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Adrenal Gland Involvement in Synchronizing the Preovulatory Release of LH in Rats[1] (38985)

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(Introduced by John J. Christian)

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From the studies of Wyman (1) and Kroc and Martin (2) it can be concluded that estrous cycle regularity in rats depends on normal adrenal cortical function. Following adrenalectomy, estrous cycles ceased, and this effect could be prevented by the administration of adrenal cortical extracts (3). However, these results could be attributed to the nonspecific effects of adrenalectomy since such animals when maintained on physiological saline exhibited normal cycles (4). More recently, Feder *et al.* (5) observed that adrenalectomy performed during metestrus or diestrus blocked the next expected ovulation in 50% of the animals, but they also noted that sham-surgery had a similar action. When adrenalectomized animals were maintained on physiological saline for 12–15 days, cycle length became less predictable and the "critical period" for the LH release was prolonged. On the other hand, stressful surgical procedures such as sham-ovariectomy or ovariectomy, if performed on the morning of proestrus can advance the release of LH by several hours (6). Presumably, such stress involves the secretion of adrenal steroids which, once released, facilitate LH discharge. Adrenal removal on proestrous morning seemed to prevent the release of the gonadotropin, at least in concentrations which could be measured by bioassay procedures. However, in such animals, complete ovulations had occurred by the following day (estrus) suggesting a lack of adequate sensitivity in the LH assay method employed in these studies.

The purpose of the present studies was to examine more critically the effects of adrenal gland secretions on the temporal patterns of LH released during the afternoon of proestrus in both acutely and chronically adrenalectomized rats, and to ascertain what effects the administration of adrenal cortical steroids would have in such preparations.

Materials and methods. Adult Sprague–Dawley female rats were maintained in a temperature and light controlled room (lights on 0400–1800 hr). Only those animals that had demonstrated at least two consecutive 4-day estrous cycles were used in the following studies.

Acute study. This investigation was an attempt to determine the effects of adrenalectomy on the morning of proestrus on the subsequent release of LH that afternoon. Animals were bilaterally adrenalectomized (ADX) under ether anesthesia between 0900–1000 hr. At this time a polyethylene cannula (OD, 0.038 in.) was inserted into the right external jugular vein and coursed caudally into the heart. Sequential blood samples (0.25 cc) were collected on an hourly basis beginning at 1300 hr and continuing until 1800 hr. Animals were sacrificed at 1200 hr (estrus) and the Fallopian tubes were then examined for presence of ova. Cannulated intact and sham-ADX animals served as controls.

Other groups of rats were bilaterally adrenalectomized and were given either 500 μg/100 g body wt of corticosterone (B) in steroid suspending vehicle (National Cancer Institute), or 2 mg progesterone (P) in oil or both immediately following surgery. All steroids were administered subcutaneously.

To determine whether or not the stress of cannulation and sequential blood sampling altered LH release and ovulation in ADX rats, a group of noncannulated-animals were adrenalectomized on proestrous morning and were sacrificed by decapitation at 1300 and 1700 hr that afternoon or on estrus. In addition, several ADX animals which had

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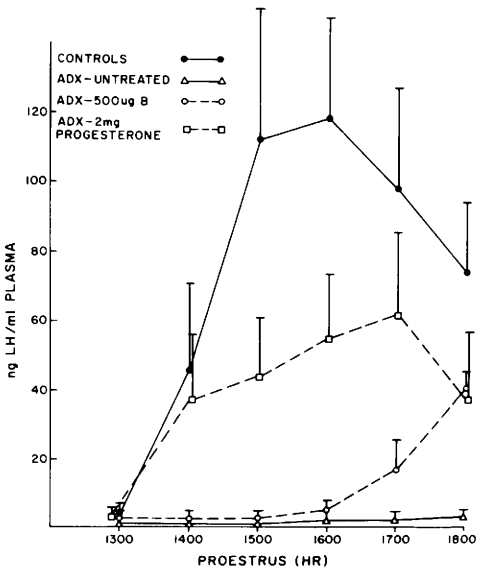

FIG. 1. Plasma LH concentrations on the afternoon of proestrus in rats adrenalectomized (ADX) between 0900 and 1000 hr on proestrous morning. The effects of the administration of 500 μg corticosterone (B)/100 g body wt and 2 mg progesterone (P) immediately following surgery on plasma LH levels in ADX is also illustrated. The number of animals represented by each group: Controls (n = 7); ADX-untreated (n = 8); ADX-B (n = 14); ADX-P (n = 15). Vertical lines represent standard error of mean.

been cannulated, but not sequentially bled, were killed at estrus and the Fallopian tubes were examined for the presence of ova.

