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Circadian Component in the Fattening and Reproductive Responses to Prolactin in the Hamster[1] (38263)

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Circadian rhythms of prolactin and adrenal corticosteroids have important roles in the regulation of body fat stores and reproduction in many of the lower vertebrates (1, 2). Prolactin injections may have either stimulatory or inhibitory effects on fat stores and the weights of reproductive organs depending on the time of day when the injections are made. The photoperiodic entrainment of the daily rhythms of fattening and reproductive responses to prolactin appears to be mediated by the interrenal system. In animals held in continuous light, injections of adrenal corticosteroids can entrain daily rhythms of responses to prolactin. The studies of the female hamster reported herein demonstrate that daily injections of prolactin and corticosterone in various temporal relations can also have strong influences on the fat stores and the reproductive system of a female mammal.

Materials and Methods. Female golden hamsters (*Mesocricetus auratus* Waterhouse) were maintained on a 12-hr daily photoperiod (0800–2000) from birth until they were 2.5–3 mo old. The animals used in the experiments were selected on the basis of their having a regular estrus cycle. Those which failed to exhibit two consecutive 4-day cycles were excluded. The body wt ranged from 120–150 g. They were caged in pairs and maintained at room temperature (24° ± 2°).

Experiment 1 was carried out with 40 hamsters retained on a 12-hr daily photoperiod (0800–2000) with an intensity of 250 lumens/cm^2. Four groups of six hamsters each were injected (ip) with ovine prolactin (100 μg/animal/injection) at 0800, 1400, 2000, or 0200 for 8 consecutive days. Four additional groups (4 hamsters/group) were used as controls. They received saline injections at one of the 4 times of day. Vaginal smears were taken daily between 0800 and 1000. The animals were killed at one time 24 hr after the final injection, and the ovaries, uteri, and abdominal fat were removed and weighed.

Experiment 2 was carried out with 24 female hamsters adapted to continuous light for 15 days. Injections (ip) of corticosterone (100 μg/injection/animal) were made on alternate days. Twelve of the hamsters received the injections at 0600 and the other 12 received them at 1800. Both groups were subdivided into three groups (4 animals/group) and given daily injections of ovine prolactin (100 μg/animal/injection) at 0600, 1000, or 1400. Thus, there were six groups that received prolactin at different intervals after the time of corticosterone injection (0, 4, 8, 12, 16, and 20 hrs). The injections of prolactin were initiated 1 day after the first injection of corticosterone and continued for 8 days. Vaginal smears were taken daily at 0900–1000. The animals were killed at one time (1400) 24 hrs after the final injections of prolactin. The ovaries, uteri, and abdominal fat were weighed. Liver fat was extracted by petroleum ether (B.P.: 30–60°) using Soxhlet apparatus.

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Results. As indicated by the examinations of the vaginal smears and by the absence of postovulatory discharge, the estrus cycle was delayed in five of six hamsters that received daily injections of prolactin at 0200 (6 hr after the onset of dark) (Experiment 1). If the animal was in proestrus or estrus when the hormone injections were begun, the normal anticipated postovulatory discharge and characteristic vaginal smear appeared. Thereafter the cycle was lengthened and postovulatory discharge did not reoccur. The cycles appeared to be arrested in late diestrus or in early proestrus. Thus in five of six hamsters that received prolactin injections at 0200, ovulation had not occurred for 6–10 days at the day of autopsy. All of the animals in all the other groups receiving either prolactin or saline (control) injections exhibited normal 4-day cycles during the experimental period.

At autopsy, the ovaries were examined with a dissecting microscope. Although corpora lutea were discernible in many of the other hamsters, none could be found in the animals with lengthened cycles. The ovaries of the animals with lengthened cycles grossly resembled ovaries of lactating animals.

The ovarian weights were lowest in the animals which received prolactin at 0200 and highest in the animals which received prolactin at 0800 (Table I). The uterine weights, on the other hand, were highest in the animals given prolactin at 0200 and lowest in the animals treated at 0800. An analysis of variance by randomized block design indicated that the daily variations in ovarian and uterine responses to prolactin were both highly significant ($P < 0.01$). In the hamsters injected with saline, there were no statistically significant nor apparent rhythms of ovarian and uterine responses.

There were no daily rhythms of responses to prolactin in the abdominal fat weights in either the prolactin- or saline-injected animals (Table I). However, the abdominal fat was heavier in the animals treated with prolactin than in those treated with saline ($P < 0.01$: Student's t).

The hamsters injected with prolactin 12 hr after the time of corticosterone injections (12-hr group) exhibited lengthened estrus cycles (Experiment 2). None of these hamsters ovulated during the last 6 days of an 8-day regimen of hormone treatment. All animals in the 12-hr group were in late diestrus or in early proestrus at the time of killing. On the other hand, all the animals in all of the other groups exhibited normal 4-day cycles. Thus the estrus cycle is arrested in early proestrus or late diestrus by injections of prolactin given at 12 hr after the time of corticosterone injections (Expt 2) or at 6 hr after the onset of dark (Expt 1).

