BIOLOGY OF REPRODUCTION 24, 100–104 (1981)

Pulsatile LH Release During the Ovulatory LH Surge on Proestrus in the Rat[1]

ROBERT V. GALLO

Department of Physiology,
University of California, San Francisco,
School of Medicine,
San Francisco, California 94143

ABSTRACT

The present experiments were carried out to determine if LH release during the ovulatory LH surge in the rat was pulsatile. Unanesthetized rats with 4-day estrous cycles were bled through jugular vein cannulae at a rate of 10 μl whole blood/2–3 min from 1400 to 1930 h on proestrus during the ovulatory LH surge. Ovulation occurred in all rats that had surges of LH release. These surges consisted of ascending, plateau, and descending periods. LH release was pulsatile during the plateau portion as indicated by a significant increase in the percent coefficient of variation for individual bleeding periods when compared with assay variation, and during the ascending and descending phases as evidenced by pronounced nadir-to-peak changes in blood LH levels which were greater than the minimum significant change detected by radioimmunoassay. Blood LH levels increased during the ascending phase and then plateaued because of high amplitude LH pulses (538 ng/ml in the ascending phase and 572 ng/ml in the plateau phase), which occurred with LH interpulse intervals of 16 and 23 min, respectively. Blood LH levels declined during the descending phase since the pulse amplitude (386 ng/ml) significantly decreased, and in comparison with the ascending phase the LH interpulse interval lengthened significantly to 29 min. These studies indicate that pulsatile LH release occurs during the ovulatory LH surge on proestrus in the rat, and that the rise, plateau, and fall in blood LH levels can be accounted for by changes in the LH pulse amplitude and frequency during different phases of the surge.

INTRODUCTION

The midcycle ovulatory LH surge in humans (Midgley and Jaffe, 1971; Yen et al., 1972; Kapen et al., 1973) and cows (Rahe et al., 1980) is composed of high amplitude pulses of LH release. The aim of the present experiment was to determine if pulsatile LH secretion occurred during the cyclic or ovulatory surge of LH on the afternoon of proestrus in the rat. If LH release was pulsatile, the second objective was to characterize it to determine if variations in LH secretory episodes (i.e., LH pulse magnitude and/or frequency) occurred with different phases of the ovulatory surge.

MATERIALS AND METHODS

Adult female Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) weighing 260–320 g were maintained on a 14L:10D schedule (lights on 0500–1900 h) and fed Purina rat chow and water ad libitum. Daily vaginal smears were taken up to and including the day of the experiment, and all rats showed two or more consecutive 4-day estrous cycles before that time.

Bleeding Procedure

Rats were cannulated via the external jugular vein between 0900–1030 h on diestrus Day 2, and all rats had nucleated epithelial smears characteristic of proestrus the next AM. On the morning of proestrus the cannula was connected to one end of a piece of flexible tubing which was inserted into a peristaltic pump. The other end of the tubing was connected to a Hamilton microliter syringe kept on ice for the collection of blood samples. Animals were injected with 350 U heparin 20 min prior to being bled continuously at a rate of 10 μl whole blood/2–3 min from 1400–1930 h. At the end of this period all rats were returned to the animal quarters overnight. The following morning (estrus AM) the ovaries were removed, the oviducts dissected free, and ovulation verified by counting the ova under a dissecting microscope.

Analysis of Data

The surge of LH release was found to consist of ascending, plateau, and descending phases. To determine if the variation in blood LH levels in the plateau

Accepted October 1, 1980.
Received July 14, 1980.
[1] These studies were supported by grants from NIH (HD05577 and AM06704).

phase was indicative of changes in the secretory pattern of LH rather than assay variation, the percent coefficient of variation (CV; standard deviation ÷ mean × 100) was determined for LH values during these plateau periods and then compared (unpaired t test) with the intraassay CV determined for a similar range of mean LH values, i.e., ∿1200–2500 ng/ml. This CV was based on 18 determinations done in three assays, each determination consisting of 25 replicates of 12, 20, or 25 ng NIAMDD Rat LH RP-1/10 μl, which corresponds to the 10 μl samples taken from the animals. The intraassay variation at these LH levels was 7.0 ± 0.6%, did not differ whether 12, 20, or 25 ng LH was used in the determination, and was significantly different from the CV for the plateau phase (12.3 ± 0.9%; $P<0.001$). Thus, LH release was said to be pulsatile during this phase. The CV was also determined for all LH values composing the rising (nadir to peak) as well as the declining portions of each potential LH pulse in the plateau phase, and during this phase of the LH surge a pulse was defined as occurring when its CV was at least 10% (∿1.5 × intraassay variation).