Chronic study. Female rats were adrenalectomized and maintained on either 500 μg/100 g body wt of B daily (administered 1000 hr) or 0.9% saline in their drinking water. Vaginal histories were charted and on the third proestrus day following surgery animals were decapitated at 1000, 1300, 1400, 1500, 1600, 1700, and 1800 hr and blood samples were collected and measured for changes in concentrations of plasma LH and progesterone.

In all groups, plasma LH was determined by radioimmunoassay using the NIAMD rat LH kit. This kit contained the LH-RP-1 standard which has been shown to have a potency of 0.03 × NIH-LH-S1. All values obtained have been converted to the NIH-LH-S1 standard. Statistical analysis was performed using 6 × 6 factorial analysis of variance for repeated measures and the Neuman–Keuls test for *a posteriori* multiple comparisons (7). Plasma progesterone concentrations were determined by radioimmunoassay using an antibody prepared against progesterone-6-BSA and supplied by Dr. Gordon Niswender. The specificity of this antibody is such that progesterone measurements can be made without prior chromatography (8).

Results. Acute study. Figure 1 summarizes the plasma LH concentrations observed in animals sequentially bled from jugular catheters on proestrous afternoon. When the plasma LH concentrations were analyzed, it was found: (a) that there was an overall significant difference ($P < .001$) in plasma LH levels among groups; (b) that overall LH levels were significantly changing with time ($P < .001$); (c) that there are significant differences ($P < .001$) in the way plasma LH changes with time among the groups. The pattern of LH release observed in the cannulated control animals (intact and sham-ADX) was similar to that observed in untreated rats decapitated at various times on the afternoon of proestrus. Since no difference was noted between intact cannulated controls and sham-ADX controls, only the LH data for intact control animals appears in Fig. 1. Plasma LH levels rose significantly ($P < .001$) from a baseline at 1300 hr (2.5 ± 1.1 ng/ml) to a peak value at 1600 hr (119.5 ± 29.6 ng/ml) in the intact control animals. All of these rats were found to have ovulated (13.0 ± 0.5 ova/rat) when sacrificed on estrus. Conversely, seven of nine ADX rats sequentially bled on the afternoon of proestrus failed to show a plasma LH surge nor had they ovulated the next day. The remaining two animals ovulated, but peak plasma LH values observed on proestrus (1300–1800 hr) were only 20% of levels obtained for controls. Moreover, the rise in plasma LH was delayed by 3–4 hr in these animals. When ADX was followed immediately by the administration of 500 μg/100 g body weight of B the number of animals that had ovulated on estrus rose to 71% (10.9 ± 0.8 ova/rat). However, the temporal pattern of LH release was different in comparison to

that found in the controls, although a significant elevation of plasma LH ($P < .001$) was observed on the afternoon of proestrus. Plasma levels of LH were just beginning to rise at 1700 hr, whereas in control rats peak values had been obtained by 1600 hr and were beginning to fall by 1700 hr. A retarded discharge of pituitary LH was not observed in ADX animals which received 2 mg P either alone or in combination with B. Since comparable results were obtained with these two groups, their plasma LH data has been combined in Fig. 1. Although the time course of LH release in progesterone treated ADX rats was similar to that observed in control animals, peak plasma concentrations obtained were still well below those observed in the controls (62.8 ± 17.1 versus 119.5 ± 29.6 ng/ml). When animals were laparotomized on estrus, it was found that 83% of those receiving P had ovulated (11.5 ± 0.7 ova/rat).

Animals adrenalectomized on the morning of proestrus and then decapitated rather than sequentially bled during proestrous afternoon exhibited a significant rise ($P < .001$) in plasma LH levels between 1300 (0.74 ± 0.19 ng/ml) and 1700 hr (142.8 ± 22.2 ng/ml). Moreover, eight of nine animals treated in a similar fashion but sacrificed on estrus ovulated (12.4 ± 0.9 ova/rat). Two ADX rats which also were cannulated but not bled were found to have ovulated on estrus.

