Detailed Profile of Prolactin Secretion in the Immature Female Rat: Evidence for the Existence of an Ultradian Rhythm

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Abstract

The detailed profile of prolactin (PRL) secretion in 22-24 and 29-31 days old female rats was investigated by serial blood sampling through an intracardiac cannula at 15-min intervals for each of the 9 or 10-h periods beginning at 09.00 or 10.00 and 22.00 h. By analysis of the power spectrum and the least squares method the time series of PRL concentrations which were measured by RIA were found to have approximately a 3-h period ultradian rhythm in either sampling period of both the 22-24 and 29-31 days old rats. The peak times calculated based on the acrophase estimated through the calculation of periodicity were concentrated around 12.00, 15.00 and 18.00 h for the sampling period 10.00-19.00, and 24.00, 03.00 and 06.00 h for the sampling period 22.00-07.00 h. However, in more than half of the animals at 22-24 days of age, one secretory episode around 12.00 h, and two secretory episodes around 24.00 and 03.00 h had markedly small amplitudes, making the remaining secretory episodes distinct diurnal and nocturnal surges, respectively. In the animals at 29-31 days of age, the amplitudes of the PRL episodes occurring around 12.00 h were markedly small, making the remaining two episodes as diurnal surges, whereas the amplitudes of PRL secretory episodes during the period 22.00-07.00 h were analogous to each other. These findings indicate that the semicircadian rhythm of PRL secretion is established on the basis of PRL secretion with the 3.0-h period ultradian rhythm.

Materials and Methods

Female rats of Wistar strain which were delivered at the age of 22 days or raised in our own animal quarters were placed 4-5 animals to a cage under controlled environmental conditions. The room was...
lighted from 05.00 to 19.00 h and food and water were freely available.

Animals were implanted with intracardiac cannulae under anesthesia with ether at least 24 h before the blood sampling experiment. A silastic tube cut down to 7.5 cm was filled with heparinized 0.9 % saline (10 U/ml) and inserted via the right external jugular vein into the superior vena cava-right atrium. The other side was brought out subcutaneously to the back of the neck. After the surgery, the animals were kept in individual transparent plastic cages, allowed to behave ad libitum, and then removed to the experimental room controlled as described above.

Animals were bled at 22-24 or 29-31 days of age. Sequential 30 µl blood samples were collected with a 100 µl microsyringe and replaced with an equal volume of heparinized 0.9% saline. Unanesthetized and unrestrained rats were bled at 15-min intervals for the 9 or 10-h periods beginning at 09.00 or 10.00 h and 22.00 h. The blood was added directly to assay tubes containing 70 µl of the buffer solution and stored at -20 \(^\circ\)C until assayed.

Whole blood samples were analyzed for PRL with the double antibody radioimmunoassay method using materials supplied by the NIAMDD. The values are stated in terms of NIAMDD rat PRL-RP-1. The minimally detectable amount of PRL (95% confidence limits of buffer controls) on 10 assays was 0.7 ± 0.6 (SD) ng/ml. The within- and between-assay variance for the 10 assays, which were calculated for the quintuple determinations in each assay for a pool of rat serum containing approximately 40 ng/ml were 4.8% and 10.8%, respectively.

The PRL time series obtained were applied to the statistical analysis of the power spectrum to find out periodical characteristics. The spectrum was computed by means of the Fourier transform of the autocovariance function of the series and the precise periodicity was identified by using the least squares method (Halberg et al., 1964). The peak time was calculated on the basis of the acrophase estimated through the calculation of periodicity.

Results

The individual examples of PRL time series for each 9 or 10-h period as well as the mean values for PRL concentrations at each sampling point are shown in Fig. 1–4.

1. **PRL secretory profiles in the 22–24 day-old female rat**

As supposed simply by observing the diagrammatic representation of PRL time series in Fig. 1 and 3, an ultradian rhythm with a 2.17–4.42 h periodicity was consistently revealed in the individual time series of PRL concentrations by the analysis of power spectrum and the least squares method. The mean periodicity for 22 rats bled from 09.00 or 10.00 h to 19.00 h was 3.16 ± 0.11 (SE) h and that for 13 rats bled from 22.00 to 07.00 h was 3.30 ± 0.14 (SE) h. Consequently, in either sampling period, each animal had on the average three secretory episodes, peaking around 12.00, 15.00 and 18.00 h during the former sampling period, and around 00.00, 03.00 and 06.00 h during the latter sampling period, respectively.

