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Adrenal Gland Rhythmicity and Pituitary Regulation of Adrenal Steroid Secretion (39953)

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Introduction. The adrenal glands of the female rat secrete corticosterone in a diurnal pattern resulting in peak plasma concentrations shortly after lights off and a nadir a few hours after lights on (1, 2). The amplitude of this rhythm varies during the estrous cycle. Plasma fluctuations of this hormone are greatest on proestrus when circulating levels of estrogen are at a maximum (1). A similar diurnal pattern of peripheral plasma concentrations of progesterone has been reported in the cycling female rat (3-5), although other investigators insist that such a rhythm does not exist (6, 7). One purpose of this study was to reexamine peripheral plasma levels of progesterone and corticosterone over a 24-hr interval using ovariectomized rats bearing Silastic implants of estradiol. This particular animal was utilized in order to eliminate the ovarian contribution of progesterone to the peripheral plasma pool. In these animals diurnal changes of plasma LH and prolactin were also examined.

It has recently been suggested that prolactin may play an integral role in the regulation of adrenal progesterone secretion (8). In order to further test this hypothesis, plasma concentrations of prolactin, corticosterone, and progesterone were examined in dexamethasone-blocked ovariectomized rats, following the iv administration of various pituitary hormones.

Materials and methods. Diurnal rhythm study. Sprague-Dawley female rats (50-55 days old) from our colony (lights on, 0400 to 1800 hr; LD) were bilaterally ovariectomized (Ovx) under ether anesthesia. Three weeks later, a Silastic implant of estradiol (E_2) was implanted sc in each animal according to the procedure of Legan *et al.* (9). Blood levels of E_2 are maintained at approximately 100 pg/ml for several weeks by these implants (2). Animals were sacrificed by rapid decapitation at 4-hr intervals on the fifth day following placement of the implant. All decapitations were carried out in a room adjacent to the animal vivarium within 2 min of entering the vivarium. No more than two animals were killed during any one decapitation period. Blood samples collected were assayed for progesterone (P) by a slightly modified radioimmunoassay procedure of Niswender (10). The competitive protein binding assay of Murphy (11) served to measure plasma corticosterone (B).

Plasma progesterone concentrations were measured without prior chromatography using a highly specific antibody prepared against BSA-6β-P and generously provided by Dr. G. D. Niswender. The specificity of this antibody was tested by Niswender (10), and has also been examined in our laboratory (12). The intra- and interassay coefficients of variation for P were 5.7 and 8.8%, respectively. The between-assay coefficient of variation for B was 14.2%, and the within-assay coefficient was 7.8%.

Plasma LH was assayed using the ovine:ovine procedure of Niswender *et al.* (13). All LH values are expressed in terms of the NIH-LH-S1 standard. The NIAMDD kit was utilized to measure plasma concentrations of prolactin. Values are expressed in terms of the RP-1 standard. In our hands the interassay and intraassay coefficients of variation have been 9.6 and 10.4% for LH, and 12.9 and 13.5% for prolactin. These analyses were based upon multiple replicates of different volumes of a plasma pool.

In a second study, female rats were maintained in constant illumination (LL) until they exhibited at least 10 consecutive days

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of vaginal estrus or proestrus. At that time each animal was ovariectomized and the identical experimental procedures were followed as described in the previous study.

Pituitary hormone regulation of adrenal steroid secretion. Female rats, which had been ovariectomized 3 weeks earlier at 50–55 days of age, were fitted with indwelling jugular catheters at 1000 hr. Twenty-four hours later, each rat received an ip injection of 25 μg of dexamethasone acetate/100 g body weight to block endogenous ACTH and prolactin secretion. This dosage of dexamethasone has been shown to totally suppress ACTH release for at least 12 hr (8), and we found it to be effective in blocking prolactin secretion which accompanies bleeding stress (Fig. 1). Four hours after dexamethasone was administered (1400 hr), a blood sample (0.50 ml) was taken from each animal (time 0). Immediately thereafter, the following pituitary hormones or combinations of hormones were administered iv: 5 mU of porcine ACTH (Sigma), 10 IU of ovine prolactin (NIH-S11, 26.4 IU/mg), 10 μg of ovine LH (NIH-S19, 1.01 IU/mg), 25 μg of FSH (NIH-S11, 1.15 IU/mg), or a combination of 5 mU of ACTH and 10 IU of prolactin. The described dosages were administered per 100 g body weight. Animals were bled (0.50 ml) 15, 30, and 60 min following hormone administration. Blood samples collected were utilized to measure P, B, and prolactin as previously described. The animals in this study were not pretreated with E_2.

