

偽妊娠ラットのプロラクチンサーージに及ぼす制限給餌の効果

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Restricted Feeding Regimen Affects the Diurnal Prolactin Surge but not Nocturnal Surge in Pseudopregnant Rats

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ABSTRACT. The influence of food restriction on the prolactin surges and circadian adrenocortical rhythms was investigated in pseudopregnant rats. Two groups (G-1 and G-2) of rats were maintained under 14L:10D (G-1: lights on 0500–1900 h, G-2: lights on 1700–0700 h), and allowed to feed *ad libitum* or to feed restrictedly only between 0900 and 1100 h every day for 4 weeks. Pseudopregnancy was induced 23 days after the initiation of the food restriction by uterine cervical stimulation performed on the proestrous day. Under *ad libitum* feeding, nocturnal and diurnal surges occurred at 0300 and 1800 h (G-1), and at 1500 h and 0600 h (G-2) respectively, suggesting that these prolactin surges are adjusted to lighting conditions. Restriction of food availability to 2 hr did not affect the nocturnal surge (G-1, 2). In contrast, the diurnal surge in individual rats occurred either irregularly or disappeared, and no significant diurnal surge was detected from the pooled data. The circadian adrenocortical rhythm adjusted to altered feeding time progressively, with the peak level immediately before the feeding time. These results are discussed in relation to the hypothesis that multiple neural oscillators may control the bimodal prolactin surges in pseudopregnant rats.—**KEY WORDS:** circadian rhythm, corticosterone, food restriction, prolactin surge, pseudopregnancy.

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Stimulation of the rat uterine cervix during proestrous day induces a series of twice daily surges of prolactin secretion; one occurring in the late afternoon (diurnal surge) and the other in the morning (nocturnal surge) [4, 6, 26]. Both surges are endogenous circadian rhythm, because 1) they are entrained to an alternating photoperiod after a certain period of transient time; 2) they show “free-run” in constant dark [1] and 3) they are abolished by lesioning suprachiasmatic nuclei [2, 16] which are considered to be the pace-maker for the circadian oscillation system in mammalian species [4, 20, 25]. However, whether the periodicity of both surges is regulated by one oscillator is unknown [12]. Though a master circadian pacemaker, probably suprachiasmatic nuclei, may control both surges, the other subsequent neural mechanisms may separately regulate the occurrence of the nocturnal or diurnal surge [7, 11].

A restricted feeding regimen entrains the circadian adrenocortical rhythm but not the plasma melatonin rhythm, suggesting that multiple oscillators might be involved in the organization of the former [13, 17, 22, 29]. Thus, food restriction may provide a means of assessing the internal connection of the control mechanism regulating prolactin surges.

In the present study, we examined the effect of food restriction on the nocturnal and diurnal prolactin surges in the pseudopregnant rats.

MATERIALS AND METHODS

Adult female Sprague-Dawley rats bred in our laboratory were kept in a constant temperature room (23–25 C) under white cool fluorescent lighting with a light regimen of 14 hrs of light and 10 hrs of darkness (14L: 10D). In

one group (G-1), a lighting period was from 0500 h to 1900 h, and the other (G-2) from 1700 h to 0700 h. Vaginal smears were taken every day, and only the animals that showed 2 consecutive 4 day cycles were used. Two groups of rats were kept on a *ad libitum* or a restricted feeding regimen (free access to food was permitted between 0900 and 1100 h) for 28 days. Twenty three days after the initiation of the food restriction, pseudopregnancy was induced by cervical stimulation 2 hr before the light-off on the proestrous day, which had been determined by vaginal cytology. The day following the stimulation was designated as day 1 of pseudopregnancy.

Blood samples were taken from individual rats at 3-hour intervals on day 5 of pseudopregnancy, starting at 0900 h, for 33 hrs by the tail tip incision method [27], with the following modification. One day before the sampling, the tail tip was incised about 2 mm in width with a razor blade. A 60 μ l blood was taken from the re-opened incision by aspiration with a heparinized micropipette. A 10 μ l blood was stored at -20°C for the measurement of corticosterone, and the remaining 50 μ l blood was immediately centrifuged for 3 min at 12000 rpm with a hematocrit-centrifuge. Then 20 μ l plasma was aspirated from micropipette with Pedersen pipette, and stored at -80°C for the prolactin measurement. The sample collection during a dark period was performed under dim red light. To compare the pattern of prolactin secretion obtained by the tail tip incision method with that by decapitation, a group of rats were killed by decapitation at 3 hr intervals for 24 hr on day 5 of pseudopregnancy.