However, there was an apparent tendency in 55% of the animals sampled during the period 09.00–19.00 h for the peak amplitude of an earlier episode, i.e., the one occurring around 12.00 h, to be markedly smaller than the later two episodes (Fig. 1). The amplitudes of the earlier two episodes occurring around 00.00 and 03.00 in 70% of the animals sampled during the period 22.00–07.00 h were smaller than the later episode (Fig. 3).

Similarly, approximately a 3.0 h-periodicity rhythm was found for every 9 or 10-h period time series of mean PRL concentrations (Fig. 4). The periodicity for the period 09.00–19.00 h was 3.08 h, and peaks of PRL episodes were estimated to be at 09.20, 12.25, 15.30 and 18.35 h. The periodicity for the period 22.00–07.00 h was 3.40 h and peaks were at 23.26, 02.50 and 06.14 h. Further, as found in the individual PRL time series, amplitudes of secretory episode at 12.25 h as well as those at 23.26 and 02.50 h were smaller than the remaining episodes. The secretory episodes in which the amplitude remained high were considered to constitute the diurnal and nocturnal surges previously observed (Kimura and Kawakami, 1980), based on the time when the episodes peaked, i.e., 15.30 and 18.35 h, respectively.
ULTRADIAN RHYTHM OF PRL SECRETION IN RATS

Fig. 1. PRL profiles determined by serial blood collection at 15-min intervals through the intracardiac cannula during a 9-h period from 10.00 to 19.00 h in female rats at 22-24 days of age. Individual profiles in 8 representative animals are shown. Left 4 show profiles with almost the same amplitude secretory episodes and right 4 show those with small amplitude secretory episodes around 12.00 h. The number in parenthesis following the animal number indicates the periodicity (hours).

2. PRL secretory profiles in the 29–31 day-old female rat

The power spectrum analysis and the least squares method revealed a 2.37–4.67 h periodicity in the individual time series of PRL concentrations during the period 09.00 or 10.00–19.00 h in 29–31 day old rats, and the peaks were also estimated to be around 09.00, 12.00, 15.00 and 18.00 h. The mean periodicity for 17 rats examined was 3.37 ± 0.14 (SE) h. As seen in Fig. 2, however, in 90% of 17 animals the amplitudes of PRL episodes occurring around 09.00 and 12.00 h were not as pronounced as those occurring around 15.00 and 18.00 h.
Fig. 3. PRL profiles determined by serial blood collection at 15-min intervals through the intracardiac cannula during a 9-h period from 22.00 to 07.00 h in female rats. Individual profiles in 4 representative animals at 22–24 days of age (left) and in 4 representative animals at 29–31 days of age (right) are shown. See Fig. 1 for other details.

12.00 h were quite small, and thus only the episodes that occurred around 15.00 and 18.00 h were conspicuous. These latter secretory episodes were considered to correspond to the diurnal surge described previously (Ramaley and Campbell, 1977, Alvarez, 1979, Kimura and Kawakami, 1980), based on the time they occurred. This was similarly apparent in the time series of mean PRL concentrations (Fig. 4). The time series was revealed to have a 3.53 h periodicity, with peaks estimated at 10.00, 13.31 and 17.40 h.

In the individual time series of PRL concentrations obtained in 16 rats during the period 22.00–07.00 h, an ultradian rhythm with a 1.97–4.70 h periodicity was revealed. Most peaks occurred around 00.00, 03.00 and 06.00 h, too. The mean periodicity was 3.31±0.26 (SE) h. However, as apparently seen in Fig. 3, in most animals there were no significant differences among peak amplitudes of PRL episodes occurring during this sampling period. Peak amplitudes around 00.00 and 03.00 h were as high as those around 06.00 h. This is more apparent in Fig. 4 which shows the mean values of PRL concentrations over the period. The time series had a rhythm with 3.47 h periodicity. There were observed three distinct secretory episodes with almost the same amplitudes that peaked at 23.31, 02.59 and 06.27 h.

Comparing the amplitudes of secretory episodes for two ages, apart from the rhythm, it seemed that the amplitudes in the 29–31 day old rat was higher than those in the 22–24 day old rat.

