Light-and-Dark Signal for the Initiation of Prolactin Surges in Cervically Stimulated Rats

HIROYUKI NAITO**, MICHIO TAKAHASHI* AND YOSHISUKE SUZUKI***

Laboratory of Veterinary Physiology, Faculty of Agriculture, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

Abstract

Ovulation and pseudopregnancy (psp) can be induced by cervical stimulation in persistent estrous rats maintained in a constant lighting condition (LL-psp rats). Different from the pattern of prolactin (PRL) secretion in usual psp rats maintained in a daily light-dark cycle (LD-psp rats), PRL secretion in these rats during the psp period does not occur in the form of two daily surges. The object of this study was to investigate the role of the external LD cycle for the expression of PRL surges.

A large amount of PRL was released in LL-psp rats immediately after cervical stimulation, which is in contrast to LD-psp rats whose first secretion of PRL after cervical stimulation is known to depend on the time of day rather than the time of stimulation.

The effect of the change in the lighting condition on the pattern of PRL secretion was investigated in LD- and LL-psp rats. Two surges of PRL were induced in LL-psp rats on Day 5 (Day 0 = day of cervical stimulation), if they were moved from LL to LD on Day 0. Conversely, PRL surges diminished in LD-psp rats on Day 5 if they were moved from LD to LL on Day 0. When the change in lighting conditions was made on Day 5, different results were obtained: In LL-psp rats two daily surges were not established on Day 8 in spite of an application of LD condition from Day 5. In LD-psp rats the two surges were still observed on Day 8, though the time of occurrence was shifted.

These results suggest that there is a critical period at and shortly after the time of cervical stimulation during which external LD cycle can work as an entraining agent for the expression of PRL surges. Once PRL secretion is coupled to the external LD cycle, the oscillation continues even in LL condition in a free-running manner. Exposure to LL condition during this critical period inhibits the establishment of two daily PRL surges throughout a psp period.

Taking cervical stimulation as a cue, a series of two daily prolactin (PRL) surges starts in the rat (Freeman et al., 1974; Smith et al., 1975; Beach et al., 1975). The daily occurrence of these surges involves a phase relationship with an environmental light-dark cycle (Freeman et al., 1974; Pieper and Gala, 1979). The application of a constant lighting condition to cycling rats previously kept in LD results in an anovulatory persistent estrous state. Though cervical stimulation effectively induces ovulation and pseudopregnancy (psp), the pattern of PRL secretion during their pps
period is reported to be erratic and completely different from the typical two daily surges (Bethea and Neill, 1979; Pieper and Gala, 1979; Yogev and Terkel, 1980). PRL secretion in the form of two daily surges does not seem to be a prerequisite for the initiation and maintenance of psp.

In order to clarify the role of light-dark signal for the expression of particular pattern of PRL secretion during psp period, we tried to answer the following two questions. 1) Is it possible to induce two daily surges of PRL in persistent estrous rats, if an environmental light-dark condition is provided after cervical stimulation? 2) Is it possible to erase two daily surges of PRL in normal psp rats by providing a constant lighting condition?

Materials and Methods

Animals
Adult female Wistar rats (2-3 month-old) bred in our laboratory housed in 14L:10D lighting schedule (LD; lights on 0500-1900 hr) were used. After animals were found to have at least two consecutive 4-day recurring estrous cycles, they were divided into two groups: one group of animals continued to be kept in LD (LD-rats), and the others were transferred to an isolated room where constant lighting (LL) was supplied by fluorescent bulbs (LL-rats).

Experimental groups using LD-rats
LD-rats were cervically stimulated with a glass rod at 1700 hr on the day of proestrus (LD-psp rats). The day of stimulation was designated as Day 0. They were allotted to the following experimental groups: 1) controls kept in LD, 2) animals transferred to an LL room immediately after cervical stimulation, and 3) animals transferred to an LL room on Day 5. A series of blood samples for PRL analysis was obtained from each group during a 30 hr-period by decapitating animals on Day 5-6 or Day 8-9 at 3 hr or 6 hr intervals. Six to 8 animals were sacrificed at each point.