The ovarian weights were lowest whereas the uterine weights were highest in the 12-hr group (Table II). The variations in ovarian and uterine responses resulting from the different temporal patterns of injections of prolactin and corticosterone are both highly significant ($P < 0.01$).

The amount of fat stores also varied among the treatment groups ($P < 0.01$ for both abdominal fat and liver fat). Lowest amounts of abdominal and liver fat were found in the 20-hr group (Table II). There was about half as much abdominal fat in the 20-hr group as in the 0-hr group.

Discussion. A comparison of the results of Experiments 1 and 2 supports the conclusion that the photoperiodic entrainment of the daily variations in reproductive responses to prolactin in the hamster are mediated by a daily rhythm of plasma corticosterone (Fig. 1). When the time of corticosterone injection in Experiment 2 is equated to 1400 in Experiment 1, the patterns of ovarian and uterine responses are similar for the two experiments. In addition, injections of prolactin result in a lengthened or arrested estrus cycle when given 12 hr after 1400 (Expt 1) or after the time of corticosterone injection (Expt 2). Thus one might predict that the daily increase of plasma corticosterone in a hamster would begin at midday of a 12-hr daily photoperiod.

Prolactin appears to have a stimulatory effect on fat stores at most times of day. The absence of a fattening response rhythm in Experiment 1 is probably due to the few times of day checked. Judging from a com-

TABLE I. Daily Variations in the Responses to Prolactin and Saline Injections (8 Consecutive Days) in Female Hamsters Held on a 12-hr Daily Photoperiod (0800–2000). The Results are Expressed as Means ± Standard Error. Numbers in Parentheses Indicate the Number of Hamsters Studied. The Significance of Daily Variations in Responses were Tested by an Analysis of Variance by Randomized Block Design of All Values.

Injections	Measurement	Responses to injections (time of injections) 0800	1400	2000	0200	Statistical evaluation
Prolactin (100 μg/inj) (24)	Ovaries (mg/100 g B.W.)	31.3 ± 4.3	29.7 ± 2.2	28.3 ± 1.4	23.1 ± 2.9	$P < 0.01$
	Uteri (mg/100 g B.W.)	284 ± 41	314 ± 42	307 ± 45	445 ± 70	$P < 0.01$
	Abdominal Fat (g/100 g B.W.)	1.4 ± 0.4	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	NS[a]
Saline (0.02 ml/inj) (16)	Ovaries (mg/100 g B.W.)	27.5 ± 1.9	27.8 ± 2.9	28.5 ± 2.1	28.5 ± 0.5	NS
	Uteri (mg/100 g B.W.)	323 ± 54	344 ± 42	328 ± 39	335 ± 28	NS
	Abdominal Fat (g/100 g B.W.)	1.0 ± 0.2	0.9 ± 0.2	1.0 ± 0.1	0.9 ± 0.1	NS

[a] No daily variations in responses were obtained but the values for all times were higher than those of the saline-injected groups ($P < 0.01$: Students' t).

TABLE II. Circadian Responses to Prolactin Injections (8 Consecutive Days) Entrained by Corticosterone Injections in 24 Female Hamsters Held in Continuous Light. The Results are Expressed as Means ± Standard Error. The Significance of Daily Variations in Responses were Tested by an Analysis of Variance by Randomized Block Design of All Values.

Measurement	Responses to prolactin injections (hours after time of corticosterone injections)						Statistical evaluation
	0	4	8	12	16	20	
Ovaries (mg/100 g B.W.)	28.0 ± 2.5	25.8 ± 1.6	26.7 ± 1.5	21.5 ± 2.0	28.5 ± 2.0	30.8 ± 2.1	$P < 0.01$
Uteri (mg/100 g B.W.)	310 ± 30	366 ± 33	331 ± 25	437 ± 18	300 ± 25	304 ± 17	$P < 0.01$
Abdominal Fat (g/100 g B.W.)	1.8 ± 0.4	1.1 ± 0.2	1.6 ± 0.2	1.2 ± 0.1	1.4 ± 0.1	0.8 ± 0.2	$P < 0.01$
Liver Fat (% dry liver weight)	4.9 ± 0.1	4.9 ± 0.1	4.2 ± 0.2	3.8 ± 0.4	4.7 ± 0.6	3.4 ± 0.3	$P < 0.01$

