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Daily Luteinizing Hormone Release in Ovariectomized Hamsters: Effect of Barbiturate Blockade[1]

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ABSTRACT

In the absence of the ovary, female hamsters exhibit a daily surge of LH between 1500 and 1900 h (lights on 0600–2000 h). Phenobarbital (100 mg/kg, s.c.) injected at 1400 h completely suppressed the LH surge in females that had been castrated 15 days previously. Phenobarbital was also effective in long-term (15 week) castrate animals. In both cases, surge release of LH was delayed 24 h, occurring at the expected time, the day after injection. In a final experiment, phenobarbital was injected at various times in the afternoon in females castrated for 3 weeks. Phenobarbital effectively prevented LH release when administered at any time from 1100 to 1500 h. Injections at 1600 h partially suppressed the LH peak, which occurred at about the same time.

INTRODUCTION

In Stetson et al. (1978) we documented daily LH release in cyclic hamsters following ovariectomy. Similar results were reported by Shander and Goldman (1978); however, Vomachka and Greenwald (1980) reported that the 1500 h maximum in serum LH of their animals was not significantly elevated over levels obtained at other times of the day. In ovariectomized animals, FSH levels increase dramatically (castration response) and serum levels are of too great a magnitude to determine the existence of a rhythmic release pattern. For LH, however, daily surges persist for many weeks (Stetson et al., 1978) and are similar temporally, though of lesser magnitude than the proestrous surge.

Cyclic gonadotropin (LH and FSH) release also occurs in lactating and photoperiod-induced anovulatory (PIA) hamsters (Seegal and Goldman, 1975; Bridges and Goldman, 1975). In these instances the periodicity of hormone release is decreased from once every 4 days as in ovulatory hamsters to a daily release, temporally correlated with the time of the proestrous surge (DiPinto and Stetson, 1978). We have shown (Smith and Stetson, 1980) that one of the events in puberty onset in female hamsters is the induction of daily LH and FSH release. This commences on Day 16 of life (birth = Day 1) and appears to persist up to the time of first ovulation (Days 30–35). The timing of the daily prepubertal LH and FSH surge is similar to that of the proestrous surge in adults on the same photoperiod. The nature of the oscillator that times daily LH and FSH release in PIA and prepubertal female hamsters is unknown, though we assume it is neural in nature and probably identical to that timing the estrous cycle. In lactating hamsters we have characterized the oscillator as neural, the system responding to barbiturate blockade with a precise 24 h delay in hormone release (DiPinto and Stetson, 1978) similar to that seen in cyclic hamsters (Siegel et al., 1976; Stetson and Watson-Whitmyre, 1977). In this paper we examine, in ovariectomized hamsters, the response of the system timing daily LH release to barbiturate blockade. Delay of the LH surge for 24 h would indicate the involvement of a neural oscillator, similar to that timing estrous cyclicity in intact animals.

MATERIALS AND METHODS

Adult female hamsters were raised in our laboratory. They were maintained on a 14L:10D photo-

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period (lights on 0600–2000 h) at 22–24°C. They were housed five or six per cage with food and water always available. Females having shown four consecutive 4-day estrous cycles were bilaterally ovariectomized under ether anesthesia (Stetson et al., 1978). Postoperative mortality was less than 1%. Results reported here are derived from three separate experiments performed over a period of 3 years.

Experiment 1

Phenobarbital (100 mg/kg BW) was injected s.c. in 100 ovariectomized hamsters 15 days after ovariectomy between 1330 and 1430 h (mean injection time 1400 h). The time of injection was the same as previously used for the intact hamster (Stetson and Watson-Whitmyre, 1977). At the same time, 100 ovariectomized control hamsters received s.c. injections of 1.0 ml normal saline. The animals were sacrificed by decapitation at the times indicated in Fig. 1. Trunk blood was collected, allowed to clot, centrifuged, and the serum stored frozen for radioimmunoassay of LH and FSH. The uteri were removed, debrided, weighed (wet), and examined for ovarian fragments. Sera from animals with detectable ovarian tissue were discarded from the sample groups.