Whole blood corticosterone levels were determined by the method of Murphy [24] with modification as follows [28]; as corticosterone-binding globulin crossly bound to progesterone, progesterone was removed from samples with petroleum ether. The intrassay and interassay variances were 7.3% and 10.6%, respectively. Plasma pro-

lactin concentrations were measured using NIADDK-rat-prolactin radioimmunoassay kit, obtained from National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, (NIADDK), U.S.A. by the method previously described [21] with a following modification. The antisera, the reference preparation for the standard and the preparation for the iodination of the NIADDK kit were anti-rPRL-S-9, rPRL-RP-3 and rPRL-I-5, respectively. The intra- and inter-assay coefficients of variation were 3.4% ($n=6$) and 8.9% ($n=4$), respectively. The sensitivity for prolactin was 39 pg per tube.

One or two way analysis of variance was used to analyze the serial sampling data. Student's *t* test was employed for statistical comparisons between two mean values.

RESULTS

The prolactin surges in pseudopregnant rats fed *ad libitum* are shown in Fig 1. In the samples obtained by decapitation, the peak of nocturnal and diurnal surges were observed at 0300 and 1800 h, respectively (Fig 1A). Similar secretory pattern was observed by the tail tip incision method (Fig 1B). Two way analysis of variance for repeated measures revealed no significant difference in mean prolactin levels over the time between these two groups (Fig 1A and 1B). Under the different lighting regimen (lights on 1700–0700 h), the nocturnal and diurnal surges were observed at 1500 h and 0600 h, respectively (Fig 1C). The amplitude of nocturnal surge was larger than that of diurnal surges in all groups.

Although there was an initial body weight loss during the first 5 days of food restriction (*ad libitum*; 178.5 ± 3.2 g vs restricted; 169.0 ± 2.5 g), no significant difference was observed by 2 weeks (*ad libitum*; 212.6 ± 4.1 g vs restricted; 209.5 ± 3.8 g). Food restriction affected the vaginal cycles in approximately a half of the rats, but not their establishment of

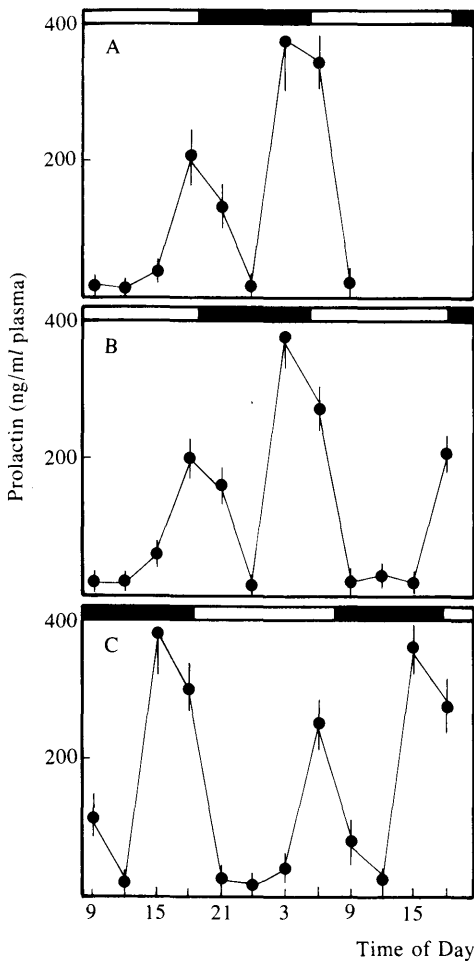


Fig. 1. The secretion of plasma prolactin levels in the pseudopregnant rats fed *ad libitum*. Samples were obtained by decapitation (A) or the tail tip incision method (B, C). Each symbol and vertical line represents the mean and SEM levels of 9 rats. The lighting regimen is 14L:10D (A and B: lights on 0500–1900, C: lights on 1700–0700 h).

pseudopregnancy.

