of synthesized LH into granules and/or intracellular transport of the LH to make it available for release with LHRH stimulation remains to be determined.

It is also possible that other mechanisms may be responsible for refractoriness to LHRH with extended long-term LHRH infusions. A progressive decline in serum LH and FSH concentration has been observed after 4 hr of continuous LHRH infusion in cyclic women (21, 22). As an acute injection of 25 $\mu$g LHRH at the end of an 8-hr continuous LHRH infusion caused a substantial increase in serum LH and FSH (22), it was concluded that refractoriness or decreased sensitivity may be the result of negative feedback of estradiol or other ovarian steroids at the pituitary. While ovarian negative feedback may indeed be involved in refractoriness to LHRH in the human since the LHRH infusion raised the serum estradiol concentration (22), this cannot explain refractoriness in the rat under the conditions of this study. Acute ovariectomy just prior to the onset of LHRH infusion in the phenobarbital-blocked proestrous rat did not alter the plasma LH or FSH responses including the decline in the plasma LH indicative of refractoriness to the LHRH. It has also been suggested that continuous LHRH stimulation of the pituitary may not permit the regeneration of LHRH receptors in the rhesus monkey (20). Although such a mechanism could be involved in pituitary refractoriness during the declining phase of the LH surge in the rat, its role at this time would likely be minor. The pituitary will respond rapidly and well to LHRH at this time if the LHRH stimulus is raised. We feel pituitary refractoriness to LHRH during the declining phase of the LH surge in the rat is more likely a reflection of a reduction in the (i) rate of LH synthesis, (ii) the rate of packaging of LH in granules, and possibly (iii) the transport of LH to make it available for release with LHRH stimulation (17).

In a previous study (8), pituitary FSH refractoriness to LHRH during LHRH infusion in phenobarbital-blocked proestrous rats was only suggested at high LHRH infusion rates. In the present study, pituitary FSH refractoriness was not demonstrated. Plasma FSH concentration did not fall during a 4.5-hr LHRH infusion at 50 ng/hr. Moreover, injection of a large dose of LHRH at 3.5 hr after the start of infusion at the time the pituitary was already refractory to LHRH in terms of LH release elevated the plasma FSH as well as it did at 2.0 hr after the start of infusion. It therefore appears that the pattern of the plasma FSH during the early evening of proestrus during the latter part of the first phase of FSH release is not modulated to any appreciable extent by pituitary refractoriness to LHRH. Rather the decline in plasma FSH concentration which is observed occasionally between the two phases of FSH release spontaneously (3) and when the second phase of FSH release is selectively blocked

(23, 24) is likely due to a decrease in the release of LHRH by the hypothalamus (8).

The authors thank Dr. Leo Reichert, Jr., Gordon D. Niswender, and Albert F. Parlow as well as the National Pituitary Agency of NIAMDD for the gifts of materials used for the radioimmunoassay of LH and FSH.

1. Gay, V. L., Midgley, A. R., and Niswender, G. D., Fed. Proc. **29**, 1880 (1970).
2. Daane, T. A., and Parlow, A. F., Endocrinology **88**, 653 (1971).
3. Ashiru, O. A., and Blake, C. A., Life Sci. **23**, 1507 (1978).
4. Koch, Y., Chobsieng, P., Zor, U., Fridkin, M., and Lindner, H. R., Biochem. Biophys. Res. Commun. **55**, 623 (1973).
5. Arimura, A., Debeljuk, L., and Schally, A. V., Endocrinology **93**, 323 (1974).
6. Sarkar, D. K., Chiappa, S. A., Fink, G., and Sherwood, N. M., Nature (London) **264**, 471 (1976).
7. Blake, C. A., Endocrinology **98**, 451 (1976).
8. Blake, C. A., Endocrinology **98**, 461 (1976).
9. Eskay, R. L., Mical, R. S., and Porter, J. C., Endocrinology **100**, 263 (1977).
10. Elias, K. A., and Blake, C. A., J. Endocrinol. **83**, 331 (1979).
11. Niswender, G. D., Midgley, A. R., Jr., Monroe, S. E., and Reichert, L. E., Jr., Proc. Soc. Exp. Biol. Med. **128**, 807 (1968).
12. Blake, C. A., Norman, R. L., and Sawyer, C. H., Biol. Reprod. **8**, 299 (1973).
13. Blake, C. A., Clin. Obstet. Gynaecol. **5**, 305 (1978).
14. Blake, C. A., Endocrinology **98**, 445 (1976).
15. Blake, C. A., Endocrinology **102**, 1043 (1978).
16. Schuiling, G. A., DeKoning, J., Zurcher, A. F., Gnodde, H. P., and van Rees, G. P., Neuroendocrinology **20**, 151 (1976).
17. Garner, L. L., and Blake, C. A., Biol. Reprod. **20**, 1055 (1979).
18. Chakraborty, P. K., and Reeves, J. J., J. Anim. Sci. **37**, 304 (1973).
19. Piper, E. L., Perkins, J. L., Tugwell, D. R., and Vaught, W. G., Proc. Soc. Exp. Biol. Med. **148**, 880 (1975).
20. Belchetz, P. E., Plant, T. M., Nakai, Y., Keogh, E. J., and Knobil, E., Science **202**, 631 (1978).
21. Jewelewicz, R., Dyrenfurth, I., Ferin, M., Bogumil, J., and Vande Wiele, R. L., J. Clin. Endocrinol. Metab. **45**, 662 (1977).
22. Pinto, H., Wajchenberg, B. L., Lima, F. B., Goldman, J., Comaru-Schally, A. M., and Schally, A. V., Acta Endocrinol. **91**, 1–13 (1979).
23. Butcher, R. L., Collins, W. E., and Fugo, N. W., Endocrinology **96**, 576 (1975).
24. Gay, V. L., and Tomacari, R. L., Science **184**, 75 (1974).

Received November 20, 1979. P.S.E.B.M. 1980, Vol. 164.