Experiment 2

Blood samples (1 ml) were drawn by cardiac puncture from ovariectomized hamsters 5, 10, and 15 weeks after ovariectomy, five or six animals each sampling time, each animal sampled only once each sample day (Fig. 2). Five days after the last cardiac puncture, the animals were injected with phenobarbital as in Experiment 1. Samples were taken by cardiac puncture on the day of injection and the animals were sacrificed the following day. Autopsies were performed as in Experiment 1.

Experiment 3

Ovariectomized hamsters (288) were divided into eight groups of 36 animals each 3 weeks after ovariectomy. Seven groups received phenobarbital (dosage as above), one group per hour, between 1100 and 1700 h. The control group was injected with normal saline. Six animals from each group were sampled (1 ml) by cardiac puncture every second hour between 1200 and 2200 h of the day of injection, and then sacrificed by decapitation the next day (six per group per time) at 0900, 1200, 1500, 1700, 1800, or 2100 h. The serum was prepared as described above.

Serum titers of LH were measured by RIA (kits for the homologous rat hormones supplied by NIAMDD through the National Pituitary Agency and Dr. A. F. Parlow). All samples from an experiment were run in a single assay. Antibody used was A-rat-LHS-3. The results are expressed in terms of nanogram-equivalents of NIAMDD-rat-LH-RP-1 per milliliter serum. This assay has been extensively characterized for use in the hamster (Berndtson and Desjardins, 1974; Bast and Greenwald, 1974) and is routinely used in our laboratory. Interassay variability for our LH RIA is low (<10%), while intraassay precision is high (CV 5–7%; Stetson and Watson-Whitmyre, 1977.)

RESULTS

Experiment 1

The results of phenobarbital administration to short-term (15 day) castrates are given in Fig. 1. Serum LH in saline-injected controls peaked at 1900 h on the day of injection and at 1500–1600 h the next day. Phenobarbital completely suppressed the LH surge on the day of injection. Serum LH in these animals peaked the following day at 1600–1700 h (Fig. 1).

Experiment 2

Serum LH profiles 5, 10, and 15 weeks after ovariectomy are shown in Fig. 2. On Weeks 5 and 10, LH peaked sharply at 1600 h, while on Week 15 the peak was extended over a 3-h period (1500–1700 h). In 15–16 week castrates, phenobarbital administration at 1400 h blocked the afternoon surge of LH (Fig. 3) for 24 h.

Experiment 3

In this experiment we examined in greater detail the "critical period" for daily LH release in hamsters ovariectomized 3 weeks before phenobarbital treatment. Phenobarbital administered before 1600 h blocked the daily LH surge, while injections at 1600 and 1700 h either partially suppressed the peak or were without effect (Fig. 4). As in the previous experiments (Figs. 1 and 3), the LH surge was detectable in every group the following day (Fig. 4). The LH surges of each group did not

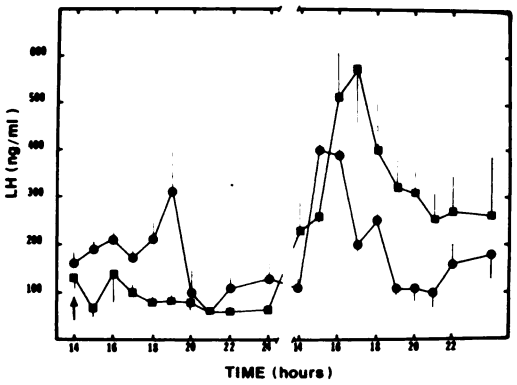

FIG. 1. Serum LH levels in saline-injected (solid circles) and phenobarbital-injected (solid squares) ovariectomized female hamsters. The arrow defines the time of injection. Each point is the mean of five animals. Vertical lines depict the SEM.

