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Timing of the Estrogen-Induced Release of LH in Ovariectomized Rats Under an Altered Lighting Schedule[1] (37965)

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In 1950 Everett and Sawyer (1) advanced the hypothesis of a daily periodicity in mechanisms controlling LH release in the rat. This proposal has received further support from experiments demonstrating that the estrogen-induced release of LH in ovariectomized rats occurs only during the afternoons under standard lighting conditions, e.g., lights on 0500–1900 hr (2). These observations have recently been confirmed in the rat (3, 4) and hamster (5).

The role of light as a synchronizer of the cyclic ovulatory surge of LH has also been documented (6, 7). Therefore, it was of interest to study whether changing the standard lighting schedule would alter the timing of the estrogen-induced LH release in ovariectomized animals. Should this be the case, it would reemphasize the similarities in mechanisms subserving spontaneous and estrogen-induced LH surges in the rat.

Materials and Methods. Adult (200–250 g) female Sprague–Dawley (Simonsen) rats with established 4-day estrous cycles were used. They were given a standard laboratory diet and water *ad lib.* and subjected to an 8-hr shift in lighting schedule (lights on 2100–1100 hr) for a minimum of 4 weeks. The standard daily period of light in our animal room is 0500–1900 hr. Animals were caged in pairs and bled according to the following schedules: (1) 0700, 0930, 1230, 1530, and 1830 hr, or (2) 0800, 1100, 1400, 1700, and 2000 hr. Blood samples (0.6 ml) were taken from the jugular vein under light ether anesthesia and collected in heparinized tubes. After centrifugation, the plasma was separated and stored frozen for subsequent radioimmunoassay (RIA) of LH. In order to avoid possible effects of light during the bleeding procedures, during the dark periods rats were anesthetized in the animal room. Two experiments were conducted:

Experiment I. This study was designed to establish the timing of the cyclic LH surge under our modified lighting schedule. Sequential bleedings were performed throughout the day of proestrus at the times indicated above.

Experiment II. After the animals had recovered from the proestrous sampling and had shown at least two consecutive 4-day estrous cycles, they were ovariectomized at 1000 on the second day of diestrus. Exactly 48 hr later they were given subcutaneous injections of estradiol benzoate (Eb) (5 μg/100 g body wt) or oil (0.1 ml/100 g body wt). On the second day after the estradiol or oil injections, the animals were bled according to one of the schedules outlined above. The second day after injection was selected on the basis of data reported by Ramirez and Sawyer (4).

LH was measured in duplicate by RIA using the NIAMDD Rat LH kit. Values are expressed in terms of ng/ml of NIAMDD-LH-RP-1 standard which has a potency of 0.03 × NIH-LH-S1 (OAAD assay).

Results. Experiment I. The spontaneous LH surge in proestrous rats exposed for 4

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weeks or more to the advanced lighting schedule was observed to peak 7–8 hr earlier when compared with the results obtained in this laboratory (8) under standard light conditions (Fig. 1). The timing of the shift in the LH surge is directly comparable with the advancement in the lighting schedule. Under either condition, plasma LH peaks close to the beginning of the dark period (Fig. 1), such that LH levels under the modified light regime are markedly reduced by the time of the LH peak under standard illumination. Figure 1 also shows that the percentages of animals with their highest plasma LH levels at given time periods were maximal at 1100 and 1230 hr (Fig. 1).

Experiment II. Plasma LH levels fluctuated greatly in ovariectomized animals receiving oil injections, with individual values ranging from 50 to 400 ng/ml (Fig. 2). This group did not reveal a preferential time for LH release. On the other hand, two days after Eb injection in ovariectomized rats, significant increase in plasma LH occurred at 1100 and 1230 hr. In a few animals maximum LH levels attained before 1100 hr but none after 1230 hr. Actual plasma LH increases in the Eb-treated group ranged from 4- to 20-fold.

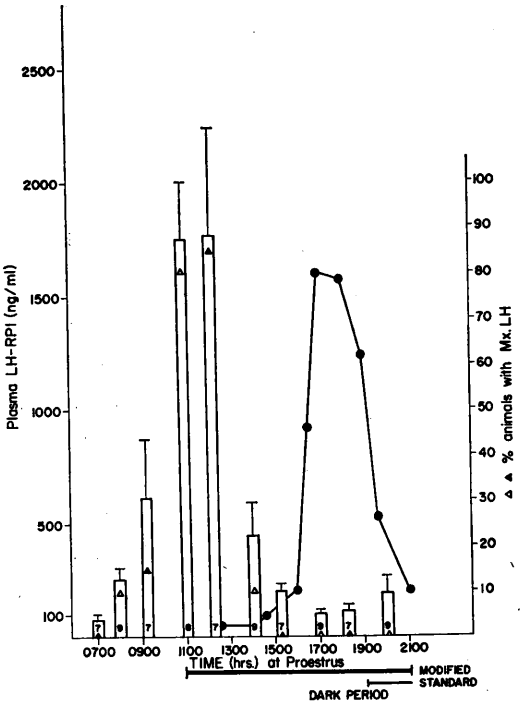

FIG. 1. LH surges during proestrous in animals under modified (open bars, mean ± SD) and standard (curve with closed circle points, from Blake *et al.* (8) lighting schedules. Number of animals per group is as indicated inside bars. Open and closed triangles within bars represent percentages of maximal LH values at each bleeding time in two different groups of animals.

