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Effects of Limiting Water Intake on the Rat Estrous Cycle with Observations on LH, Prolactin, and Corticosterone (39237)

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Introduction. Recently it has been reported that restricting water (1) or water and food (2) intake to certain periods during the day will alter the normal daily rhythm of the pituitary-adrenal system. Water restriction also has been observed to disrupt the timing of gonadotropin release and onset of sexual behavior and lengthen the normal estrous cycle in the rat (3). It was concluded that the changes in reproductive function were probably secondary to alterations in the diurnal rhythms of adrenal corticoids. Other studies in various vertebrate species (4) have indicated that the timing of adrenal glucocorticoid secretion may be very critical for the initiation and maintenance of reproduction and other endocrine functions. The present investigation was undertaken to gain additional information on the effects of restricted water intake (RWI) to determine its feasibility as a model for the study of interactions between adrenal and gonadal rhythms.

Materials and Methods. Adult female Sprague-Dawley rats (Simonsen Laboratories, Gilroy, Calif.) weighing 220-280 g and showing consistent 4-day cycles were maintained in a temperature and light-controlled room (22°; lights on 0500-1900). The animals had free access to Purina laboratory rat chow. Approximately 2 weeks prior to water restriction, 40 rats were paired and given water *ad libitum*, after which they were watered daily for only 15 min at 1100 for 8-10 weeks. Vaginal smears were taken daily and body weights weekly throughout the entire experiment. Body weight gains were similar in control and experimental animals. After approximately 8 weeks of restricted water intake (RWI), animals showing consistent 5-day cycles (3-day diestrus) were decapitated at various times on the day of proestrus within 30 sec of removal from their cage. The blood was collected in heparinized tubes and the plasma transferred to three separate tubes and stored frozen (−20°) for subsequent radioimmunoassay (RIA) of prolactin and LH and fluorometric determination of corticosterone as described elsewhere (5). No more than two animals were killed at any one time period during a given day. Control animals were treated identically except that they were given access to food and water *ad libitum*. A second group of eight rats was treated initially in the manner described for the 1100 RWI animals except that the 15-min watering period was at 1800 rather than 1100. After approximately 5 weeks of RWI at 1800, the animals were returned to water *ad libitum* for 30-35 days before terminating the experiment. In this latter group of animals only the daily changes in vaginal cytology were monitored, i.e., no blood was taken.

Results. Although a few animals (5/40) showed an immediate effect of RWI at 1100 during the ongoing cycle (Fig. 1), a change in cycle length was most evident in the subsequent cycle in which 50% (20/40) of the animals had a 5-day cycle. The 5-day cycle almost always included 3 days of diestrus; a few showed 2 days of cornification and 2 of leukocytes, but this type of 5-day cycle was rare and was never seen in consecutive cycles. Many animals (15/40 on the second cycle after RWI) showed irregular cycles, i.e., 6-14 days, shortly after water restriction, but then returned to regular 5-day cycles or fluctuated between 4- and 5-day cycles. Animals showing irregular cycles usually had extended periods of diestrus, but

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occasionally some animals' smears were cornified for several days. Approximately 5 weeks after RWI at 1100, a large majority of animals (31/40) were exhibiting regular 5-day cycles while a few (3/40) were showing 6- to 7-day cycles with an occasional 5-day cycle. Regular 4-day cycles were recorded at this time in only 15% (6/40) of the animals treated. Of these, two animals had consistent 4-day cycles throughout the entire duration of the experiment.

Plasma corticosterone was observed to have two peaks, one at 1100 just prior to the 15-min watering period, and a second at 1900 (Fig. 2). The values at these two times

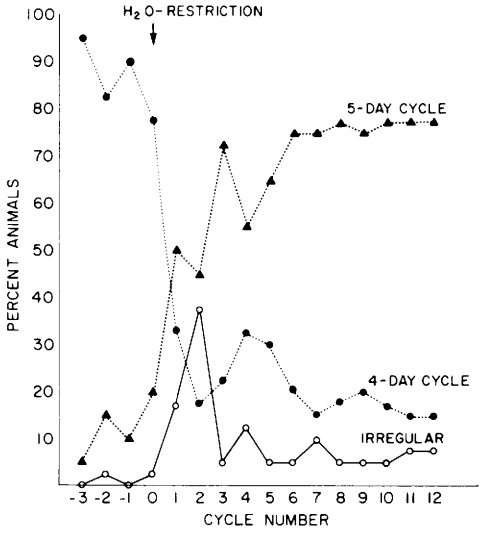

FIG. 1. Percentages of animals showing 4-day, 5-day, and irregular (greater than 5 days) cycle lengths before (minus) and during restriction of water intake at 1100. $n = 40$.