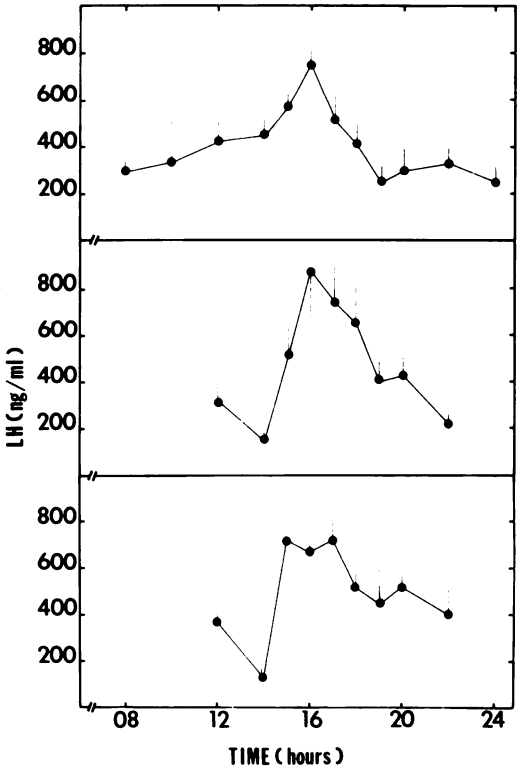

FIG. 2. Serum LH in hamsters 5 (top), 10 (middle), and 15 (bottom) weeks after ovariectomy. Each point is the mean value from five or six individuals. Vertical lines depict SEM.

differ appreciably in terms of time and magnitude and have therefore been pooled for ease of presentation (Fig. 4).

DISCUSSION

Clock-timed gonadotropin release in spontaneous ovulators like the rat and hamster is well documented (Everett, 1977). In hamsters, a single injection of phenobarbital on the afternoon of proestrus blocks preovulatory LH and FSH release and ovulation for 24 h (Alleva and Umberger, 1966; Norman et al., 1972; Siegel et al., 1976; Stetson and Watson-Whitmyre, 1977). Two or three consecutive daily injections of phenobarbital cause a 48 or 72 h delay, respectively, of gonadotropin release and ovulation (Stetson and Watson-Whitmyre, 1977; Terranova, 1980). We have further characterized the neural oscillator timing the proestrous surge of LH and FSH in hamsters as a circadian clock, capable of reentrainment to an altered photoperiod (Stetson and Gibson, 1977) and of freerunning under conditions of constant darkness (Stetson and Anderson, 1980). Other aspects of the hamster's estrous cycle have been shown to freerun under conditions of continuous dim light (Alleva et al., 1968; Fitzgerald and Zucker, 1976; Stetson et al., 1977), and their timing bears a fixed phase-relationship to the circadian activity rhythm. Ablation of the suprachiasmatic nucleus (SCN) of the hypothalamus results in cessation of both estrous cyclicity, the hamsters becoming persistently estrous, and locomotor rhythmicity (Stetson and Watson-Whitmyre, 1976; Watson-Whitmyre and Stetson, 1977). Coincidental loss of estrous and locomotor rhythmicity also occurs under constant light (Stetson et al., 1977). We have interpreted the induction of persistent estrus as synonymous with loss of rhythmicity of the oscillator driving the rhythm. That is, the rhythmic component of LH and FSH release is no longer manifest, and under tonic, or at least an altered pattern of gonadotropin stimulation, the ovary becomes polyfollicular with an increased output of estradiol (E_2), resulting in the persistent estrous state. We should state, however, that neither we, nor to our knowledge, anyone else has compared serum gonadotropin and E_2 levels in SCN-lesioned or constant light-induced persistent estrous hamsters.

The intent of this investigation was to determine the nature of the mechanism timing daily LH surges in ovariectomized hamsters, and to compare it to that timing the proestrous surge in ovulatory hamsters. We have shown

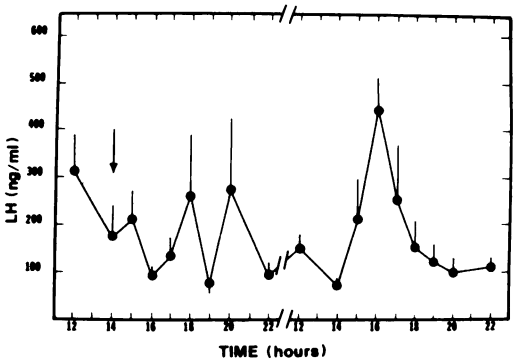

FIG. 3. Serum LH following phenobarbital injection (arrow) in the animals from Fig. 2, bottom. Each point is the mean value from five or six individuals, and the SEM is depicted by vertical lines. Note that on the day of phenobarbital injection serum LH was extremely variable but that the next day a typical surge occurred.

