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Pulsatile Plasma LH Rhythms in Normal and Androgen-Sterilized Ovariectomized Rats: Effects of Estrogen Treatment (37902)

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(Introduced by S. E. Greisman)
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While the effects of estrogen on adenohypophyseal luteinizing hormone (LH) synthesis and release have been extensively evaluated, only recently have some of the sex steroid feedback actions on the dynamics of LH secretion become known. In rats (1), rhesus monkeys (2), and ewes (10), it has been demonstrated that ovariectomy results in elevated plasma LH levels which are due in large measure to pulsatile discharges of gonadotropin from the pituitary. Furthermore, in the primate, such pulsatile discharges can be blocked by single intravenous injections of estradiol 17-β and such treatment also results in depressed plasma LH concentrations (3).

In a previous study we examined the changes in pituitary and plasma LH concentrations produced by a single injection of estrogen in ovariectomized normal and androgen-sterilized rats employing the ovarian ascorbic acid depletion assay method (OAAD) (4). In androgen-sterilized rats, high normal plasma LH concentrations were reported which decreased after ovariectomy and thereafter remained unaltered through a wide range of estrogen dosages, although pituitary concentration increased markedly. These studies further suggested that it requires approximately 5× the estrogen dosage to alter plasma LH in the sterile as compared to the normal rat (4).

On reevaluation of these LH changes by use of the radioimmunoassay technique (RIA), it immediately became apparent that the high plasma LH concentration reported for the sterile rat using the OAAD method was not consistent with the low plasma LH concentration obtained on radioimmunoassay. Consequently, in the current investigation, we have reevaluated the alterations produced by ovariectomy in the normal and androgen-sterilized rat employing RIA methods. Further, as a consequence of the episodic LH release observed in rats (1), plasma LH concentrations in sequential blood samples obtained prior to and following estrogen replacement were analyzed. Such studies should provide greater insight into the differences which may exist in the tonic release of LH in the normal vs the sterile rat.

Materials and Methods. Normal rats. Adult Sprague–Dawley rats were purchased and maintained in a temperature- and light-controlled room (14 hr light–10 hr dark) for 2 weeks prior to use. Only those animals which had demonstrated cyclic vaginal behavior were studied.

Androgen-sterilized rats (ASR). As previously described (5), single sc injections of 1.25 mg testosterone propionate in oil were administered to 5-day-old Sprague–Dawley female rats obtained from litters within our breeding colony. This treatment produces sterility in 99.9% of the treated animals. At 120 days of age, vaginal lavages were taken and all rats exhibited persistent vaginal cornification. These animals were used for experimentation between 130 and 150 days of age.

Surgical manipulation. Ovariectomy was performed under ether anesthesia during the

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morning hours in 10 normal cycling rats and 9 ASR. After gonad removal from the ASR, the ovaries were examined macroscopically to insure absence of corpora lutea. On Day 20 postsurgery, all animals were anesthetized with ether during the morning hours and a polyethylene cannula was inserted into the external jugular vein. Beginning at approximately 1300 hr, serial 0.3-ml blood samples were collected every 10 min for a total of 90 min. The animals then were sacrificed and the anterior pituitaries were removed, weighed, and flash frozen.

Estrogen-treated groups. Two groups of 6 normal ovariectomized rats received single injections of either 0.1 or 1.0 μg estradiol benzoate (EB) in oil sc/100 g body wt on Day 17 and blood was sequentially collected via a jugular cannula throughout the afternoon on Day 20 postovariectomy. Similarly, two groups of ASR (n = 6–7) were injected with either 0.1 or 1.0 μg EB/100 g body wt on Day 17 and sequential blood samples were taken at 15-min intervals for a total of 120 min during the afternoon hours on Day 20 postcastration. Following the last blood collection, all animals were sacrificed and the pituitaries were collected. Plasma and pituitary LH concentrations were determined by radioimmunoassay according to the ovine:ovine procedure of Niswender *et al.* (6) using the sheep antigen (LER-1056-C2) generously supplied by Dr. Leo Reichert, Jr., and the ovine LH antibody (GDN-15) obtained from Dr. Gordon Niswender. A crude rat pituitary extract prepared against a B160 standard supplied to us by Dr. V. L. Gay was used as standard reference material; this preparation has been shown to have a LH potency of 0.17 × NIH-LH-S1. The Student's t test was employed to evaluate significant differences between groups.

