OBSERVATIONS ON SUCCESSIVE PRO-OESTROUS LH SURGES IN INDIVIDUAL 4-DAY CYCLING AND POST-PSEUDOPREGNANT RATS

By

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ABSTRACT

The internal variations (i.e. in timing, duration and amplitude) of the LH surge mechanism within individual rats were examined by monitoring from 3 to 7 successive pro-oestrous LH surges in each of 5 regular 4-day cycling rats fitted with chronic intravenous cannulas. On each successive pro-oestrus blood was collected (0.5–0.6 ml hourly from 14.00–21.00) for radioimmunoassay of LH. The surgery of cannulation had no long-term effect on the regularity of the oestrous cycle. Two rats did, however, show briefly irregular cycles, including one with a 9-day period of anoestrus (pseudopregnancy). In three of the five rats successive pro-oestrous plasma LH curves (4 in one and 3 each in the other two) were internally very consistent in timing, shape and amplitude, however, between each 2 of these 3 animals there were distinct differences in the LH secretory patterns, by as much as 2 h in timing of the onset of the surge and its peak amplitude. The first two surges of the other two rats were atypical of their subsequent surges, which were mostly consistent in timing and amplitude. The pro-oestrous LH surge following the 9-day period of anoestrus was advanced by 2 h and elevated to twice the mean peak amplitude of the cyclic LH surges in that rat. Subsequently, post-PSP surges were studied in rats made pseudopregnant by mechanical stimulation of the cervix. In all cases the immediate post-PSP surge occurred earlier in the afternoon and with a greater peak amplitude than the subsequent cyclic LH surge in the same rat.

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Ovulation in the rat is preceded by an abrupt elevation in plasma LH concentration, an event referred to as the pre-ovulatory LH surge (Barraclough 1973). In the 4-day cycling rats this surge begins during the 2 h “critical period” on the afternoon of pro-oestrus (Everett & Sawyer 1950). Circulating LH attains peak values 1–3 h after the initiation of the surge and declines to basal concentration by midnight of that same day (Blake 1976). It is now well accepted that the LH surge among different rats is highly variable with respect to time of initiation, magnitude and duration (Blake 1976; Turgeon & Barraclough 1973; Everett et al. 1973); however, little is known about the uniformity of this hormonal event during successive oestrous cycles in the same animal.

The present study was designed to investigate the variations in timing and amplitude of the LH surges within individual female rats during several successive periods of pro-oestrus. As a result of some unexpected findings obtained during the course of these experiments, another experiment was designed to investigate the LH surge on the day of pro-oestrus that follows a period of pseudopregnancy.

MATERIAL AND METHODS

Animals used in this study were adult female Sprague-Dawley rats (200–300 g Simonson) which were initially housed 5–10 per cage under controlled lighting (lights on from 05.00 h to 19.00 h). Under this lighting schedule the critical period is defined as 14.00–16.00 h (Everett & Sawyer 1950). Purina lab chow and water were available ad libitum. Vaginal smears were taken daily and only those animals that had maintained at least two consecutive 4-day oestrous cycles were selected for the study.

Cannulation procedure

To study the pre-ovulatory LH surge, a cannula was inserted into the right external jugular vein during the afternoon of dioestrus II (unless otherwise stated). The cannulation was done under Brevital (sodium methohexitol) anaesthesia, following the technique of Harms & Ojeda (1974) with the following modifications: (a) the use of a smaller diameter silastic tubing (Dow-Corning No. 602-105, I.D. 0.012 in., O.D. 0.025 in.); (b) use of a smaller implantation needle (29-gauge stainless steel hypodermic needle inside a 23-gauge needle); and (c) replacement of the silicone sheet with surgical silk that was loosely tied to the cannula (4 cm from the intravenous tip) and bonded to it with Silastic brand silicone adhesive type A. The loose ends of this ligature were then sutured to the strap muscles of the neck, thus securing the cannula. After cannulation, the animals were housed one per cage, and the cannulas were flushed twice daily with heparinized saline (200 units/ml) to maintain patency.

