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Light Synchronization of the Preovulatory LH Surge in Adrenalectomized Rats (40539)[1]

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It is well established that the timing of the spontaneous preovulatory LH surge in intact or the estrogen-induced LH surge in ovariectomized rats is closely synchronized to the prevailing light–dark cycle (1–4). For example, animals maintained on a standard light schedule of 14 hr light:10 hr dark have a plasma LH surge which begins approximately 3–4 hr after the midpoint of the light period. When the onset of the light period is shifted, but the light to dark ratio is held constant, the LH surge will shift according to the advancement or delay of the light period.

Little is known about the mechanisms relating the effect of light to the timing of the LH surge. It is clear that an estrogen background is necessary for the expression of the LH rhythm, but it has been shown that the timing of the LH surge is synchronized with the light cycle rather than with the time of estrogen administration (4). Others have suggested that the rhythm of adrenal steroids, particularly adrenal progesterone, which is also synchronized to the light:dark cycle, may be important for the timing of the onset of the LH surge (5–7). A recent report by Mann *et al.* (8) has shown that acute adrenalectomy (i.e., day of proestrus) had no effect on the LH rhythm, whereas chronic adrenalectomy resulted in an asynchronous release of LH during the afternoon of proestrus when compared with intact control animals. These results again suggest that the adrenal gland secretes a substance responsible for synchronizing the timing of the LH surge. In a later study, based upon observations on steroid-induced changes in LH secretion in the ovariectomized rat, Mann *et al.* (9) suggested that the light–dark cycle played an important role in maintaining the critical period of LH release independent of the adrenal glands. The adrenal steroids, on the other hand, were considered to be an additional synchronizing component.

In an earlier report (10), we found that although the length of an ongoing estrous cycle might be influenced by removal of the adrenal glands, all animals returned to regular 4-day estrous cycles beginning on the next cycle after surgery. These observations suggested that a preovulatory surge of LH occurred every 4 days in the absence of the adrenal glands, but did not indicate whether the timing of the surge was altered after adrenalectomy. The purpose of the present study was to determine the influence of the adrenal gland on the timing of the preovulatory LH surge as induced by changes in the light–dark cycle in the 4-day cycling female rat.

Materials and methods. Adult female Sprague–Dawley rats (Simonsen Laboratories, Gilroy, Calif.) weighing 200–250 g were maintained in temperature- and light-controlled rooms (22°C; 14 hr light). Purina laboratory chow and water were available *ad libitum.* All animals had shown at least two consecutive 4-day cycles as determined by daily vaginal smears before use. Blood samples (0.8–1.0 ml) were collected from the jugular vein under light ether anesthesia with a heparinized syringe and 24-gauge needle. Plasma was collected and stored frozen (−20°C) for subsequent radioimmunoassay (RIA) of LH.

Adrenalectomy was performed under ether anesthesia by making a single incision along the midline of the back and entering the abdominal cavity through small incisions near the kidney. Adrenalectomized (adx) animals were maintained on 0.9% saline solution for drinking water throughout the duration of the experiments. Completeness of adx

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was checked at the end of the experiment by inspection for any remaining adrenal tissue. Any animal suspected of having adrenal tissue remnants was excluded from the study. The occurrence of ovulation was checked the day following the night of expected ovulation (i.e., approximately 17 hr after lights off on proestrus) by microscopic examination of the ampullae for the presence of ova. All animals which failed to ovulate were also excluded from the study. This represented 8 of the 62 adx and 1 of the 66 intact rats examined.

Two experiments were conducted to determine the effect of light on the timing of the LH surge in adx animals. In the first experiment, animals were placed under standard lighting conditions (lights on 0500–1900 hr) and after at least two 4-day estrous cycles were observed, animals were adx. Normal intact animals served as controls. Rats showing consecutive 4-day estrous cycles (i.e., two–four cycles) were bled at 1400, 1600, 1800, or 2000 hr on the day of proestrus. Only one blood sample was taken from any one animal (normal and adx) since we had found in an earlier pilot study that if more than one sample was taken from adx animals, there was a high percentage of animals not ovulating. All animals, adx and intact controls, were checked for ovulation the following day.