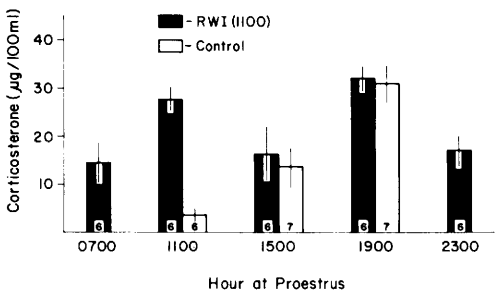

FIG. 2. Plasma corticosterone levels at (4-hr intervals) during proestrus in controls and animals on a restricted water intake (RWI) at 1100. Each bar represents the mean and the vertical line represents the SEM. The number at the base of the bar represents the number of animals.

were not significantly different from each other, but both were significantly higher ($P < 0.05$) than the RWI levels at 0700, 1500, and 2300. Plasma corticosterone levels at 1100 in the control animals were significantly lower ($P < 0.001$) at 1100, but did not differ from the respective RWI animals when measured at 1500 and 1900.

Plasma LH levels during proestrus were not significantly different between the RWI and control animals (Fig. 3). However, the levels at 1500 in RWI animals were considerably more variable than controls due to an extremely high level observed in one animal (877 ng/ml) and a moderately high level in another (264 ng/ml); whereas the maximum level observed in control animals was 134 ng/ml.

Prolactin levels during proestrus in the RWI animals (Fig. 4) were found to be low at 0700 and just prior to watering at 1100. Maximum levels were observed at 1500 and 1900, with a slight decline ($P < 0.05$) at 2300. Plasma prolactin concentrations in the control animals were similar to RWI animals at 1100 and 1500, but at 1900 and again at 2300 control animals had significantly lower ($P < 0.001$) levels than the RWI rats. By 2300, the prolactin values in control animals had returned to levels near those found prior to the rise in prolactin while values for the RWI animals remained elevated. Thus, it was concluded that the RWI animals had an extended period of prolactin release during the afternoon of proestrus.

The effect of restricting the water intake at 1800 (Fig. 5) was similar to the animals on RWI at 1100, i.e., 6/8 animals had prolonged cycles within 10 days after water restriction. After 40 days of RWI, 75% (6/8) showed 5-day cycles and one animal had irregular cycles (6–9 days). One animal maintained consistent 4-day cycles throughout the entire RWI period. Within 10 days after restoration of an unlimited water supply, 7/8 of the animals had returned to regular 4-day cycles. One animal showed an extended period of cornified vaginal epithelium, but by 30 days of water *ad libitum* all eight animals had regular 4-day estrous cycles.

Discussion. Limiting the daily watering period to 1 hr or less during the early part of

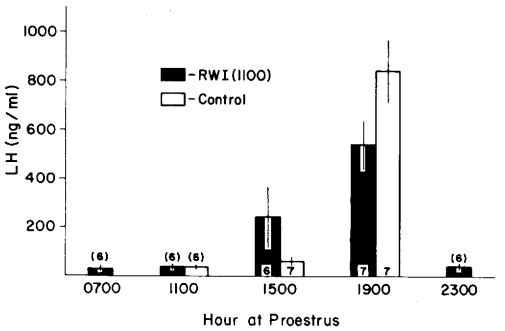

FIG. 3. Plasma LH levels during proestrus in controls and animals on a restricted water intake at 1100. See legend in Fig. 2 for further details.

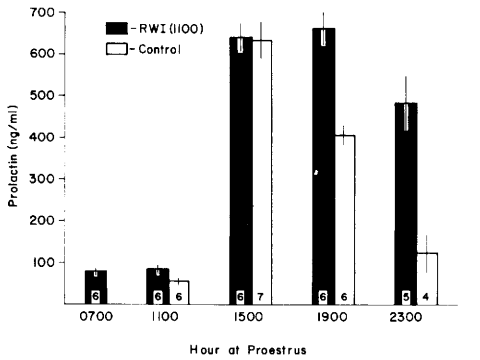

FIG. 4. Plasma prolactin levels during proestrus in controls and animals on a restricted water intake at 1100. See legend in Fig. 2 for further details.