The CV method of analysis could not be applied to the ascending and descending phases of the surge since LH pulses were superimposed on a changing baseline concentration. It was therefore necessary to determine which nadir-to-peak changes in blood LH levels (i.e., pulse Δ blood LH), if any, could be defined as pulses during these phases of the ovulatory surge. To do this it was first necessary to determine the minimum significant difference between nadir and peak blood LH levels that could be detected by radioimmunoassay, and thus indicate that a pulse of LH release had occurred. Therefore, comparisons were made between LH solutions whose mean concentrations differed by 1, 2, or 2.5 ng NIAMDD Rat LH RP-1/10 μl. Comparisons consisted of 10 replicates of 10 μl samples from each solution, and this was done for those values in the RIA dose-response curve corresponding to the blood LH levels seen during the ascending and descending phases of the LH surge. With $P<0.001$ as the level of significance (unpaired t test), in six assays 25% (6 to 24), 86% (24 of 28), and 93% (39 of 42) of the comparisons differing by 1, 2, and 2.5 ng, respectively, were significantly different. Therefore, the minimum significant change reliably detected by radioimmunoassay is a difference of 2.5 ng/10 μl, or 250 ng/ml. During the ascending and descending phases a pulse was thus defined as occurring when the pulse Δ blood LH (i.e., the difference between the nadir and ensuing peak in blood LH levels) was greater than 250 ng/ml. Pulses of such magnitude occurred during both phases.

All pulses in the plateau phase had a pulse Δ blood LH greater than 250 ng/ml. Therefore, comparisons of pulsatile LH release during all three phases of the ovulatory surge could be made. LH interpulse intervals and the LH pulse amplitude (pulse Δ blood LH) were determined for all three phases of the surge, and values compared by one-way analysis of variance followed by Duncan's multiple range test.

Radioimmunoassay

Whole blood samples were analyzed for LH by a slight modification (Osland et al. 1975) of the ovine-ovine rat LH double-antibody radioimmunoassay of Niswender et al. (1968). The minimum sensitivity of this assay is 2.5 ng. Anti-ovine LH #15 was diluted 1:40,000, and 24 h later 50,000–60,000 cpm of [^{125}I]-ovine LH were added to each assay tube. Second antibody was added 24 h later. The intraassay CV was 7% and the interassay CV for pools of rat plasma containing 488 ± 22 ng/ml and 101 ± 5 ng/ml (n = 5) were 10% and 11.8%, respectively. Samples obtained from an individual rat were all assayed in a single assay, but samples from all animals were not measured in the same assay. LH values (ng/ml whole blood) are expressed in terms of the NIAMDD Rat LH-RP-1 which has a biological potency equivalent to 0.03 × NIH-LH-S1. Values given in the text represent the mean ± SEM.

RESULTS

Eleven rats bled on the afternoon of proestrus showed marked surges in LH secretion which caused each animal to ovulate, yielding 11.5 ± 0.8 ova per rat. The ovulatory surge had ascending, plateau, and descending phases. Mean blood LH levels at 1700 and 1800 h for the group as a whole represented the composite of points taken from individual rats during different phases of the surge, predominantly coming from the descending and secondarily from the plateau portions. These levels were 1194 ± 97 and 1005 ± 43 ng/ml, respectively, which is similar to the level of 1000 ng/ml (plasma LH levels converted to whole blood values) reported to occur at these times (Blake, 1976a).

In general, the ascending phases began between 1430–1600 h, lasted from 60–80 min, and were followed by plateau periods lasting about 60–90 min. As previously stated, the CV for blood LH levels during the plateau phase (12.3 ± 0.9%) was significantly greater than assay variation (7.0 ± 0.6%), and therefore LH release was said to be pulsatile during this phase. Pulsatile release was also evident during the ascending and descending phases of the surge. The LH interpulse interval and LH pulse magnitude for each phase are summarized in Fig. 1. Blood LH levels increased in the ascending period of the surge and then plateaued because of high amplitude LH pulses (538 ± 50 ng/ml in the ascending phase and 572 ± 29 ng/ml in the plateau phase) which occurred with LH interpulse intervals of 16 ± 1 and 23 ± 3 min, respectively (Fig. 1). In comparison with both the ascending and plateau portions of the LH surge, during the descending period the LH pulse amplitude decreased significantly to 386 ± 28 ng/ml ($P<0.01$ vs either preceding phase).