3. Time schedule for PRL secretory episode in the 22–24 and 29–31 day old female rat

The peak time calculated as described
in the Materials and Methods was plotted against the time of day to make the time series of the number of peak at 5-min intervals. The time series so obtained was again applied to the analysis of power spectrum and the least squares method in order to find if there was a periodicity in the time series of the number of PRL peak and to know the time where the PRL secretory peaks concentrated. Those calculations were done combining data for all the PRL time series obtained in the present study.

It was revealed that the time series of the number of PRL peak had a 3.17-h periodicity. Furthermore, a time schedule for PRL secretory rhythm which had the first peak at 00.00 h in a day was obtained on the basis of the acrophase calculated; peaks were estimated to be concentrated at 00.02, 03.12, 06.22, 09.32, 12.32, 15.52, 19.02 and 22.12 h

**Discussion**

The results of the present study indicate that PRL secretion in the immature female rat expresses a clear ultradian rhythm with approximately 3-h periodicity at a time before puberty. Because even at the stage where the semicircadian rhythm was established as a predominant secretory rhythm, there underlay a 3-h period ultradian rhythm, it seemed likely that the semicircadian rhythm of PRL secretion would have been established on the basis of the PRL secretion with the 3-h period ultradian rhythm. Taking into consideration the fact that approximately 50% animals sampled during the period 09.00–19.00 h and 70% animals sampled during the period 22.00–07.00 h showed the diurnal surge- and nocturnal surge-like secretory episodes, respectively, on the basis of the 3-h period ultradian rhythm, it seemed likely that the ultradian rhythm of PRL secretion had been
developed at the latest by the weaning age.

In the present experiment, the nocturnal surge-like secretory episode disappeared in most animals at 29–31 days of age. In our previous experiment, we found nocturnal surges with the same amplitude as that for diurnal surges by the decapitation sampling method even at 33 days of age, whereas by the cannulation sampling method we found nocturnal surges of smaller amplitude than the diurnal ones at 28–29 days of age (Kimura and Kawakami, 1980). It is possible, therefore, that the amplitude of the PRL secretory episode at 06.00 h in the 29–31 day old rat in the present experiment could be larger than that actually recorded, indicating the existence of a nocturnal surge-like secretory episode.

As a typical 3-h period ultradian rhythm, Tannenbaum and Martin (1976) have demonstrated the secretory rhythm of GH in the adult rat. We have found a similar 3-h period ultradian rhythm existing even in the immature rat (Kawakami et al., 1983 a, b). In the immature rat, we have also demonstrated that the rhythm was linked to the time of day; i.e., the secretory episode of GH occurred eight times a day around 03.00, 06.00, 09.00, 12.00, 15.00, 18.00, 21.00 and 24.00 h. A quite interesting fact discovered in the present experiment is that the PRL secretory episode seems to occur in almost the same time schedule as that for the GH secretory episode. Since it was shown that the GH secretory episode coincided with the sleep cycle in the immature rat (Kawakami et al., 1983a), it is possible that PRL secretion also coincides with sleep. Experiments on this are currently under way.

In a study aimed at obtaining detailed secretory profiles of LH, we have found that LH secretion in the immature female and male rats also occurred episodically (Kimura and Kawakami, 1982) with approximately 3-h periodicity (Kimura, 1983). Taking these findings into account, it seems possible that the 3-h period ultradian rhythm is a basic secretory rhythm for many anterior pituitary hormones. The rhythm specific for adulthood would develop on the basis of this ultradian rhythm. It is quite possible, therefore, that the semicircadian rhythm of PRL secretion found at a stage during maturation is an intermediate phase which should eventually mature into circadian rhythm of the adult type. The mechanism of this maturation is not yet known, but it is probable that the development of certain neural element contributes to the process. For instance, the medial preoptic area is a candidate. First of all, electrical stimulation of the medial preoptic area exerted a strong inhibitory influence on PRL secretion in both the immature (Kawakami et al., 1973) and the mature female rat (Kimura and Kawakami, 1978). Further, in the adult female rat, the elimination of the function of this area resulted in the appearance of a nocturnal PRL surge (Freeman and Banks, 1980, Arita and Kawakami, 1981), and in the adult male rat, anterior deafferentation of the hypothalamus did permit almost normal PRL secretion (Turpen and Dunn, 1976, Willoughby et al., 1977).

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References


Turpen, C. and J. D. Dunn (1976). The effect of surgical isolation or ablation of the medial basal hypothalamus on serum prolactin levels in male rats. *Neuroendocrinology* 20, 224-234.