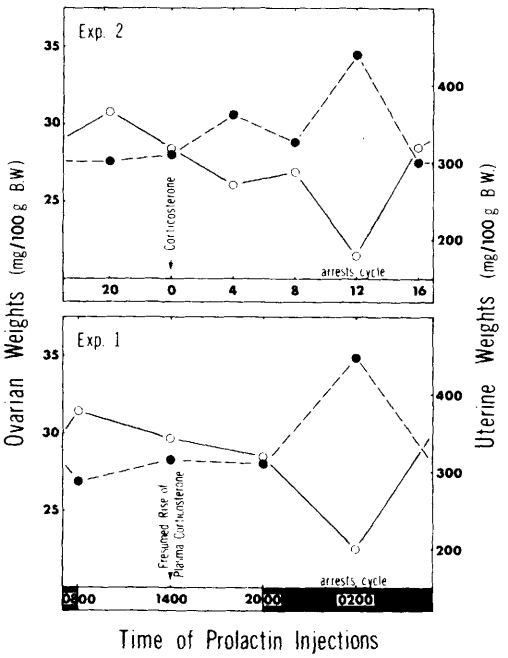

FIG. 1. A comparison of the daily variations of ovarian (open circles) and uterine (closed circles) responses to prolactin in hamsters held on a 12-hr daily photoperiod (Expt 1) with hamsters held in continuous light and injected with corticosterone (Expt 2).

parison of the reproductive responses to prolactin in Experiments 1 and 2 (Fig. 1) and from the rhythm of fattening responses in Experiment 2 (Table II), an inhibitory effect by prolactin on fat stores should occur at about 2 hr after the onset of a 12-hr daily photoperiod. No tests were made at that time of day in Experiment 1. Daily peaks or troughs of fattening responses to prolactin may occur during short intervals in some species. For example, in a fish held on different photoperiods, prolactin stimulated large gains in body fat when injected at 8 hr after the onset of light, but not at 0, 4, 12, or 16 hr after "dawn" (3). The role of prolactin rhythms in the regulation of fat stores in vertebrates has been discussed (1).

With regard to the reproductive responses, the most interesting effects were obtained when prolactin was injected at 6 hr after the onset of dark (Experiment 1) or 12 hr after the time of corticosterone injections (Experiment 2). The mechanisms by which prolactin induces uterine enlargement and ovarian reduction and arrests the estrus cycle in late diestrus or early proestrus are not determined by these studies. There are some indications that these responses to prolactin are similar to several conditions found during pseudopregnancy and early gestation. In pseudopregnant rats, the daily release of prolactin occurs late during the dark (4) and is thought to be responsible for the early morning surge of progesterone, the daily decrease in ovarian weights, and the daily increase in uterine weights (5). In addition, mating initiates a second daily peak in plasma prolactin concentrations in rats that occurs at the end of the dark period during the first week of gestation while permitting the continuation of the normal peak occurring at the end of the light period in the cycling rat (6). Perhaps the presence of prolactin at a specific time might inhibit the rise of estrogen that normally occurs during the afternoon of diestrus 2 and is necessary, at least in rats, to stimulate the release of ovulating hormone and ovulation (7). The principal argument against this explanation is that no corpora lutea were observed in the groups in which the estrus cycle was delayed. They should be evident for at least 8–9 days during pseudopregnancy in the hamster (8). Further studies are called for in order to understand the possible roles of the daily rhythms of corticosteroids and prolactin in regulating the female reproductive system.

The prevention of ovulation by this relation of corticosterone and prolactin for extended periods could have considerable significance. Other methods of experimentation may be necessary, however, because the disturbances of injection alone have significant effects on the reproductive systems and fat stores in vertebrates, including a mammal (white mouse), when they are carried out daily at specific times for 2–3 weeks (9). The responses to the timed disturbances are also believed to depend on alterations of the temporal patterns of adrenal corticoids and prolactin.

Inasmuch as the pineal gland exerts a strong influence on the reproductive system

of female hamsters (10), it is conceivable that its action is mediated by prolactin. Melatonin stimulates the release of prolactin in male rats (11) and pinealectomy abolishes the daily increase in plasma prolactin in rats (12). However, prolactin given at specific times has important influences on the reproductive systems of all the vertebrates tested (1, 2) whereas the importance of the pineal gland has been demonstrated in a relatively few species. In addition, prolactin has marked influences on fat stores and the pineal apparently does not.

Although considerable research needs to be done to elucidate the mechanisms involved, it is now apparent that circadian hormone systems are involved in regulating reproduction and fat storage. The rhythms of prolactin and corticosterone appear to be important parts of those mechanisms in a mammal as well as in other vertebrates.

Summary. Prolactin has variable effects on the weights of ovaries and uteri of hamsters depending on the time of day when the injections are made. The photoperiodic entrainment of these daily variations in responses to prolactin is apparently mediated by the adrenal cortex because corticosterone injections can entrain similar response rhythms in hamsters held in continuous light. The estrus cycle appears to be arrested in late diestrus or early proestrus by injections of prolactin at 18 hr after the onset of a 12-hr daily photoperiod or at 12 hr after the injection of corticosterone. The body fat stores are also increased or decreased depending on the temporal pattern of injections of prolactin and corticosterone.

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