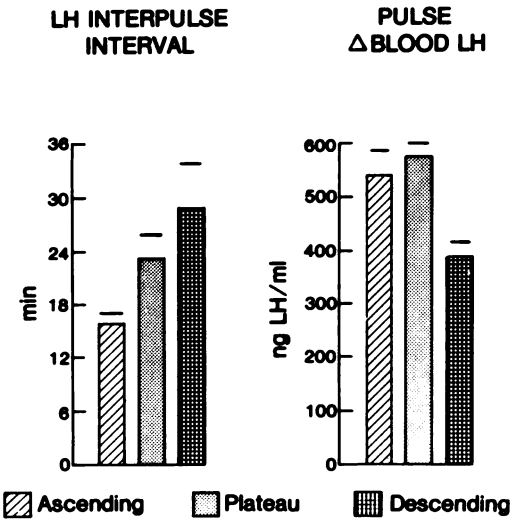

FIG. 1. LH interpulse interval and LH pulse amplitude (pulse Δ blood LH) during the ascending, plateau, and descending phases of the ovulatory LH surge on proestrus in the rat. Each bar represents the mean ± SEM for 9–11 rats. LH interpulse interval: ascending vs descending phase, $P<0.01$; Pulse Δ blood LH: descending vs ascending or plateau phase, $P<0.01$.

Moreover, in contrast to the ascending phase, the LH interpulse interval lengthened significantly to 29 ± 5 min ($P<0.01$). These factors accounted for the decline in blood LH levels. Representative examples of pulsatile LH release in individual rats during all three phases of the LH surge are given in Fig. 2.

DISCUSSION

These studies indicate that pulsatile LH release occurs throughout the ovulatory LH surge on proestrus in the rat. Moreover, the rise, plateau, and fall in blood LH levels can be accounted for by changes in the LH pulse amplitude and frequency during different phases of the surge. On the morning of proestrus, the LH pulse amplitude is 16 ng/ml and the interpulse interval is 63 min (Gallo, 1981). Blood LH levels increased in the ascending period of the surge and then plateaued, due in part to a 35-fold increase in pulse amplitude. In addition, these pulses occurred at intervals of 16 min in the ascending phase and 23 min in the plateau phase, and this is far shorter than the circhoral rhythm observed on the morning of proestrus. Therefore, both factors contribute to the elevation and subsequent maintenance of high blood LH levels during the ovulatory surge. LH levels decline as the LH pulse amplitude markedly decreases during the descending phase of the surge. The LH interpulse interval also lengthens as the pulses occur much less frequently than during the ascending phase. Both factors contribute to the decline in blood LH levels. In contrast, the human ovulatory LH surge is due only to a doubling in the LH pulse amplitude, as the periodicity of LH release remains the same as during the follicular or early luteal phases of the cycle (Yen et al., 1972).

In view of the present data it is reasonable to postulate that LHRH may also be secreted in a pulsatile fashion throughout the ovulatory surge, with the frequency of these pulses varying with different stages of the surge. The secretion of LHRH is increased during the ovulatory surge (Sarkar et al., 1976; Kalra and Kalra, 1977; Oshima et al., 1978), and the large variations observed in the concentration of LHRH in the portal blood are suggestive of pulsatile release (Sarkar et al., 1976). The content of LHRH in the medial basal hypothalamus also fluctuates during the ovulatory surge (Kalra and Kalra, 1977), although with a periodicity (60 min) longer than suggested by the present report (Barr and Barraclough, 1978). Measurement of LHRH content, however, does not necessarily reflect the actual frequency of LHRH release.

The present data support the speculation, offered in an elegant description of the ovulatory LH surge in the rat, that the plateau and descending phases may be composed of pulses of LH release (Blake, 1976a). However, with a sampling interval of 5 min the rapidly ascending phase was indicated to be linear (Blake, 1976a). In experiments preliminary to those reported in the present paper, pulsatile LH release was evident during the ascending phase in two of five rats bled at 5 min intervals. By sampling a signal more frequently, the possibility of reproducing the original signal increases (Ackerman and Gatewood, 1979). Therefore, during the ascending phase, when LH pulses are superimposed on a changing baseline concentration, it would appear that a sampling interval shorter than 5 min is required to measure an LH interpulse interval which is only 16 min.