Chronic study. Figure 2 illustrates the effects of adrenal gland ablation on temporal pattern of plasma LH on the third proestrus following surgery. Analysis of variance (3 × 6 factorial) demonstrated that: (a) there was no overall difference in plasma LH concentrations among the three groups (intact control and the two ADX groups); (b) overall plasma LH levels changed with time ($P < .001$); (c) the temporal pattern of plasma LH was not the same in the three groups ($P < .001$). In other words, although there was no difference between controls and ADX rats in the absolute concentrations of LH achieved on the afternoon of proestrus, the time course of the pituitary discharge of the hormone was altered by ADX. Trend analysis (7) for the plasma LH concentrations demonstrated a significant fit of the LH data from control animals to a quadratic function ($P < .001$). Both groups of ADX animals failed to demonstrate this orderly progression of LH values. This suggests that adrenalectomy caused an asynchronous pituitary LH release. Despite this difference in the plasma LH pattern, ADX animals sacrificed on estrus had ovulated a normal complement of ova (10–14 ova). Although ADX did not significantly alter the cycle length (last cycle

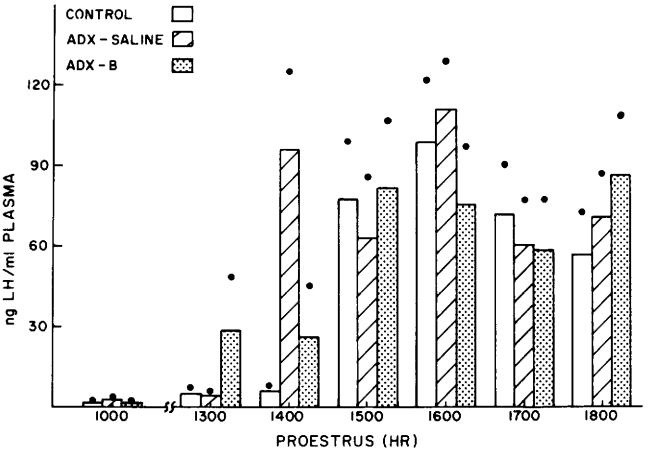

FIG. 2. Plasma LH concentrations on proestrus in adrenalectomized (ADX) rats maintained on 500 μg corticosterone (B)/100 g body wt or physiological saline. Blood samples were collected by decapitation on the third proestrus subsequent to surgery. Each bar graph represents the mean value of 7–12 rats. Dots above bar graphs represent standard error of mean.

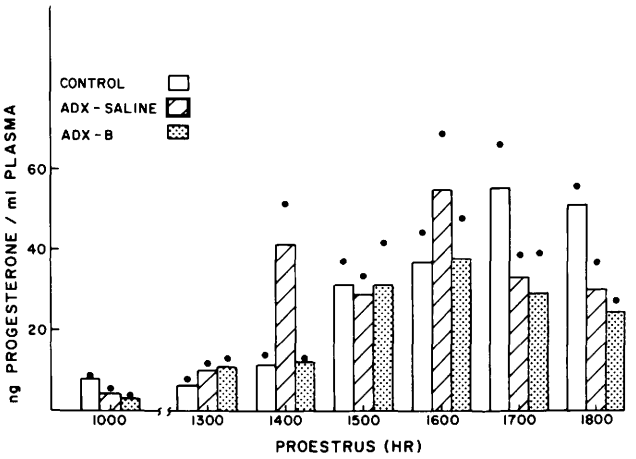

FIG. 3. Plasma progesterone concentrations on proestrus in adrenalectomized (ADX) rats maintained on 500 μg corticosterone (B)/100 g body wt or physiological saline. Blood samples were collected by decapitation on the third proestrus subsequent to surgery. Each bar graph represents the mean value of 7–12 rats. Dots above bar graphs represent standard error of mean.

prior to sacrifice), ADX rats maintained on B exhibited a tendency toward increased cycle length (4.92 $\pm$ 0.11 days) when compared to control rats in our colony (4.20 $\pm$ 0.12 days).

Figure 3 illustrates the effects of adrenal ablation on the preovulatory levels of plasma progesterone. Plasma progesterone changes closely paralleled those observed in LH. Although no significant overall differences were observed in progesterone among the three groups, plasma levels of this hormone did change with time ($P < .001$) and the temporal pattern of plasma steroid levels was not the same in the three groups ($P < .05$).