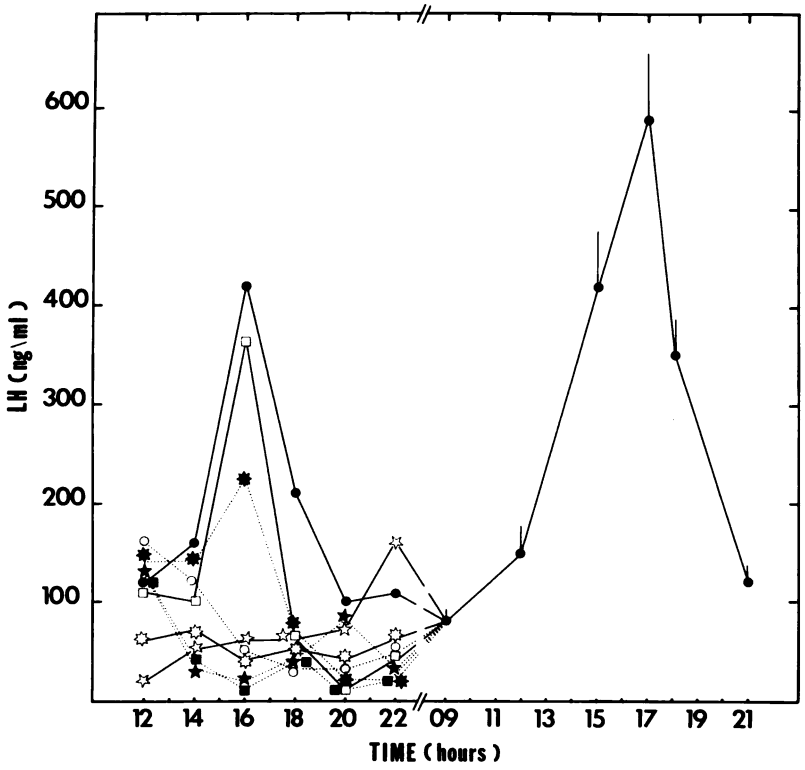

FIG. 4. Serum LH in ovariectomized hamsters injected with saline (solid circles) or with phenobarbital at 1100 (open stars), 1200 (open asterisks), 1300 (solid stars), 1400 (solid squares), 1500 (open circles), 1600 (solid asterisks), or 1700 (open squares) h. Each point between 1200 and 2200 h of the injection day represents the mean LH value from six animals. SEM bars are omitted for clarity. Serum levels in all groups sampled the next day (0900–2100 h) were not statistically different so the data have been pooled for ease of presentation. Therefore each point represents the mean value of 48 animals; vertical lines represent SEM.

that 1) phenobarbital is capable of blocking the daily surge of LH in ovariectomized hamsters for 24 h; 2) the LH surge in ovariectomized animals occurs at the same time of day as that in intact animals, and the timing of this surge is not altered following recovery from barbiturate block; and 3) the effective injection time for successful barbiturate block appears to be longer in ovariectomized females than in intact females.

The first two findings noted above are strong indications that the daily surge of LH in ovariectomized hamsters is timed by a 24 h neural clock. Furthermore, since the response of ovariectomized animals to phenobarbital is similar to that of intact animals, the same oscillator may be involved in determining the timing of peak LH release in intact and in castrated animals. A recent study by Kawakami et al. (1980), gives strong evidence that this neural oscillator, at least in rats, is the SCN. These investigators reported that the daily cyclic release of prolactin, LH, and FSH, which is induced by estradiol administration in ovariectomized rats, was abolished by lesions which destroyed the SCN. Taken along with the finding that SCN lesions abolish estrous cyclicity in intact rats, the data of Kawakami et al. (1980) implicate the SCN as the site of neural regulation of daily cyclic gonadotropin surges.