The decrease in plasma LH concentration during the initial declining phase of the spontaneous LH surge is reported to be slower than the decrease in LH seen after stopping a constant infusion of LHRH in proestrous rats

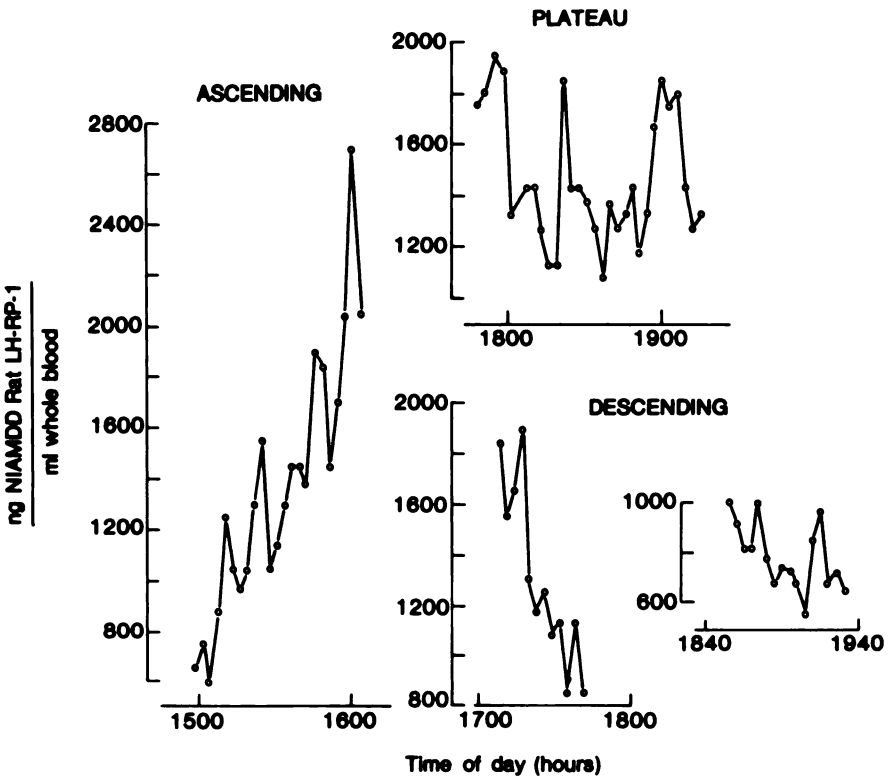

FIG. 2. Representative examples of pulsatile LH release in individual animals during the ascending, plateau, and descending phases of the ovulatory LH surge on proestrus in the rat.

(Blake, 1976b). In addition, during the declining phase of the LH surge the half-life for LH is greater than the reported half-life for LH in rats (Blake, 1976a; Schuiling et al., 1976a,b). These findings suggest that LH release continues during the declining phase of the surge. This conclusion is supported by the present indication that pulses of LH release do occur during this declining phase. Moreover, since LH secretion stops completely following infusion of LHRH in proestrous rats (Blake, 1976b), these pulses cannot be due to residual LHRH activity remaining at the pituitary gland. The factors that contribute to a decreased LH pulse amplitude in the declining phase may include decreases in the secretion of LHRH, in the pituitary responsiveness to LHRH, and in the ability of pituitary LH cells to synthesize and package LH in response to LHRH (Aiyer et al., 1974; Blake, 1976b; Garner and Blake, 1979; Blake and Garner, 1980).

While rats can be made to ovulate by a single injection of LHRH (Humphrey et al., 1972), or to release large amounts of LH over several hours in response to constant-rate infusions of LHRH (Blake, 1976b; Schuiling et al., 1976b), this does not necessarily mean that the pulsatile aspect of LH release during the surge is unrelated to the ovulation process. Moreover, there are other ovarian processes, such as meiosis, cumulus maturation, follicular development, luteinization, and progesterone production, which are also dependent on the LH surge (for references see Turgeon, 1979). The physiological response of another endocrine target organ, the pituitary, is clearly dependent on its being stimulated by a pulsatile signal as opposed to a prolonged steady hormonal input (Belchetz et al., 1978). The importance of the pulsatile character of LH release during the ovulatory surge to the above ovarian events remains to be determined.

ACKNOWLEDGMENTS

I want to thank Cindy Voytek and Geoffrey Lewis for their skilled technical assistance in these experi-

ments, Annette Lowe for preparation of figures, and Nancy Lyon and Jeanne Connolly for typing the manuscript. Thanks also to Dr. G. D. Niswender for antiovine LH #15, Dr. H. Papkoff for highly purified ovine LH G3222B for iodination, Dr. J. Garcia for goat antiserum against rabbit gamma globulin, and Dr. A. F. Parlow and the NIAMDD for the rat LH used as a reference preparation.

REFERENCES

Ackerman, E. and Gatewood, L. C. (1979). Mathematical Models in the Health Sciences. A Computer Aided Approach. University of Minnesota Press, Minneapolis. p. 127.

Aiyer, M. S., Fink, G. and Greig, F. (1974). Changes in sensitivity of the pituitary gland to luteinizing hormone-releasing factor during the oestrous cycle of the rat. J. Endocrinol. 60, 47–64.

