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Ovariectomy at Different Stages of the Estrous Cycle Modifies the Pulsatile Secretory Pattern of Prolactin in Rat

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The role of estrogens in the synchronization of pulsatile prolactin secretion throughout the estrous cycle in the adult female rat is studied. Mean values of prolactin, in sham-operated animals increased from diestrus-2 to proestrus and then decreased to the values found at diestrus-1. Ovariectomy did not modify the pattern which follows mean serum levels of the hormone throughout the estrous cycle; although increased values of prolactin were found at both proestrus and estrus, when compared to control animals. No changes in mean values of the hormone were observed at any other phase of the estrous cycle. The number of peaks was fairly constant in sham-operated rats in any phase of the estrous cycle, but a decrease in its number, after ovariectomy, was observed at proestrus and estrus. The absolute amplitude of the peaks increased numerically but not statistically significant from diestrus-2 to proestrus, then decreasing to diestrus-1 in control animals whereas an increase in the absolute amplitude of the prolactin peaks was detected in proestrus and estrous after ovariectomy. The duration of the prolactin peaks was not changed either by the phase of the estrous cycle or by ovariectomy. The relative amplitude of the prolactin peaks was only changed in rats ovariectomized at diestrus-1, as compared to sham-operated animals. All these data indicated that changes in estrogen secretion by ovariectomy throughout the estrous cycle of the rat induced a differential effect on the pulsatile pattern of prolactin, being more marked in proestrus and estrus, associated with the higest circulating estrogen levels.

Key words: Prolactin, Pulsatile release, Estrous cycle, Ovariectomy.

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There is increasing evidence that prolactin is secreted in an episodic fashion (5-7, 9, 15), although it is incompletely characterized, and the regulation of this mode of prolactin secretion is not understood. In this regard, different neuromodulators have been observed to exert complementary effects on the characteristic parameters, which define prolactin pulsatility (4), and suggest that the episodic release of prolactin is the final result of interactive effects of all endogenous substances involved in its regulation.

Among all these modulators, estrogens play an important role in the regulation of the episodic prolactin secretion (13, 19). During the estrous cycle estrogen secretion increases from diestrus to estrus (14), and these modifications are followed by changes in prolactin secretion (3, 10, 11). To avoid estrogen changes throughout the estrous cycle for the characterization of the episodic secretion of prolactin, most of the work in females of any species studied, was performed in ovariectomized animals (9). However, most of them did not explain the phase of the estrous cycle in which the ovariectomy was performed (13).

This work was designed to study the effects of estrogens in the synchronization of the episodic prolactin secretion throughout the estrous cycle. For that purpose, female rats were ovariectomized in each phase of the estrous cycle and 24 hours later the pulsatile secretory pattern of prolactin was evaluated. A group of sham-operated rats in each phase of estrous cycle were also used also as controls. Sampling was performed in the morning.

Materials and Methods

Animals.– Adult female Sprague-Dawley CD rats, weighing 240-280 g, were used in all experiments. They were maintained in a room with controlled photoperiod (14 h light/10 h darkness; lights on from 06.00 to 20.00 h), and temperature (22 ± 2 °C) and with rat chow and water available *ad libitum*. Vaginal smears were taken daily, and only rats showing at least two consecutive 4-day estrous cycles were used in this study.

Ovariectomy and cannula implantation.– Rats in the morning of each phase of the estrous cycle were anesthetized with tribromoethanol (2.5 % tribromoethanol, 1 ml/100 g), and ovariectomized or sham-operated, and atrial cannulas were implanted through the external jugular vein according to procedures used in previous studies (1). This procedure allows the animals to move freely in their cages during the period of bleeding.

Experimental Design and blood sampling.– Eight groups of rats were studied: ovariectomized or sham-operated animals in the morning of each phase of the 4-day estrous cycle. Eight rats per group were studied.