In the second experiment, animals were placed under standard lighting conditions (lights on 0500–1900 hr) until 4-day estrous cycles were established. One group of rats were then adx independent of the stage of the cycle and a second group remained intact. Both groups were immediately transferred to an altered lighting schedule in which the onset of the light period was advanced by 7 hr (i.e., lights on 2200–1200 hr). After at least 3 weeks under the altered light schedule and the reestablishment of regular 4-day cycles, animals were bled at 0700, 0900, 1100, 1300, or 1500 hr on the day of proestrus. As in the first experiment all animals were checked for ovulation the following day.

Plasma LH was determined by using the NIAMDD Rat LH RIA system obtained from the Rat Pituitary Hormone Program of the NIAMDD, NIH. The reference preparation was NIAMDD-Rat LH-RP-1, which has a biological potency of 0.03 × NIH-LH-S1 (OAAD). All samples were run in duplicate at two different volumes.

The data were analyzed using Student's *t* test.

Results. The plasma LH levels at 2-hr intervals during the afternoon and evening of proestrus in intact and adx animals exposed to standard lighting conditions (lights on 0500–1900 hr) are shown in Fig. 1. There were no significant differences in LH levels between intact and adx animals at any of the time periods tested. In the two groups of animals, the times of onset of the preovulatory LH surges and peak LH levels were practically coincidental. Peak concentrations were obtained 1–3 hr prior to the end of the light period. Both groups of animals ovulated a normal quota of ova (11.5 ± 0.3 vs 9.8 ± 0.3, intact vs adx); however, as previously reported by Peppler and Jacobs (11) the mean number of ova was significantly lower ($P < 0.05$) in the adx animals.

The effect of advancing the onset of the light period by 7 hr (i.e., lights on 2200–1200 hr) on the preovulatory LH surge is illustrated in Fig. 2. The light-induced advancement in the timing of the LH surge was quite similar in intact and adx animals. Similar LH concentrations were observed at all time periods for the two groups of animals, with peak concentrations occurring near the end of the 14-hr light period. Both intact and adx rats

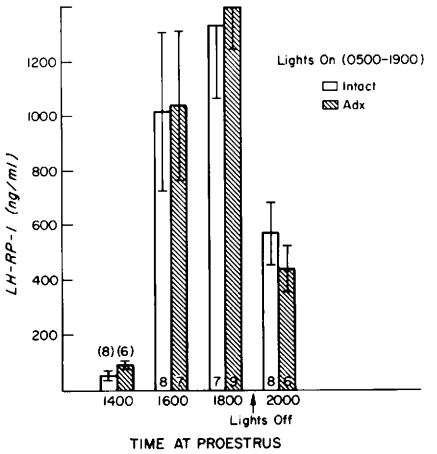

FIG. 1. Plasma LH concentrations during proestrus in cycling intact and adrenalectomized (adx) rats maintained on 14-hr light:10 hr dark (lights on 0500–1900 hr). Each bar represents the mean and the vertical line the standard error. The number in parentheses or at the base of the bar represents the number of observations.

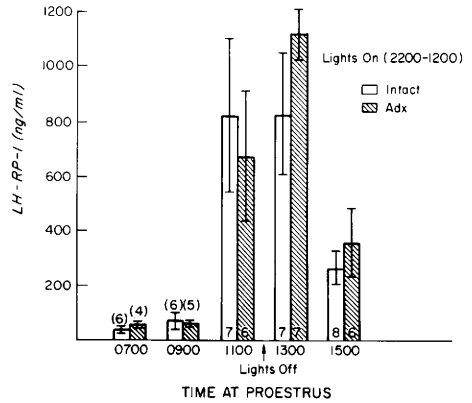

FIG. 2. Plasma LH concentrations during proestrus in cycling intact and adrenalectomized (adx) rats after 3 or more weeks in an altered light environment. Intact or adx animals were placed in a 14-hr light period which was advanced by 7 hr (lights on 2200–1200 hr; changed from 0500–1900 hr) immediately after adx. For other details, see legend to Fig. 1.

released similar numbers of ova (10.8 ± 0.3 and 10.3 ± 0.3, respectively). In this experiment, it was found that subjecting animals to the altered light schedule resulted in a disruption of the regular 4-day estrous cycle. The average time required to return to a regular 4-day cycle was 17 ± 0.9 days (mean ± SEM) in control animals. On the other hand, when animals were adx just prior to being placed in the altered light environment, the average time required to return to regular 4-day estrous cycles (27.5 ± 2.4 days) was significantly ($P < 0.01$) longer than in the intact controls. This is in contrast to results in animals adx and maintained under standard lighting conditions, in which only an occasional animal exhibited a 1-day extension of the ongoing cycle during which the surgery was performed.