Fig. 2 shows prolactin secretion for 33 hr on day 5–6 of pseudopregnancy in G–1 (Fig. 2A) and G–2 (Fig. 2B) groups. No diurnal surge was observed in rats receiving the restricted feeding, when the individual data were pooled. In G–1, however, a minor peak elevation (above 150 ng/ml) resulting the diurnal surge was observed at 1800 h in 3 of 9 rats (data not shown). In the remaining six

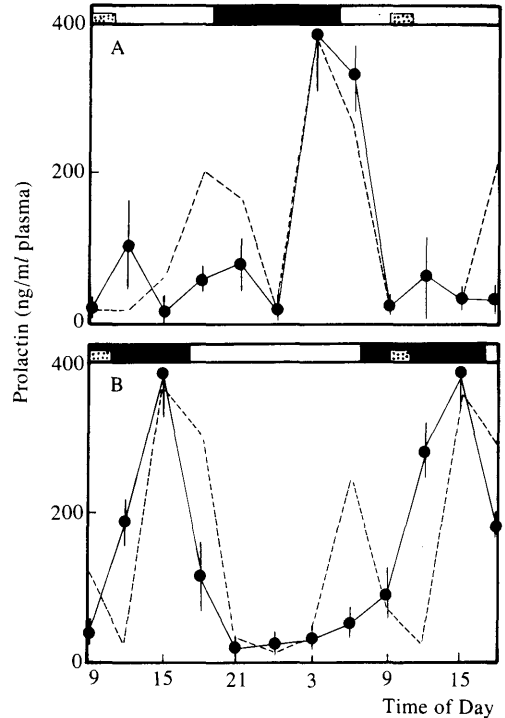


Fig. 2. The effect of the food restriction on the prolactin secretion (solid line) in the pseudopregnant rats kept under different lighting condition (A: lights on 0500–1900, B: lights on 1700–0700 h). Free access to food was permitted between 0900 h and 1100 h for 4 weeks, is represented as dotted bar. Each symbol and vertical line represents the mean and SEM levels of 9 rats. The dotted line represents the pattern of mean prolactin levels in rats fed *ad libitum* shown in Fig. 1.

rats, prolactin was maintained at low levels throughout a period between 0900 h and 2100 h. In contrast, the nocturnal surge was not affected by food restriction.

In the rats kept under a reversed photoperiod (G–2), a diurnal surge was not observed after food restriction (Fig. 2B). Only 2 of 9 rats showed a surge of small amplitude other than the nocturnal surge (data not shown). The other rats showed no surges equivalent to the diurnal surges observed in normal pseudopregnant rats. The time of appearance and the amplitude of the nocturnal surges were indistinguishable between the group fed restrictedly and the group fed *ad libitum*. The prolactin level at 1200 h and 1800 h in rats

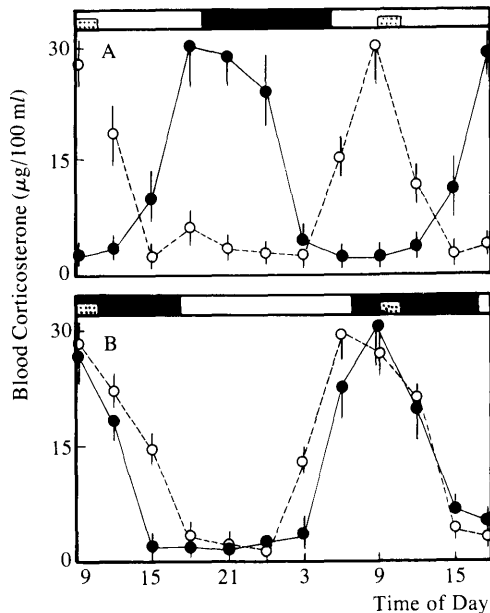


Fig. 3. Blood corticosterone levels in the rats fed *ad libitum* (solid line) and the rats fed restrictedly (dotted line). Each symbol and vertical line represents the mean and SEM levels of 9 rats. The food restriction, if any, was performed for 2 hours (between 0900 h and 1100 h) for 4 weeks, and is represented as dotted bar. The rats were kept under different lighting condition (A: lights on 0500–1900 h, B: lights on 1700–0700 h).

fed restrictedly was significantly higher and lower, respectively, than those in rats fed *ad libitum* (Fig. 2B).

Blood corticosterone levels during a 33 hr period in rats receiving the two different feeding are illustrated in Fig. 3. The blood corticosterone levels in rats fed *ad libitum* showed a clear circadian change, with the peak during a dark period, in spite of the different lighting regimens. Under the food restricted condition, however, a single peak elevation of blood corticosterone levels appeared just before the feeding time irrespective of the lighting regimen.

DISCUSSION

The nocturnal and diurnal surges were observed at 0300 and 1800 h under commonly used lighting condition (lights on 0500–1900

h). The time of the occurrence of both surges confirmed our previous report with different rat colony [21]. The reversed occurrence of nocturnal and diurnal surges under the reversed lighting condition suggests that these surges had been entrained to a changed photoperiod.