Rats were bled 24 h after the ovariectomy-or sham operation and cannula implantation. On the experiment day, conscious and freely moving rats from each group were continuously infused with 0.9 % saline (0.5 ml/h) for 4 hours, beginning at 09.30 h. One hour after the intravenous infusion of saline and 15 minutes after the administration of 300 I.U. of heparine, rats were bled continuously through a peristaltic pump at a flow rate of 50 µl every 5 minutes. Blood samples were collected in Hamilton microliter syringes every 5 minutes for three hours from 10.30 h to 13.30 h. The samples were collected into assay tubes kept on ice and containing phosphate buffer (0.01 mol/l) with 0.1 % gelatin. Hematocrits remain stable with this bleeding protocol (36-41

%). Samples were centrifuged at 1,500 x g for 15 min at 4 °C and the serum was kept frozen at -20 °C until analyzed. The sampling schedule was chosen in accordance with previous work of this group which was carried out during the morning in order to analyze serum plasma prolactin levels (5, 17).

Prolactin radioimmunoassay.– Serum prolactin levels were measured using a specific double antibody radioimmunoassay technique. The reagents were kindly supplied by the National Hormone and Pituitary Program (Baltimore, MD). Prolactin values (µg/l) are expressed in terms of the rat NIADD rat PRL RP-3 reference preparation. The sensitivity of the assay was 5 pg/tube. Samples were measured within the same assay to avoid interassay variation. The intraassay coefficient of variation was 6.5 %.

Data analysis.– To identify and characterize pulses appearing in the hormonal profile of each rat, a computer program (Ultra-analysis) was used (18), a pulse is defined as a significant increase exceeding a multiple of the dose-adjusted coefficient of variance (CV), followed by a significant decrease. The intraassay CVs were calculated from values of five different concentrations of prolactin in its standard curve. Thus, the CV and the mean hormone level were determined for prolactin values which comprised the ascending and descending phases of each potential pulse. The pulse was defined when this CV was twice that of the intraassay CV determined at a comparable mean prolactin level. To test the specificity of pulse detection, a series of 26 samples from a pool of serum was analyzed using a threshold of 2 CV for prolactin peaks.

Pulsatile prolactin secretion pattern was characterized by the mean hormone level, absolute and relative amplitude of the peaks, their frequency and pulse duration. The absolute pulse amplitude was defined as the difference between the hormone level at the maximum of the peak and the hormone level at the preceding nadir. The relative pulse amplitude was calculated as the quotient between absolute pulse amplitude and preceding nadir value. Pulse frequency was the number of pulses/3 h. Pulse duration was the time between the beginning of the ascending phase and the end of the descending phase of the peak.

The mean hormone level was calculated by the mean of all samples collected from each rat during the 3 h period, and the average for the experimental group from the individual means.

Statistical analysis of the data.– Comparison of values for the pulsatile parameters was done by analysis of variance followed by Duncan's multiple range test or Student's t test. The results were considered significant at $p < 0.05$. All values represent the mean ± SEM.

Results

A representative profile of the pulsatile pattern of prolactin, of one rat from each group, is given in figure 1. The main changes in prolactin pulsatility by ovariectomy were observed when the surgery was performed in proestrus and estrus, whereas no differences were seen in diestrous-1 and 2 phases of the estrous cycle.

Ovariectomy in all phases of the estrous cycle increased serum prolactin levels, measured as a mean value of all points studied during the bleeding period, being significantly different in proestrus and estrus ovariectomized rats (table I). The number of prolactin peaks was significantly reduced only in rats ovariec-