Our finding that the effective injection time for phenobarbital block of LH surges is not identical in ovariectomized and intact animals may indicate that the sensitivity to phenobarbital is perhaps modified by the presence or absence of ovarian steroids. In intact animals, the effectiveness of phenobarbital administered after 1430 h is much diminished; at a dose of 65 mg/kg, phenobarbital injected between 1330 and 1430 h prevented ovulation in 75% of the animals, while by 1515 h this value declined to 37.5%, and phenobarbital given at 1600 h was

totally ineffective in preventing ovulation (Stetson and Watson-Whitmyre, 1977). In the present study, using a dosage of 100 mg/kg, which, in our hands, is 100% effective in blocking ovulation if administered between 1330 and 1430 h, we found that injections at 1500 h were fully effective and injections at 1600 h were partially effective in suppressing the LH peak in castrates. The extension of the effective injection time may be due to the absence of steroid feedback or of the influence of some other substance of ovarian origin. The exact mechanism and site of action of phenobarbital in blocking the LH surge is not known.

The evidence from this study and from previous work implies that a neural oscillator, or clock, capable of timing daily gonadotropin surges, operates in intact and in ovariectomized hamsters. It appears to emit a daily signal which results, in ovariectomized (this study), lactating (Bridges and Goldman, 1975; DiPinto and Stetson, 1978), prepubertal (Smith and Stetson, 1980) and photoperiod-induced anovulatory (Seegal and Goldman, 1975) hamsters, in an FSH and/or LH surge within a prescribed time period or "gate." If the surge does not occur within the gated period it is delayed until the clock emits its next signal, the following day. The gate corresponds in time and definition to Blake's "potential activation period" (Blake, 1974). Studies similar to those of Blake (1974) must be done in hamsters before we can determine the precise dimensions of the gate. From the data reported here we know it is of similar duration to that in the rat, extending in ovariectomized hamsters from 1500 to at least 2200 h (Fig. 4).

Daily surges of LH and FSH appear to be the rule rather than the exception in the intact female hamster. Why then do ovulatory females exhibit gonadotropin surges only 1 in 4 days? In Stetson et al. (1978) we showed that exogenous estradiol (E_2) administered to short-term castrates from constant-release implants enhanced the magnitude of the following daily LH surge and depressed the magnitudes of the surges on subsequent days. These results differ from those observed after injection of E_2 in chronic castrates at 0900 h in which there appears to be an immediate elevation of serum LH followed by a rapid decline in serum levels (Vomachka and Greenwald, 1980). As no samples were taken in late afternoon, we do not know whether injections of E_2 are as effective as implants in enhancing the LH surge, but their results suggest that E_2 has an immediate effect on LH release that is probably independent of the circadian system. As we failed to examine serum LH in castrates immediately after receipt of an E_2 Silastic capsule (Stetson et al., 1978), we have no indication of the immediate effect of this type of procedure on LH release.

Presumably, circulating ovarian E_2 and progesterone (Norman et al., 1973) as well as other factors interact with neural control mechanisms to produce the characteristic 4-day estrous cycle of hamsters. A substance of follicular origin has been shown to depress FSH release in rats (Campbell and Schwartz, 1979; DePaulo et al., 1979; Marder et al., 1977). The concentration of this substance, or one(s) of similar function, in the ovaries of ovulatory hamsters changes throughout the estrous cycle (Chappel, 1979), being greatest on the afternoon of proestrus. Its role, if any, in the generation of estrous cyclicity remains to be defined. In our view, the neural oscillator (SCN) in the ovulatory female provides a daily signal which would result in a daily surge of LH and FSH were it not for the inhibiting effects of ovarian substances. Inhibition is manifest every day but the day of proestrus. That other rhythmic phenomena also timed by the SCN are not affected during the estrous cycle or after ovariectomy suggests that the site of inhibition is not the oscillator but rather some other level of the control system, perhaps the medial basal hypothalamus or the medial preoptic region.

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