LH surges in different animals

A group of rats were cannulated, and on the second or third post-operative pro-oestrous day each animal was placed in a sampling chamber and the cannula was connected to a polyethylene (PE 50) extension tubing. Just prior to 14.00 h, 200 units of heparin were injected through the cannula, and blood samples (0.5–0.6 ml) were
collected every hour from 14.00 h to 21.00 h. Each blood sample was centrifuged and the plasma was separated and frozen at −20°C for subsequent LH radioimmunoassay (RIA). Red blood cells were resuspended in physiological saline (0.2–0.3 ml) and returned to the animals. On the next day (oestrus) the uterine tubes were examined for the presence of ova.

**Successive LH surges in the same animal**

In another group of rats the oestrous cycle was closely monitored during the post-cannulation period and on the morning of each successive pro-oestrus, the animal was placed in a sampling chamber, and blood samples were collected as described above. On the morning of oestrus which followed the last period of pro-oestrus sampled (3 to 7), uterine tubes were examined for ova.

**The post-pseudopregnancy LH surge**

Pseudopregnancy was induced in a third group of rats by mechanical stimulation of the cervix on the mornings of pro-oestrus and oestrus. Each rat was stimulated for 60 sec with a vibrator as described by DeFeo (1966), and the second day of stimulation was designated as day 0 of pseudopregnancy. The duration of the resulting pseudopregnancy was 13–18 days. On days 10–11 the animals were cannulated and during the first two pro-oestrous periods after pseudopregnancy, blood samples were taken. On the morning of the second oestrus uterine tubes were checked for ova.

**Radioimmunoassay**

Plasma LH was measured using the kits provided by NIAMDD (A. F. Parlow) with rat LH-RP1 as a reference preparation which has a biological potency of 0.03 × NIH-LH-S1. Samples from each individual rat were determined in a single assay. A total of 2 assays were run. Samples (50 μl) were assayed in duplicate and the values were averaged. In order to determine the interassay variation, samples (50 μl) from a plasma pool obtained from castrated female rats were measured for LH. Mean ± SE for the pool from both assays was 712 ± 18 ng/ml and the interassay coefficient of variation was 11.38%.

**RESULTS**

In rats cannulated to be bled during only one pro-oestrous period the surgery of cannulation appeared not to exert any long-term deleterious effect on the regularity of the oestrous cycles, and the rats were bled during the second or third pro-oestrus after cannulation. They were autopsied the following day and examination of their uterine tubes on this final morning of oestrus revealed that all of them had ovulated normal complements (7–12) of ova. Nevertheless, as shown in Fig. 1, there was considerable variation in the time of initiation, amplitude and duration among the LH surges in these eight rats. After reaching peak values, however, most of the curves descended quite closely together.

The pro-oestrous LH profiles of two rats (Nos. 49 and 103) with chronic cannulas are plotted in Fig. 2. Both animals were cannulated on the afternoon...
Pro-oestrous plasma LH surges in 8 different 4-day cyclic rats.

Pro-oestrous plasma LH profiles in rats Nos. 49 and 103. In this and subsequent figures the letters on top of the graph depict the days of the oestrous cycle: P, pro-oestrus; E, oestrus; D, dioestrus. The LH surges corresponding to the successive periods of pro-oestrus are numbered in chronological order (lettered A, B, C for No. 103). The day of cannulation is represented by an arrow. The number of ova found on the last day of oestrus indicated in parentheses.
**Fig. 3.**
Pro-oestrous plasma LH profiles in rat No. 118. As in Fig. 2.

**Fig. 4.**
For comparison, the curves represent the means and standard errors (vertical lines) of the LH profiles of rats Nos. 49, 103 and 118 in Figs. 2 and 3. The data on No. 118 are a summary of curves 2–5 in Fig. 3.
of pro-oestrus and sampled on the following three cycles during pro-oestrus. The cannulation surgery had no apparent effect on cyclic pituitary-ovarian function, and in these animals the LH releasing mechanisms seemed to have little internal variation. However, they were very different from each other in time of initiation and amplitude, and showed no overlap in the rising phases of the LH curves. Notice that the later “take-offs” (No. 103) were associated with lower peak amplitudes, and that in spite of the differences between the two animals, both groups descended in an almost superimposable manner.