Results. Changes in LH concentration in sequential plasma samples. Following jugular vein cannulation on Day 20 postovariectomy, sequential blood samples were obtained at 10-min intervals for a total of 90 min. Shown in Fig. 1 are the variations in plasma LH concentration in 3 representative normal (nonandrogenized) castrates.

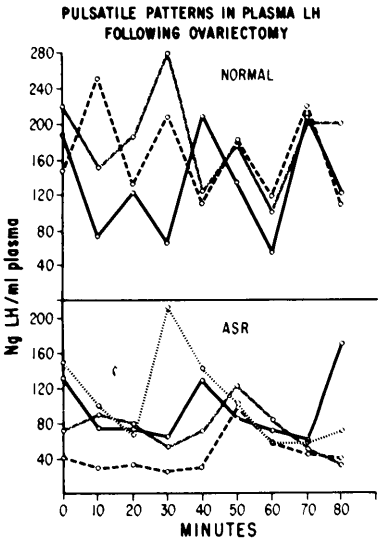

FIG. 1. Sequential plasma LH concentrations in three representative individual normal rats (above) and four representative individual androgen-sterilized rats (below) castrated 20 days earlier.

Typically there was a rapid rise in plasma LH followed by a decrease at a rate approximating the 20–30-min plasma half-life of LH. If each collection point was averaged for individual rats, plasma LH seemed to be oscillating around a 150 ng/ml point composed of rapid episodic release of LH followed by an exponential disappearance of this hormone from circulation. In comparison, the androgen-sterilized rats (ASR) exhibited more bizarre patterns of LH release in which each rat had a rhythm, but it was slower, averaging 40–60 min between peaks (Fig. 1). Again, if each point was averaged for individual rats, plasma LH was elevated but only to 75 ng/ml.

Effect of estrogen on plasma LH concentration and pulsatile rhythms. The temporal patterns and concentrations of LH were examined 72 hr following the injection of 0.1 or 1.0 μg EB. While both dosages decreased plasma LH concentration, the pulsatile rhythm in plasma LH was still discernible (Fig. 2). The principle difference in the response was that the amplitude of the pulses was markedly reduced in the 1.0 vs the 0.1 μg EB-treated group and the resultant effect was a greater reduction

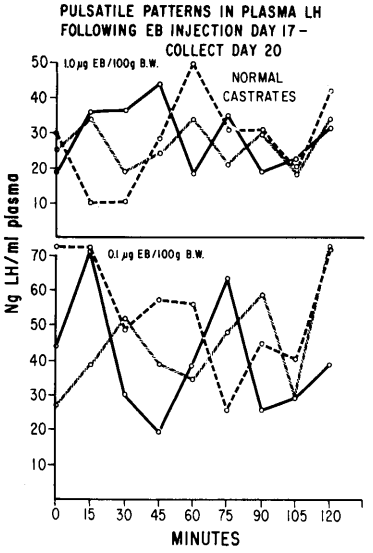

FIG. 2. Sequential plasma LH concentrations in representative normal castrated rats treated with either 0.1 μg EB (below) or 1.0 μg EB (above).

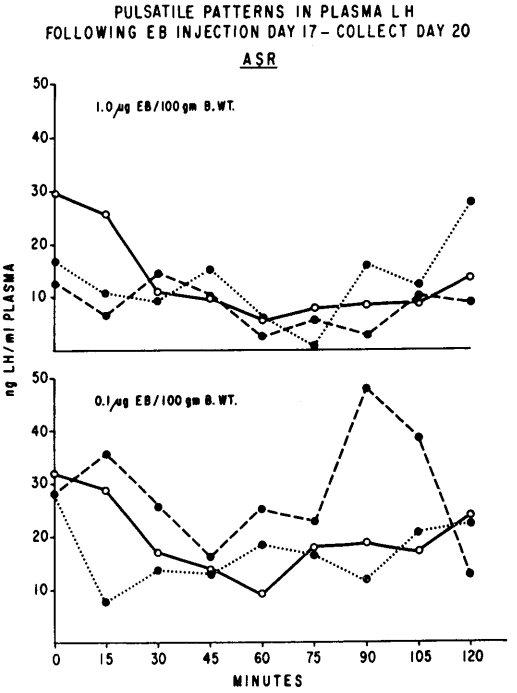

FIG. 3. Sequential plasma LH concentrations in representative androgen-sterilized castrated rats treated with either 0.1 μg EB (below) or 1.0 μg EB (above).

in plasma LH concentration in this former group.