The restricted feeding regimen affected the circadian adrenocortical rhythm and diurnal prolactin surge, but not the nocturnal surge. Although the difference in prolactin levels was observed at 1200 and 1800 h between the two different feeding groups (Fig. 2B), the effect of restricted feeding does not seem to affect the timing and magnitude of the nocturnal surge. With the restricted feeding, the peak corticosterone level was observed immediately prior to feeding. The observation implies that the corticosterone rhythm has been entrained to the altered feeding pattern, confirming previous results [17, 22, 29]. The circadian rhythm of blood corticosterone levels has been reported to be generated by suprachiasmatic nucleus [20]. Food-shifted adrenocortical rhythm, however, persists in rats with lesion of the suprachiasmatic nucleus [18]. Further, the circadian rhythm in the suprachiasmatic nucleus itself is not affected by food restriction [15, 23]. These findings suggest the existence of the anatomical locus or loci other than the suprachiasmatic nucleus responsible for controlling food-shifted rhythm. Both prolactin surges are also considered to be regulated by the suprachiasmatic nucleus, since the lesion in this nucleus abolished the rhythm pattern of the surges [2, 16]. Nevertheless, there was a marked difference in the effect of food restriction between the diurnal and nocturnal prolactin surges, which strongly suggests that the two surges may be regulated by different mechanisms. Pineal circadian rhythms are known to be unaffected by food restriction. The nocturnal surge or pineal rhythm may be tightly coupled to a master circadian clock. Our data also demonstrated that the restriction of food affect-

ed on the diurnal surge of prolactin in different way than it affected the corticosterone rhythm. It abolished the diurnal surge of prolactin, while it shifted the corticosterone rhythm. The reason of this difference is not known other than the fact that the diurnal surge, but not corticosterone, is abolished by stress. If food restriction is stress for rats, diurnal surges may be abolished by food restriction.

It was not clear whether the restricted feeding regimen directly affected prolactin secretion during the expected diurnal surge period. It has been reported that the ventromedial nucleus, which has been implicated in the regulation of feeding behavior [3], is involved in the establishment of the diurnal surge [11]. Moreover, the lesion in the ventromedial nucleus abolishes the food-shifted adrenocortical rhythm [19]. Therefore, ventromedial nucleus might be involved in the process of the ablishment of diurnal surge by the food restriction. It is also possible that the diurnal surge was influenced by the change in ovarian steroids. The food restriction resulting in irregular vaginal cycles in half of the rats suggests that the secretory pattern of ovarian steroids may have been affected by the treatment. It is well known that the diurnal surge can be regulated by the ovarian steroid [8, 10]. Besides the involvement of ventromedial nucleus or steroid hormones, several other neural mechanisms have also been demonstrated in controlling prolactin surges [5, 9]. The mechanism involved in the restricted feeding suppression of the diurnal surge remains to be elucidated.

In conclusion, separate neural mechanisms controlling nocturnal and diurnal surges of prolactin were suggested. Our observation supports the hypothesis that oscillators, which are coupled with a master circadian pacemaker, organizes the prolactin surges [10].

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要 約

偽妊娠ラットのプロラクチンサージに及ぼす制限給餌の効果: 村上 昇・高橋清久¹⁾・黒田治門・江藤禎一(宮崎大学農学部家畜生理学教室, ²⁾滋賀医科大学精神科)——偽妊娠ラットにおいて, 制限給餌のプロラクチンサージおよびコルチコステロンリズムに及ぼす効果を検討した。成熟雌ラットを, 14L:10D(グループ1: 5時点灯19時消灯, グループ2: 19時点灯7時消灯)の条件下で飼育し, それぞれのグループに自由給餌群と, 毎日9-11時のみ2時間の制限給餌群を設け, 23日後の発情前期に子宮頸部刺激で偽妊娠を誘起した。自由給餌群では, ノクターナルサージおよびダイアーナルサージは, グループ1ではそれぞれ3時および18時に, グループ2では15時および6時に認められ, プロラクチンサージがそれぞれの光条件に同調していることがわかった。一方, 制限給餌群では, 両グループともにノクターナルサージは影響を受けなかったが, ダイアーナルサージは消失した。血中コルチコステロンリズムは両グループともに, 制限給餌直前にピークを示した。以上の結果から, 偽妊娠ラットのプロラクチンサージでノクターナルサージとダイアーナルサージの成立には別の機構が存在する可能性が示唆された。