Experimental groups using LL-rats
LL-rats which were kept in LL for more than 3 weeks and showed persistent estrus for 11 to 13 days were cervically stimulated with a glass rod at 1000 hr or 1600 hr (LL-psp rats). The day of stimulation was called Day 0. A group of animals without cervical stimulation served as controls. LL-psp rats were allotted for the following experimental groups: 1) animals kept continually in LL, 2) animals transferred to an LD room immediately after cervical stimulation, and 3) animals transferred to an LD room on Day 5. Blood samples were collected similarly as in LD-psp rats. Additional blood samples were collected from cervically stimulated LL-rats at 30 min to 1 hr intervals from 30 min to 5 hr after cervical stimulation.

PRL measurements
Blood samples were allowed to clot at room temperature and sera were preserved at -20°C until the PRL assay was done. The serum PRL concentration was determined by radioimmunoassay using the method described by Murakami et al. (1979), where NIAMDD-RP-1 rat PRL (11 IU/µg) was used as the standard and radioiodinated rat PRL (48.4 µCi/µg) purchased from New England Nuclear, Boston was used as the labeled hormone. Statistical comparisons were performed using an analysis of variance.

Results

Pattern of PRL secretion in LD-psp rats
As indicated in Fig. 1, typical two PRL surges were observed during a 24 hr-period on Day 5 in rats maintained in LD.

PRL secretion immediately after cervical stimulation in LL-rats
Cervical stimulation in LL-rats applied at either 1000 hr or 1600 hr induced an immediate elevation in serum PRL levels (Fig. 2). The initial high levels were maintained for 3 hr and then decreased.

Pattern of PRL secretion in LL-psp rats
Changes in serum PRL concentrations in LL-psp on Days 5 and 6 were presented in Fig. 3. They had been cervically stimulated at either 1000 or 1600 hr on Day 0. Irrespective of the time of cervical stimulation, moderately increased PRL levels were observed at each sacrifice time. The mean values were generally accompanied by large standard errors. Evident peaks comparable to nocturnal or diurnal surge
Fig. 1. Serum PRL levels in the samples collected following decapitation at 6 hr-intervals on Day 5 in LD-psp rats. Each point represents the mean ±SEM of 6 to 8 animals. The solid bars along the abscissae indicate darkness.

Fig. 2. Patterns of PRL secretion shortly after cervical stimulation applied at either 1000 hr (●—●) or 1600 hr (○—○) in LL-rats. Each point represents the mean ±SEM. Controls (△—△) are the mean serum PRL levels in LL-rats which did not receive cervical stimulation.

Fig. 3. Serum PRL levels in the samples collected from LL-psp rats following decapitation between Days 5 and 6. Cervical stimulation was applied at either 1000 hr (●—●) or 1600 hr (○—○) on Day 0. Each point represents the mean±SEM. Controls (△—△) are the mean serum PRL levels in LL-rats which did not receive cervical stimulation.

were not appreciable. Analysis of variance of these data indicates that differences in mean PRL concentrations during a 30 hr period are statistically insignificant (p>0.05). The controls for the above two groups were LL-rats without receiving cervical stimulation. These rats did not show any significant rise in the serum PRL level during a 24 hr period.

Effect of a change in the lighting condition immediately after cervical stimulation in LD- and LL-rats

Fig. 4 illustrates changes in the serum PRL concentration in LL-rats which were moved from an LL to an LD room on Day 0. They exhibited two daily PRL surges which were similar in magnitude and periodicity to those in LD-psp rats.

Fig. 5 illustrates the results for LD-rats which were transferred from an LD to an LL room on Day 0. Their pattern of PRL
secretion was less regular. No evident peaks were observed and the mean values were accompanied by large standard errors, as in LL-psp rats. The analysis of variance of these data indicates that differences in mean PRL concentrations during a 30 hr-period are statistically insignificant (p > 0.05).