Discussion. The preovulatory release of LH in the rat is controlled by a neural mechanism that is subject to blockade by barbiturate administration. Activation of this regulatory system occurs during a precisely timed 2-hr interval on proestrous afternoon (9). This interval is referred to as the "critical period," and extends approximately from 1300–1500 hr in our colony of animals. The "critical period" for LH release is cued by the light-dark cycle, since advancing the onset of the light phase results in a corresponding shift in the "critical period" in both cycling (10) and estradiol-treated ovariectomized rats (11). In the present investigation, the temporal pattern of plasma LH on the afternoon of proestrus in chronically ADX animals was different from that observed in control rats maintained in the same environmental lighting. On the other hand, no overall differences in plasma concentrations of LH were observed between the ADX and control groups. These results suggest that although the amount of LH released is not altered by adrenalectomy, adrenal gland ablation did lead to an asynchronous discharge of LH. Adrenal steroids are known to influence the timing of the ovulatory secretion of gonadotropin. Surgical stress on the morning of proestrus results in an advancement of the surge of plasma LH by several hours; this effect can be abolished by adrenalectomy (6). Moreover, changes in the circadian rhythms of adrenal corticoid secretion induced by water deprivation lead to coincident changes in the LH surge and the "critical period" for LH release (12). Feder and his collaborators (5) have reported that the period of susceptibility to barbital blockade of ovulation was extended in ADX rats, but to the best of our knowledge the present study is the first to actually demonstrate an asynchronous LH discharge in proestrous ADX rats. Presumably the adrenal factor involved in this phenomenon is not corticosterone, because an asynchronous LH surge also was noted in ADX animals

maintained on this steroid. More likely the factor is adrenal progesterone. Shaikh and Shaikh (13) recently observed a rise in the adrenal vein concentrations of progesterone on proestrous afternoon which preceded the preovulatory surge of pituitary gonadotropins. Presumably this rise could be involved in facilitating pituitary LH release. Moreover, this investigator (14) has demonstrated that adrenal progesterone secretion also exhibits diurnal rhythmicity. It is likely that this rhythm is cued by the light–dark cycle as is the circadian rhythm for adrenal corticosterone secretion (13). Since synchronization of LH release is lost following adrenalectomy, then perhaps the photic stimulus does not directly synchronize this event but instead this effect is mediated via the circadian rhythm of adrenal progesterone.

The proestrous surge of plasma LH was associated with a sharp rise in circulating concentrations of progesterone. This response was comparable in both the control and ADX rats, indicating that the primary source was the ovaries. Since LH release on proestrus is so closely aligned with an increase in peripheral plasma progesterone concentrations, the asynchronous LH discharge in ADX rats was clearly reflected in the temporal pattern of this steroid.

Previously, Lawton (6) reported that a normal preovulatory surge of plasma LH could not be detected in animals ADX on proestrous morning. However, seven of nine ADX rats similarly treated but sacrificed the next day exhibited full ovulation. In the present investigation, animals ADX on the morning of proestrus and decapitated that afternoon, exhibited a significant rise in plasma LH between 1300 and 1700 hr. Moreover, the plasma levels obtained were comparable to those observed in decapitated controls. Perhaps the reason this study is at variance with the previous report is that our use of a radioimmunoassay rather than a bioassay method to measure plasma LH permitted us to detect smaller concentrations of this gonadotropin.

One of the more interesting findings in this study was that sequential blood sampling blocked the preovulatory release of LH and ovulation in seven of nine acutely ADX animals. Conversely, similar treatment of control animals had little or no effect on the plasma LH surge. This response of the ADX rats appears to be related to the inability of these animals to respond normally to the stress of bleeding; when these animals were given either an injection of B or P immediately following surgery the number of rats ovulating was markedly improved (71 and 83%, respectively). However, only the P-injected animals showed a plasma rise at the normal time. In the B-treated rats the plasma LH surge was delayed from 3 to 4 hr. We have reported previously that the administration of progesterone on the morning of proestrus (0930 hr) potentiated the LH response to exogenous estrogen in rats ovariectomized on diestrus Day 2 (15). More significantly, however, LH secretion appeared to be synchronized to the time of the progesterone injection. The present study again demonstrates the ability of progesterone to influence the timing of the preovulatory release of LH.

Summary. In this study we have demonstrated that acute adrenalectomy (1000 hr proestrus) has no effect on the release of LH on proestrous afternoon. However, chronic adrenalectomy results in the loss of some factor responsible for synchronizing the preovulatory LH surge. Since this investigator has shown previously (15) that progesterone can influence the timing of LH release in ovariectomized and ovariectomized–adrenalectomized animals, it is most likely that adrenal progesterone is involved in synchronizing this event.

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