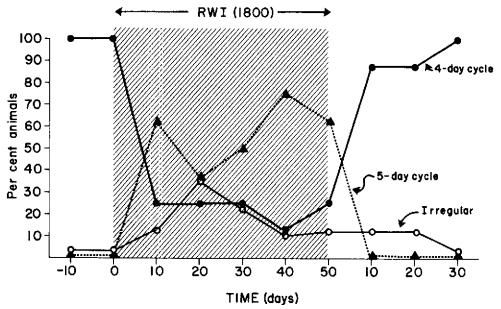

FIG. 5. Percentages of animals showing 4-day, 5-day and irregular (greater than 5 days) cycle lengths before (minus) and after restricting the water intake at 1800 (Days 0–49) followed by water *ad libitum*. The shaded area represents the period of water restriction. $n = 8$.

the light cycle (i.e., 4–5 hr after lights on in a 14-hr light: 10-hr dark schedule) results in a lengthening of the rat estrous cycle (3, and present study). We have also shown that RWI for a 15-min period near the end of the light period (1 hr before lights off) exerts a similar effect on length of the estrous cycle.

The underlying mechanism responsible for the alteration in cycle length is unknown, but it appears to be closely coupled to the RWI since the effects on cycle length are quickly reversed by returning the animals to an unlimited watering schedule. It has been suggested that the change in adrenal steroid secretions after restricted water intake (1) may be responsible for the alteration in the estrous cycle length (3). We also observed a change in normal corticosterone levels as a result of RWI at 1100 similar to that reported by Johnson and Levine (1). In other words, there was an elevation of corticosterone just prior to the watering period in addition to the normal elevation occurring around the beginning of the dark period. A recent report that a change in cycle length from 4 to 5 days as the result of injections of dexamethasone given on the morning and evening of the first day of diestrus (6) supports the suggestion that an alteration of adrenal corticoid rhythm may be involved in changing cycle length.

In addition to the alteration of the normal adrenal rhythm in the animals subjected to RWI at 1100, a change in the normal pattern of prolactin levels at proestrus was observed. RWI animals exhibited a prolonged elevation in plasma prolactin throughout the early afternoon and late evening whereas the control animals had a peak in the early afternoon with a return to normal basal levels by late evening. Whether this difference in prolactin secretion could contribute to the 1-day extension of the estrous cycle is not known. Since the duration of the luteal function has been suggested as a likely explanation for the occurrence of 4- and 5-day cycles (7), and prolactin has been reported to be luteotropic in the rat (8, 9), it seems possible that the prolonged elevation of the proestrous surge of prolactin may have resulted in the formation of corpora lutea with a longer functional life span than those formed in a normal 4-day cycle. This is supported by the fact that almost all of the 5-day cycles included 3 days of diestrus.

Plasma LH levels during RWI at 1100 were not significantly different from the normal control values although there was a tendency for levels to be higher at 1500 and lower at 1900 in RWI animals than in 4-day

cycling controls. The variation in LH levels noted in the RWI rats at 1500 would suggest that the time of onset of the proestrous surge of LH was more erratic in these animals. This suggestion is supported by the observation that pentobarbital is more effective in blocking ovulation at proestrus in water restricted animals (3).

Finally, the possibility exists that the induced change of drinking and presumably feeding activity in the RWI animals (compared to those with food and water *ad libitum*, 10) might have caused a desynchronization of several biological rhythms, and the alterations in estrous cycle length and hormone levels are merely reflections of this desynchronization. This possibility will require further experimentation.

Summary. By the fifth week of restricting water intake at 1100 to a 15-min period per day, estrous cycle lengths had shifted in most animals (31/40) from 4 to 5 days with the 5-day cycles usually consisting of 3 days of diestrus. In the 5-day cycling rats, plasma corticosterone peaked at 1100 and also at 1900 hr, the proestrous surge in plasma LH was not significantly changed, although the variation in individual animals was greater and the prolactin surge was prolonged. Similar prolonged cycles followed water restriction at 1800 hr with animals returning to regular 4-day cycles when given water *ad libitum*. These results suggest that the alteration of the normal corticosteroid rhythm and/or changes in prolactin secretion may account, in part at least, for the changes noted in cycle length after restricting the water intake.

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