The slower LH rhythm seen in castrated androgen-sterilized rats was still present following a single injection of 0.1 μg EB, but as with the normal castrates, pulse height was reduced, resulting in an overall reduction in plasma LH concentration as seen in the three representative ASR in Fig. 3. Increasing the dosage of EB to 1.0 μg further reduced plasma LH concentration and pulse height was so reduced that a pulsatile rhythm was not apparent (Fig. 3).

Effect of ovariectomy and EB treatment on pituitary LH concentration. Examination of pituitary LH concentration in normal animals following estrogen therapy (Fig. 4) revealed changes previously noted employing the OAAD method of LH analysis. Treatment of ovariectomized rats 17 days postovariectomy with either 0.1 or 1.0 μg EB resulted 72 hr later in a progressive depression in plasma LH and a concomitant significant increase ($P < 0.02$) in pituitary LH concentration. However, pituitary LH was not significantly greater in the 1.0 vs 0.1 μg EB-treated rats although plasma LH was further depressed.

In contrast to the normal rats, with increasing dosages of EB, no increase in pituitary LH was observed in the androgen-sterilized animals even though a progressive decrease in plasma LH occurred. Conversely, at the 1.0 μg dosage pituitary LH concentration was significantly less than the ASR ovariectomized control ($P < .02$).

Discussion. The results of the present studies support the observations of Gay and Sheth (1), Atkinson *et al.* (2), and Butler *et al.* (10) in rats, primates, and ewes that gonadal removal results not only in elevated concentrations of plasma LH but also in rhythmic patterns of release. Gay and Sheth (1) have demonstrated that plasma LH concentration can increase as much as 200 ng/ml during a 5-min interval and decrease at a rate approximating the 20–30-min half-life for LH in proestrous and castrated rats. In the present study, blood sampling was not performed at such frequent intervals (2–3 min), and, consequently, the rapidity of rise of LH could

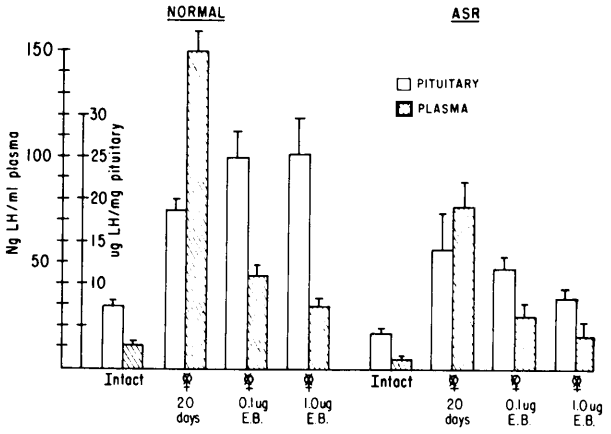

FIG. 4. Pituitary and plasma LH concentrations in normal and androgen-sterilized rats following ovariectomy and estradiol benzoate replacement therapy.

not be discerned. However, in normal (nonandrogenized) ovariectomized rats, we observed that the rise from nadir to peak and return to baseline required approximately 30 min, a time period similar to that previously reported (1). When all collection points were averaged for each rat, a value was obtained which did not vary from animal to animal within the normal ovariectomized group.

In the ASR group, each rat exhibited a rhythm but most all were of a slower 40–60-min type. In previous studies, we reported that plasma LH concentration in sterile rats was high and decreased by approximately one-half upon ovariectomy (4). The data obtained by RIA did not confirm these earlier OAAD assay observations. Rather, plasma LH concentration was within the range of a normal diestrous rat. Apparently, in the earlier assays a nonspecific depletion of ascorbic acid occurred which was unrelated to plasma LH concentration.

While the neuroendocrine correlates of the pulsatile rhythms of LH release are not known, seemingly such patterns of pituitary discharge in the absence of the negative feedback action of estrogen could be an intrinsic function of the tonic arcuate–median eminence complex. This hypothesis is suggested by the observations that both the ASR and male rat exhibit LH pulsatile rhythms after castration, and, in both, the cyclic preoptic area (POA) is nonfunctional (5). Further, Taleisnik *et al.* (7) have investigated the changes which occur in plasma LH following ovariectomy of normal rats and animals in which the cyclic POA has been surgically separated from the tonic tubero-infundibular region. The same increase in plasma LH occurred in both preparations.