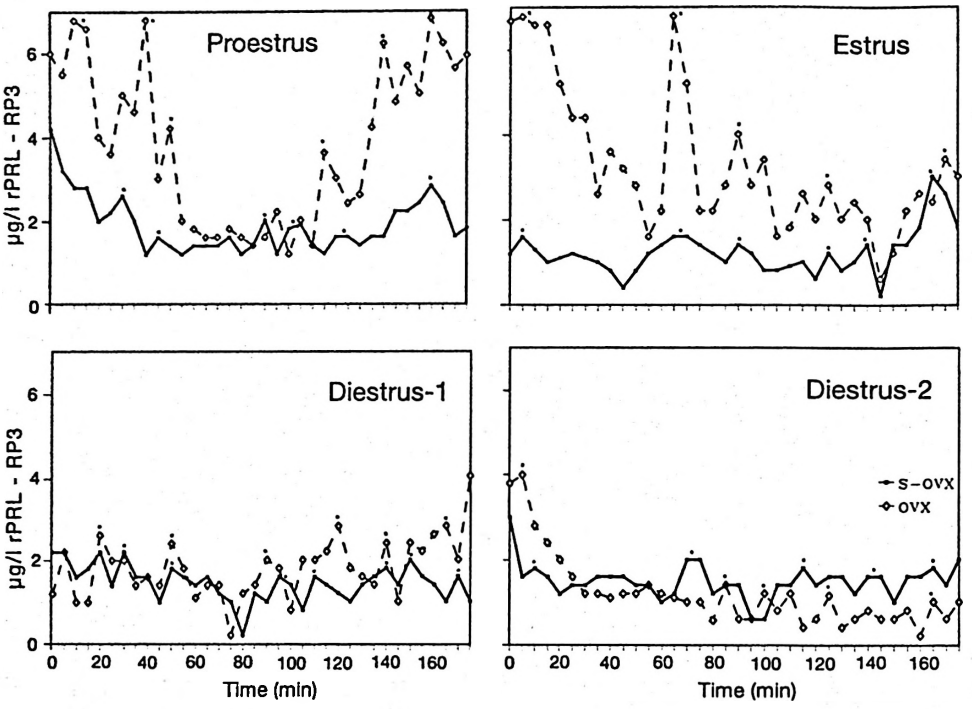

Fig. 1. *Individual pulsatile prolactin patterns 24 hours after ovariectomy or sham-operation throughout the estrous cycle at proestrus, estrus, diestrus-1 and diestrus-2.*
Values are given in terms of rPRL-RP-3. Asterisks indicate the prolactin peaks during the period studied.

tomized in the proestrous and estrous phases of the estrous cycle (table II) as compared to sham-operated rats. The absolute amplitude of the prolactin peaks significantly increased only when ovariectomy was performed in estrous and proestrous phases of the cycle (table I) in relation with that observed in sham-operated females. The relative amplitude of prolactin peaks significantly decreased on diestrus-1 ovariectomized rats (table I) as compared to control rats. Ovariectomy did not change the relative amplitude of the prolactin peaks in any other phase of the estrous cycle (table I). The duration of prolactin peaks was not significantly modified by ovariectomy in any of the phases of the estrous cycle (table II).

Discussion

The foregoing results suggest that ovariectomy in each phase of the estrous cycle differentially affect the episodic prolactin release, when compared to the pulsatile patterns shown in control animals. In fact the prolactin pulsatility of the control animals is similar to that observed in previous work from the group (6).

The main effects of estrogens on pulsatile prolactin secretion are observed at the proestrous phase of the cycle in which the secretion of estradiol is higher (6) than in any other phase of the estrous cycle.

The values of mean prolactin levels in control animals in each phase of the estrous cycle is in agreement with previ-

Table I. *Quantitative parameters of pulsatile prolactin secretion (mean serum prolactin levels, absolute pulse amplitude and relative pulse amplitude) throughout the estrous cycle and 24 h after ovariectomy.* The relative pulse amplitude was calculated as the quotient between absolute pulse amplitude and preceding nadir value. Values are means ± S.E.M.; n = 8 rats per group.

Group	Prolactin (µg/l)	Absolute amplitude	Relative amplitude
Sham-operated			
Proestrus	3.75 ± 0.74	2.77 ± 1.09	0.98 ± 0.41
Estrus	2.92 ± 0.58	1.78 ± 0.59	1.64 ± 0.67
Diestrus-1	1.60 ± 0.27	1.52 ± 0.49	2.80 ± 0.80
Diestrus-2	1.22 ± 0.17	0.72 ± 0.09	1.15 ± 0.25
Ovariectomized			
Proestrus	6.58 ± 1.98*	4.50 ± 1.77*	1.64 ± 0.69
Estrus	5.01 ± 1.03*	3.44 ± 0.86*	1.59 ± 0.44
Diestrus-1	2.97 ± 0.88	1.59 ± 0.27	1.89 ± 0.36*
Diestrus-2	1.93 ± 0.71	0.91 ± 0.47	1.06 ± 0.26

*P < 0.05 compared with sham-operated rats.

Table II. *Qualitative parameters of pulsatile prolactin secretion throughout the estrous cycle and 24 h after ovariectomy.* Values are means ± S.E.M.; n = 8 rats per group.