Barr, G. D. and Barraclough, C. A. (1978). Temporal changes in medial basal hypothalamic LHRH correlated with plasma LH during the rat estrous cycle and following electrochemical stimulation of the medial preoptic area in pentobarbital treated proestrous rats. Brain Res. 148, 413–423.

Belchetz, P. E., Plant, T. M., Nakai, Y., Keogh, E. J. and Knobil, E. (1978). Hypophyseal responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. Science 202, 631–633.

Blake, C. A. (1976a). A detailed characterization of the proestrous luteinizing hormone surge. Endocrinology 98, 445–450.

Blake, C. A. (1976b). Simulation of the proestrous luteinizing hormone surge after infusion of LH-releasing hormone in phenobarbital blocked rats. Endocrinology 98, 451–460.

Blake, C. A. and Garner, L. L. (1980). Pituitary refractoriness to luteinizing hormone releasing hormone: Its importance in ending the luteinizing hormone surge in the cyclic rat. Proc. Soc. Exp. Biol. Med. 164, 374–379.

Gallo, R. V. (1981). Pulsatile LH release during periods of low level LH secretion in the rat estrous cycle. Biol. Reprod. In Press.

Garner, L. L. and Blake, C. A. (1979). Morphological correlates of LHRH self-priming and anterior pituitary gland refractoriness to LHRH in proestrous rats: An immunocytochemical study. Biol. Reprod. 20, 1055–1066.

Humphrey, R. R., Dermody, W. C., Brink, H. O., Bousley, F. G., Schottin, N. H., Sakowski, R., Vaitkus, J. W., Veloso, H. T. and Reel, J. R. (1972). Induction of LH release and ovulation in rats, hamsters and rabbits by synthetic luteinizing hormone-releasing factor (LRF). Endocrinology 92, 1515–1526.

Kalra, P. S. and Kalra, S. P. (1977). Temporal changes in hypothalamic and serum LHRH levels and the circulating ovarian steroids during the rat oestrous cycle. Acta Endocrinol. 85, 449–455.

Kapen, S., Boyar, R., Hellman, L. and Weitzman, E. D. (1973). Episodic release of luteinizing hormone at mid-menstrual cycle in normal adult women. J. Clin. Endocr. Metab. 36, 724–729.

Midgley, A. R., Jr. and Jaffe, R. B. (1971). Regulation of human gonadotropins. X. Episodic fluctuation of LH during the menstrual cycle. J. Clin. Endocr. Metab. 33, 962–969.

Niswender, G. D., Midgley, A. R., Jr., Monroe, S. E., and Reichert, L. E., Jr. (1968). Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine LH-^{131}I. Proc. Soc. Exp. Biol. Med. 128, 807–811.

Oshima, I., Morishita, H., Omura, K. and Saito, S. (1978). Changes in hypothalamic LHRH content and blood levels of LHRH, gonadotropin and estradiol during the preovulatory stage of the rat estrous cycle. Endocrinol. JPN. 25, 607–611.

Osland, R. B., Gallo, R. V. and Williams, J. A. (1975). In vitro release of luteinizing hormone from anterior pituitary fragments superfused with constant or pulsatile amounts of luteinizing hormone-releasing factor. Endocrinology 96, 1210–1216.

Rahe, C. H., Owens, R. E., Fleeger, J. L., Newton, H. J. and Harms, P. G. (1980). Pattern of plasma luteinizing hormone in the cyclic cow: Dependence upon the period of the cycle. Endocrinology 107, 498–503.

Sarkar, D. K., Chiappa, S. A., Fink, G. and Sherwood, N. M. (1976). Gonadotropin-releasing hormone surge in pro-oestrous rats. Nature 264, 461–463.

Schuiling, G. A., deKoning, J. and Zurcher, A. F. (1976a). On differences between the preovulatory luteinizing hormone surges of 4 and 5 day cyclic rats. J. Endocrinol. 70, 373–378.

Schuiling, G. A., deKoning, J., Zurcher, A. F., Gnodde, H. P. and van Rees, G. P. (1976b). Induction of LH surges by continuous infusion of LHRH. Neuroendocrinology 20, 151–156.

Turgeon, J. L. (1979). Estradiol-luteinizing hormone relationship during the proestrous gonadotropin surge. Endocrinology 105, 731–736.

Yen, S.S.C., Tsai, C., Naftolin, F., Vandenberg, G. and Ajabor, L. (1972). Pulsatile patterns of gonadotropin release in subjects with and without ovarian function. J. Clin. Endocrinol. 34, 671–675.