A third “chronic” animal (No. 118) was cannulated on dioestrus I and bled during the afternoons of the five subsequent pro-oestrus periods (Fig. 3). The first LH curve was atypical and was followed by a five-day oestrous cycle. Thereafter, the cycles were regular four-day periods and the pro-oestrous LH surges were very consistent in timing, amplitude and duration. The lack of internal variation in rats Nos. 49, 103 and 118 is reemphasized in Fig. 4, in which their pro-oestrous LH profiles are summarized by plotting their means and standard errors on the same coordinates. Although their rising phases were essentially an hour apart, the descents of the three LH curves were practically identical.

Fig. 5 illustrates the findings from rat No. 26, which was cannulated on dioestrus II and sampled during pro-oestrus in 7 of the next 8 cycles. Although the vaginal cycles were regular, the first two LH curves were exceptional and...
Pro-oestrous plasma LH profiles in rat No. 35. As in Fig. 2, except that the asterisk (*) denotes LH level during one afternoon of dioestrus. Again, curves 1 and 2 represent extremes.

the last five quite consistent. The earliest surge (No. 1) had the highest (1967 ng/ml) peak value, and the most delayed surge (No. 2) had the lowest rise (984 ng/ml). The other five LH curves fell between these two in timing and amplitude.

Rat No. 35 (Fig. 6) was also cannulated on dioestrus II; however, unlike conditions in No. 26, its first pro-oestrous LH surge was delayed and of atypically low magnitude. Thereafter, this animal had 9 consecutive days of vaginal dioestrus or anoestrus and may have been pseudopregnant. During the afternoon of the fourth day of leucocytic smears, LH levels never exceeded 50 ng/ml. The anoestrous period terminated with an LH surge (No. 2) earlier in time and of a magnitude (2232 ng/ml) equal to twice the mean (1169 ng/ml) peak value of the succeeding LH surges. This animal then returned to regular 4-day oestrous cycles during which three out of four pro-oestrous LH surges were quite consistent in their temporal patterns and amplitudes.

The results from No. 35 suggested that the LH surge following a prolonged period of anoestrus, such as pseudopregnancy, occurs earlier in time and with a greater peak amplitude than the other LH surges. To test this possibility, PSP was induced in several rats and blood samples were taken during the afternoons of the first and second pro-oestrous surges following the period of
Plasma LH surges in 5 different rats during the two successive periods of pro-oestrus after pseudopregnancy or prolonged dioestrus. Included in this figure are two such curves from rat No. 35 shown in Fig. 6.

The results of this experiment are shown in Fig. 7, which includes the two successive LH curves from animal No. 35. Cannulation surgery during the period of PSP may have contributed to a relatively large variation in the length (13–18 days) of the PSP. However, the data from these animals confirm the hypothesis that the post-PSP LH surge has an earlier time of onset and a much greater peak amplitude than the cyclic LH surge in each rat. In all of these 5 rats, the post-PSP surge had a peak LH value of 2000 ng/ml or more, which is considerably higher than pro-oestrous peaks generally observed in this laboratory.

**DISCUSSION**

The characteristics of the pre-ovulatory LH surge have been described previously in studies involving single cycles in many rats (Blake 1976; Everett et al. 1973; Schuiling et al. 1976; Turgeon & Barraclough 1973); however, the present report is the first to our knowledge in which successive LH surges from the same animal have been monitored. This was made possible by the can-
nulation technique (Harms & Ojeda 1974) which not only enabled us to obtain non-stress blood samples for a long time (12–30 days) but also introduced a minimum of interference with subsequent cycles following cannulation surgery. Whereas these surgical procedures have been reported not to interrupt the LH surge under acute conditions (Blake 1974, 1976), very few investigators have used this technique under long-term conditions to monitor LH levels in the cycling rat.