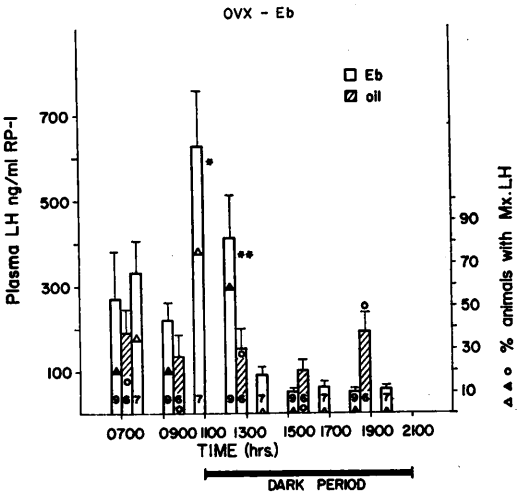

FIG. 2. Plasma LH concentrations in ovariectomized estrogen-primed rats under altered lighting conditions (dark period advanced 8 hr from standard light schedule). $*P < 0.01$ and $**P < 0.05$, compared with oil group at 1230 hr. See Fig. 1 for further explanation.

Discussion. Under controlled illumination with lights on from 0500 to 1900 hr, the "critical period" occurs between 1400 and 1600 hr and the LH surge is maximum around 1700–1900 hr. Under these lighting conditions, estradiol treatment in ovariectomized animals induces repetitive daily LH discharges at the same time as the spontaneous proestrous LH surge in cycling rats (2–4), and similar findings have been reported in the hamster (5). In the present report, animals subjected to 14 hr of light but with an 8-hr advancement in the lighting schedule also showed a posttreatment LH rise but with maximum LH levels occurring at 1100 and 1230 hr, i.e., approximately 7–8 hr earlier than in animals under standard illumination (4). Under the modified lighting

schedule, there was also a clear overlap in the times at which the estrogen-induced and cyclic LH surges occurred. In order to eliminate the possibility that the estrogen-induced LH peak was a function of the time of injection, the steroid was given at the same time as that administered by Ramirez and Sawyer (4) to rats under standard illumination.

These results indicate that the estradiol-induced LH release in ovariectomized animals is fully as dependent on the synchronizing effects of light as is the spontaneous proestrous LH surge in cycling animals. Hence, two phenomena can now be related: the induction of an LH surge by a single estradiol injection in short-term ovariectomized rats and the synchronization of that surge with the light schedule. Since the estrogen-induced LH surge can be blocked by phenobarbital or hypothalamic deafferentation (5), its effects on pituitary responsiveness to LHRH seem to be concurrent with and not determinant of the LH discharge. Attempting to consider both components (central and hypophyseal) of estrogen action, one might propose that the estrogen enables LH regulatory mechanisms to operate in a synchronous fashion resulting in a surge of LH release. As observed in this study, in animals without estrogen treatment these components operate in an "asynchronous" manner such that LH is released in an irregular pattern, thus resulting in a lower average level. Which aspects of estrogen dynamics (increasing or decreasing levels, rate of change) are related to its "synchronizing" effects on the mechanism for the LH surge remain to be established.

Regarding the mechanisms involved in the "timing" effect of light on the estrogen-induced and spontaneous LH surges, several factors should be considered. Among these it is possible that light might be acting directly upon the neural components concerned with LH surges or indirectly through given phase relationships with periodic changes occurring in other systems, such as adrenal or pineal. For example, a recent report indicates that alterations of adrenal circadian rhythms occur concomitantly with changes in the "timing" of the "critical period" for the proestrous surge of LH in animals under standard lighting conditions submitted to restricted water intake (9). At the present time, other studies are being conducted in this laboratory to investigate some of these possibilities.

Summary. Previous results from this laboratory (4) have shown that rats submitted to a 0500–1900 hr "lights-on" schedule responded to a single estradiol administration 48 hr after ovariectomy with plasma LH rises only during the afternoon (1630–1700 hr) of the following days. Under otherwise similar experimental conditions, it is now reported that if the "lights-on" period is shifted to 2100–1100 hr, the estrogen-induced LH surges occur at 1100–1230 hr. Cycling rats submitted to the altered illumination schedule show similarly altered times of the proestrous surge. The results reveal that the estrogen-induced LH rise in ovariectomized animals is synchronized by light, and suggest that similar mechanisms control this estrogen-induced LH surge in ovariectomized rats and the spontaneous proestrous LH surge in cycling rats.

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