Group	Frequency (pulses/3 h)	Duration (min)
Sham-operated		
Proestrus	8.1 ± 0.67	17.9 ± 1.13
Estrus	8.9 ± 0.65	17.9 ± 1.52
Diestrus-1	8.6 ± 0.49	17.4 ± 1.31
Diestrus-2	8.5 ± 0.62	19.1 ± 1.39
Ovariectomized		
Proestrus	6.4 ± 1.02*	23.2 ± 3.45
Estrus	6.8 ± 0.74*	22.7 ± 2.24
Diestrus-1	8.7 ± 0.92	16.6 ± 0.75
Diestrus-2	7.4 ± 0.42	19.6 ± 1.53

*P < 0.05 compared with sham-operated rats.

ous data from our group (6), with higher values during proestrous phase. Ovariectomy induced an increase in mean values of the hormone in all phases of the estrous cycle, being only significant in the proestrous and estrous stages. These effects are supported by an increase in the absolute amplitude of the prolactin peaks and the concomitant decrease in their frequency observed in this study. Similar results were described in hyperprolactinemic states of other etiology (5).

The pattern of prolactin peaks in control and ovariectomized animals showed a non-gaussian distribution, in agreement with other studies on prolactin pulsatility (5-7).

The lower values of serum prolactin, observed during diestrous 1 and 2 phases of the cycle in control and ovariectomized animals can be explained by an increased dopaminergic effect on the lactotrophs accompanied by low levels of estrogens which occur during this phase of the estrous cycle (12) and after ovariectomy.

Surprisingly the decrease in the levels of circulating estrogens induced by ovariectomy during the proestrous and estrous stages of the estrous cycle, was not

followed by the expected decrease in mean serum prolactin levels, thus suggesting that structures other than the hypothalamus are involved in the acute changes in prolactin pulsatility after the removal of estrogens.

Perhaps these effects might be mediated through not well understood changes in dopamine metabolism at the neurohypophysis, which could also influence its metabolism at the hypothalamic level (8).

The differences between other studies and ouers (2, 7) might be due to the use of different time schedules utilized to detect prolactin pulsatility and to the fact that lactotrophs have an intrinsic pulsatility which exhibits a circadian rhythm (16). This intrinsic pulsatility might be affected differently by the various levels of estrogens or ovariectomy throughout the estrous cycle which can influence the effects of other neuromodulators involved in the regulatory mechanism of prolactin release in each phase of the estrous cycle and also by the day-time timing used in this experiment.

Further experiments are needed to fully understand the mechanisms involved in the regulation of prolactin pulsatility, which seems to be a key event in controlling the reproductive cycle in the female rat.

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A. LAFUENTE, J. MARCÓ y A. I. ESQUIFINO. *La ovariectomía en las diferentes fases del ciclo estral modifica la secreción pulsátil de prolactina en rata.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (4), 211-218, 1994.

Se estudia el papel de los estrógenos en la sincronización de la secreción pulsátil de la prolactina a lo largo del ciclo estral. Los valores medios de prolactina sérica aumentan de la fase de diestro-2 a la de proestro y después disminuyen hasta los valores de diestro-1. La ovariectomía no modifica el patrón que siguen los niveles medios de prolactina a lo largo del ciclo estral, aunque se incrementan cuando se realiza en las fases de proestro y estro. La frecuencia de los pulsos de prolactina es prácticamente constante a lo largo del ciclo estral, aunque se observa un decenso después de ovariectomizar en las fases de proestro y estro. La amplitud absoluta de los pulsos aumenta numéricamente, aunque no significativamente de diestro-2 a proestro, decreciendo después hasta la fase de diestro-1 en los animales control; sin embargo, aumenta cuando se realiza la ovariectomía en las fases de proestro o estro. La duración de los pulsos hormonales no cambia a lo largo del ciclo estral ni tras la ovariectomía. Por otro lado, la amplitud relativa de los pulsos de prolactina sólo varía en las ratas ovariectomizadas en diestro-1, si se compara con los animales en las que se realiza la operación simulada en esa misma fase. Estos resultados sugieren que los cambios en la secreción de estrógenos producidos por la ovariectomía realizada en las diferentes fases del ciclo estral de la rata, inducen efectos diferentes sobre la secreción pulsátil de la prolactina, siendo más marcados cuando la ovariectomía se realiza en proestro o en estro, fases asociadas a niveles altos de estrógenos.

Palabras clave: Prolactina, Secreción pulsátil, Ciclo estral, Ovariectomía.

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