Yamaji *et al.* (3) have reported that single iv injections of estradiol 17-β arrest within minutes the pulsatile discharges of LH observed in the ovariectomized monkey. In the present study, the more prolonged effects (72 hr) of EB on plasma LH concentrations and rhythmic release patterns did not permit a comparable analysis. Yet it is apparent from these studies that increasing dosages of EB resulted in a reduction in plasma concentration without appreciably altering the pulsatile release patterns in normal ovariectomized rats. The prominent change observed by such treatment was the marked reduction in the amplitude of the pulses. However, such treatment also results in an increase in pituitary LH concentration, suggesting that LH synthesis is not impaired.

In contrast, the sterile rat appears more rather than less sensitive to estrogen when compared to the normal; not only were plasma and pituitary concentrations depressed by low estrogen dosages, but the pulsatile rhythms in plasma LH also were

abolished. Since pharmacologic dosages of androgen were used to induce sterility, it is possible that not only the cyclic but the tonic gonadotropin controlling areas of the hypothalamus were deleteriously altered by early androgen treatment. However, the extent of damage produced as well as the specific sites and mechanisms by which estrogen exerts such actions remains unresolved.

In an interesting study, Bhattacharya *et al.* (8) demonstrated that α-adrenergic blocking drugs exerted effects identical to estradiol in interrupting the pulsatile rhythm of LH discharge. They suggest that a possible action of this steroid may be to depress or block the intermittent catecholaminergic signals thought to be necessary for the release of luteinizing hormone releasing factor. Consistent with this view are the observations of Schneider and McCann (9) who observed *in vitro* that dopamine enhanced LRF release and this effect could be blocked by estradiol. From the present studies, it is apparent that this need not be an all-or-none effect, but is dependent upon the concentration of estrogen in circulation. Perhaps this is one mechanism by which the sex steroids modify the release patterns of the gonadotropins.

Summary. Plasma LH was determined in sequential venous samples obtained at 10-min intervals from rats which had been ovariectomized 20 days earlier. Normal (nonandrogenized) castrates exhibited pulsatile rhythms in plasma LH with a peak to peak time of approximately 30 min. Castrated androgen-sterilized rats (ASR) also exhibited pulsatile plasma LH rhythms, but most were of the 40–60-min peak to peak type. In normal rats, when either 0.1 or 1.0 μg estradiol benzoate (EB) was administered on Day 17 and sequential blood collections were obtained on Day 20 after ovariectomy, the pulsatile rhythm was still present; however, pulse amplitudes were reduced and the average plasma LH concentration for individual animals over a 2-hr collection period was progressively lowered as the EB dosage was increased. Pituitary LH concentration increased in these EB-treated normal castrates. Administration of either 0.1 or 1.0 μg EB to castrated ASR markedly decreased average plasma LH concentrations and, in the 1.0-μg EB group, completely abolished the pulsatile LH discharges. In contrast to normal castrates, pituitary LH concentration failed to increase following EB treatment and, in fact, decreased after 1.0-μg EB administration. These studies suggest that early androgen sterilization not only impairs the cyclic but also the tonic LH release mechanism as well.

1. Gay, V. L., and Sheth, N. A., Endocrinology **90**, 158 (1972).

2. Atkinson, L. E., Bhattacharya, A. N., Monroe, S. E., Dierschke, D. J., and Knobil, E., Endocrinology **87**, 847 (1970).

3. Yamaji, T., Dierschke, D. J., Bhattacharya, A. N., and Knobil, E., Endocrinology **90**, 771 (1972).

4. Barraclough, C. A., and Haller, E. W., Endocrinology **86**, 542 (1970).

5. Barraclough, C. A., Recent Progr. Hormone Res. **23**, 503 (1966).

6. Niswender, G. D., Midgley, A. R., Monroe, S. E., and Reichert, L. E., Proc. Soc. Exp. Biol. Med. **128**, 807 (1968).

7. Taleisnik, S., Velasco, M. E., and Astrada, J. J., J. Endocrinol. **46**, 1 (1970).

8. Bhattacharya, A. N., Dierschke, D. J., Yamaji, T., and Knobil, E., Endocrinology **90**, 778 (1972).

9. Schneider, H. P. G., and McCann, S. M., Endocrinology **87**, 330 (1970).

10. Butler, W. R., Malven, P. V., Willett, L. B., and Bolt, D. J., Endocrinology **91**, 793 (1972).

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