Discussion. The importance of light as a synchronizer of the cyclic ovulatory surge of LH is well documented (1–4). The primary focus of the present study has been to determine whether this synchronizing action of light on LH secretion is in some way coupled to the effects of light on the pituitary–adrenal system (2, 12). Several studies have indicated that the adrenal gland is involved in the regulation of the proestrous surge of LH in the rat, presumably via the secretion of adrenal progesterone (5–8, 13). Since adrenal progesterone secretion is regulated by ACTH (14), injections of progesterone can alter the timing of the LH surge (6, 15, 16), and a circadian variation of adrenal progesterone secretion has been observed throughout the rat estrous cycle (5), it has been proposed that this rhythm might be responsible for the photic synchronization of LH release (5, 6). In addition to changes in adrenal progesterone, other studies have indicated that changes in 24-hr rhythms of corticosterone secretion might alter the normal timing of the LH surge (17–19).

On the other hand, we had previously observed that adx female rats continued to show regular 4-day estrous cycles and ovulated a normal number of ova (10). These results indicated that adx did not affect the basic determinants of cycle length. In the present study, we found that removal of the adrenal glands did not alter the timing or the amplitude of the proestrous surge of LH. This is in contrast to a recent report by Mann *et al.* (8) who observed that adx rats released LH in an asynchronous manner when compared with intact control animals. The reasons for the differences between the results of our study and those of Mann and co-workers are not obvious. However, differences in experimental protocol such as time after adx, blood sampling procedures, the inclusion of only adx animals which ovulated (this study), and the time periods in which blood samples were collected, may account for some of the diversities noted between the two studies.

Our observations in the first experiment of the present study suggest that the adrenal steroids are not necessary for the characteristic light-dependent synchronization of the proestrous surge of LH. Moreover, when the light–dark cycle is advanced by 7 hr both intact and adx animals show corresponding shifts in the timing of the preovulatory surge of LH. This shift in timing is similar to that observed in a previous study in intact and estradiol-treated ovariectomized rats (4).

Thus, in agreement with an earlier suggestion by Mann *et al.* (9), our data indicate that the synchronizing effects of light on the LH surge operate via mechanisms independent of the pituitary–adrenal system. However, this does not necessarily mean that the pituitary–adrenal system fails to play a role in the light synchronization of the LH surge under more

physiological conditions. Such a role is suggested by other studies mentioned above, and by the fact that in the present study, the time interval for the return of regular 4-day estrous cycles was longer in the adx rats than in the intact control animals. Nonetheless, the predominant synchronizing effect of light on the LH surge appears to involve neuroendocrine events which are independent of any adrenal-mediated influence. As suggested by Mann *et al.* (9), the adrenal steroids appear to have secondary modulatory roles in the regulation of timing of the LH surge. Whether the influence of the adrenal steroids include a direct involvement of the synchronization by light or an indirect involvement through other actions on the brain–pituitary axis concerned with LH secretion remains to be determined.

Summary. Possible effects of adrenalectomy (adx) on light-dependent changes in the timing of the preovulatory LH surge have been investigated. In the first experiment, female rats were maintained on a standard light schedule of 14 hr light:10 hr dark (lights on 0500–1900 hr). Intact and adx rats showing at least two consecutive 4-day estrous cycles were bled at 1400, 1600, 1800, or 2000 hr on proestrus. The timing and amplitude of the preovulatory surge of LH were very similar in intact and adx animals. In a second experiment, intact animals were maintained under standard lighting conditions (lights on 0500–1900 hr) until the establishment of regular 4-day cycles. Intact rats and experimental animals immediately after adx were then transferred to an altered light regime in which the 14-hr light period was advanced 7 hr (i.e., lights on 2200–1200 hr). After 3 weeks or more in the altered light environment, animals showing regular 4-day estrous cycles were bled at 0700, 0900, 1100, 1300, or 1500 hr on proestrus. Both intact and adx rats showed an approximately 7-hr shift in the timing of the preovulatory surge of LH. However, the adx animals required a longer period of time to reestablish regular cycles when placed in the altered light environment than did the intact rats. The results indicate that the primary synchronizing effects of light on the timing of the preovulatory LH surge in the rat operate via mechanisms independent of the pituitary–adrenal system.

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