It is apparent from our results that the first or even the first two LH surges following cannulation at dioestrus may lack the consistency of later surges in timing and amplitude (rats Nos. 118, 26 and 35). This may explain some of the inter-animal variability in LH surges reported in short-term experiments (Blake 1976; Everett et al. 1973; Schuling et al. 1976; Turgeon & Barraclough 1973). However, our more consistent findings on later successive LH curves in these rats and all of the LH curves of rats Nos. 49 and 103 show remarkably little internal variation but marked contrasts between different animals when timing and amplitude of their LH curves are compared (Fig. 4). It is interesting that regardless of time of onset or elevation attained in the LH curve the descending slopes were, in most cases, closely adjacent parallel lines approaching baseline levels by 2100, the time of the last blood collection. Corollaries of this observation are that the highest peaks were usually reached in curves with early “take off” times, and that surges starting late tended to be of low amplitude and short duration. This had been shown previously in rats in which the initiation of the LH surge had been delayed pharmacologically (Blake 1974; Blake et al. 1972). Extended periods of “activation” and “potential activation” persist (Blake 1974) after the end of the critical period, which may itself have somewhat variable time limits (Everett 1964). In general, during cyclic pro-oestrus the later the LH secretory mechanism is activated the smaller the amount of LH that is released (Blake 1974).

Using pseudopregnant rats as their own controls, we noted that the first pro-oestrous LH surge after PSP or prolonged dioestrus occurred distinctly earlier in the afternoon than the next pro-oestrous surge 4 days later. In the case of rat No. 186, plasma LH levels were markedly elevated at 14.00 h, a pattern rarely seen in intact cycling rats in this laboratory. However, these findings are in agreement with those of other investigators who have reported that post-PSP (Hoffman & Schwartz 1965b) and post-partum (Hoffman & Schwartz 1965a; Ying et al. 1973) critical periods occur earlier than in the 4-day cycling rat. In partial disagreement with the present findings are two studies in which plasma LH values were reported to be already significantly elevated during the morning of the post-PSP (Bast & Melampy 1972) and post-partum (Ying et al. 1973) pro-oestrous days. Although our data indicate that the post-PSP LH surge occurs early, we have no evidence that it begins before noon on pro-oestrus.
In the post-PSP animals (Fig. 7), the LH surge not only occurs earlier in time, but also achieves an extremely high peak value. The 2000 ng/ml or higher values attained in these cases are unusual for this laboratory, although such levels have been reported by others (Blake 1976; Schuiling et al. 1976). This confirms, in individual rats, the findings of Ying et al. (1973), who reported that the peak elevations of the post-partum LH surge reached significantly higher levels than the cyclic LH surge in groups of normal 4-day cyclic animals. It is possible that these unusually high post-PSP hormone outputs are related to the higher than normal concentrations of pituitary LH content that have been observed towards the end of pseudopregnancy (Hoffman & Schwartz 1965b; Schwartz & Rothchild 1964).

In ovulation experiments involving timed hypophysectomies and retrochiasmatic deafferentiations Van Rees et al. (1972) showed that the pro-oestrous critical period started 1½ h earlier in rats with 5-day ovarian cycles than in 4-day rats, and this was confirmed by Smith et al. (1973) with radioimmunoassays of plasma gonadotrophins. Schuiling et al. (1976) observed not only that the LH surge started earlier in 5-day cyclers, but also that the peak elevation was twice as high in this group as in the 4-day animals. This was not attributable to increased responsiveness of the pituitary to LH-RH, but presumably to the central nervous control of LH-RH release (Schuiling et al. 1976), conditioned by the higher and/or more prolonged blood oestradiol peaks in the 5-day rats (Smith et al. 1973). The pro-oestrous surge in the 5-day cyclers resembles, in timing and amplitude, the post-pseudopregnancy surge in our experiments.

After recovery from the surgery of intra-atrial cannulation, which may require as much as two oestrus cycles, individual rats show successive pro-oestrous LH surges which are relatively consistent in timing and amplitude when compared with the variation among different animals. It would be advantageous, therefore, in experiments on the cyclic LH surge to have long-term cannulated rats serve as their own controls. An early and elevated LH surge appears to be characteristic of pro-oestrous following pseudopregnancy or prolonged dioestrus.

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REFERENCES


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