**Effect of a change in the lighting condition 5 days after cervical stimulation in LD- and LL-psp rats**

Fig. 6 illustrates changes in serum PRL concentrations on Day 8 in LL-psp rats which had been transferred from an LL to an LD room on Day 5. Considerably elevated PRL levels were observed throughout the period sampled, but two daily surges of PRL were not identified. All the mean values were accompanied by large standard errors. The analysis of variance of these data indicates that differences in the mean PRL concentration over a 30 hr-period are statistically insignificant (p > 0.05).

Fig. 7 represents changes in the serum PRL concentration on Day 8 in LD-psp
Fig. 7. Serum PRL levels in the samples collected from LD-psp rats following decapitation between Days 8 and 9. These animals were moved from LD to LL on Day 5. Each point represents the mean ± SEM.

Discussion

It has been shown in the study using ovariectomized rats kept under an LD condition that the appearance of the first PRL surge after cervical stimulation is related to the time of day rather than the time of stimulation applied (Smith and Neill, 1976). This seems to indicate that the mechanism for the occurrence of PRL surge is coupled to the endogenous rhythm oscillator in the first instance. Our findings in the present study, however, indicate that PRL secretion in persistent estrous rats is enhanced immediately after the application of cervical stimulation regardless of the time of the stimulation, as previously demonstrated by Smith and Ramaley (1978) in immature rats raised in constant light. Therefore, it is possible that the PRL secretion in the absence of photic cues starts without coupling to endogenous rhythm.

The pattern of PRL secretion in LL-psp rats during the middle stage of psp was not composed of two synchronized daily surges. This result agrees with the findings by Yogev and Terkel (1980) and Bethea and Neill (1979) who investigated the changes in plasma PRL levels in individual LL-psp rats and found that PRL was secreted in an erratic manner with attenuated and abbreviated elevations with a periodicity of 6–8 hr. In the present study a daily supply of an LD cycle to LL-psp rats starting on Day 5 could not cause the rhythmic PRL secretion by Day 8. This failure in the entrainment to an LD cycle cannot be attributed to the absence of an endogenous rhythm, because many fade-out rhythms in an LL condition (Bünning, 1973) are known to recover shortly after changing the lighting condition to LD (Cheifetz et al., 1968; Takahashi and Suzuki, 1969; Dunn et al., 1972; Fukuda et al., 1977). Moreover, if LL-psp rats were moved to an LD condition immediately after cervical stimulation, PRL secretion during the middle stage of psp became synchronized and changed into a form of two daily surges, suggesting that PRL secretion in these rats had been coupled to an LD cycle within 5 days.

From these results the expression of two daily PRL surges may be felt to be dependent on the presence of an external LD signal between Days 0 and 5. This hypothesis was approved in LD-psp rats in rats which had been transferred from an LD to an LL room on Day 5 in a way opposite to the previous group. The PRL level markedly fluctuated over a 30 hr-period. The analysis of variance of these data indicates that differences in mean PRL concentrations during a 30 hr-period are statistically significant (p < 0.001). Two peaks were identified at 0600 and 0000 hr. If one of the peaks observed at 0000 hr on Day 9 could be taken for the diurnal surge, the time of occurrence had apparently shifted.
which two daily surges in PRL were prevented by an application of LL condition between Days 0 and 5. Thus, there seems to be a critical period shortly after cervical stimulation during which PRL secretion is entrained to an external LD cycle. An application of LL condition during this critical period inhibits the expression of two daily PRL surges throughout a whole psp period regardless of the previous lighting condition before cervical stimulation. Once the secretion of PRL couples to an LD cycle, the rhythm continues in an LL condition in a free-running manner as shown in LD-psp rats which had been exposed to an LL condition since Day 5. The secretory pattern of these rats was still composed of two daily surges and the phase of the occurrence of the surges was shifted.

Although the two daily PRL surges bear a similarity to other circadian rhythms (Bethea and Neill, 1979; Yogev and Terkel, 1980), a major dissimilarity resides in the fact that the PRL rhythm is not being generated spontaneously but initiated only after cervical stimulation. The reason for the indispensability of an LD cycle for coupling the PRL secretion with the endogenous rhythm oscillator remains to be elucidated in connection with the peculiarity of the institution of this "circadian rhythm".

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References