Statistical significance in the first study (diurnal rhythm) was evaluated by a 2 × 6 (lighting conditions × time) factorial analysis of variance. In the second study (pituitary hormones) data were examined by a 7 × 4 (treatment × time) factorial analysis of variance for repeated measures. Once the significance of overall treatment was discerned, one-way analyses of variance were run on individual groups to determine whether plasma levels of progesterone and corticosterone changed with time within each group.

Results. Diurnal rhythm study. As illustrated in Fig. 2, in E_2-treated Ovx rats maintained in a 14 hr light:10 hr dark schedule both plasma B ($P < 0.001$) and P ($P < 0.001$) levels changed significantly with time over the 24-hr interval studied. These diurnal fluctuations in both steroids were characterized by troughs shortly after lights on (0600 hr) and peak concentrations at 2200 hr. Thus progesterone, like corticosterone, is secreted by the adrenal glands in a diurnal pattern.

Proestrous-like plasma LH ($P < 0.001$) and prolactin ($P < 0.001$) surges were observed in Ovx animals in a 14 hr light:10 hr dark schedule (Fig. 3). Plasma LH was sharply elevated at 1400 and 1800 hr, but by 2200 hr had returned to near-baseline levels. Plasma prolactin concentrations exhibited a comparable temporal pattern.

Constant light did not significantly alter overall plasma B levels (Fig. 4). However, the temporal pattern of hormone levels was

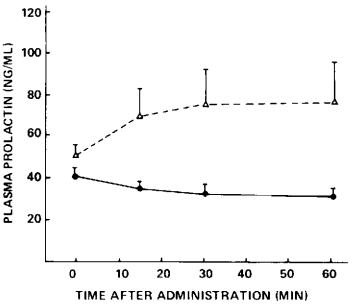

FIG. 1. Effect of bleeding stress on plasma prolactin levels in Ovx rats. The broken line represents untreated animals, the solid line rats which received dexamethasone 4 hr earlier. Each point represents the mean value of nine rats. Vertical lines above points represent standard error of mean.

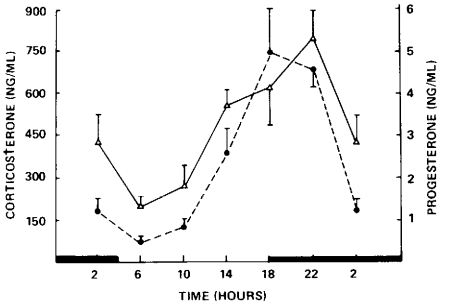

FIG. 2. Plasma corticosterone (broken line) and progesterone concentrations (solid line) in Ovx rats bearing E_2 implants and maintained in LD. Each point represents the mean plasma concentration of six decapitated rats. Vertical lines above points represent standard error of mean.

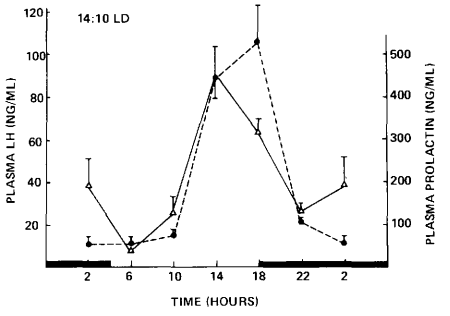

FIG. 3. Plasma LH (broken line) and prolactin (solid line) concentrations in Ovx rats bearing E_2 implants and maintained in LD. Each point represents the mean plasma concentration of six decapitated rats. Vertical lines above points represent standard error of mean.

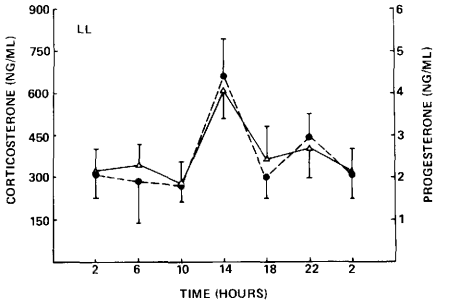

FIG. 4. Plasma corticosterone (broken line) and progesterone (solid line) concentrations in Ovx rats bearing E_2 implants and maintained in LL. Each point represents the mean plasma concentration of six decapitated rats. Vertical lines above points represent standard error of mean.

different ($P < 0.001$) than in a 14 hr light:10 hr dark schedule. Peak plasma B concentrations occurred earlier in constant light, and in these rats there was a significant daily variation in plasma B levels. Plasma P levels showed changes comparable to B in constant light-maintained rats, with one exception. P concentrations did not change significantly with time in these rats.

Constant illumination obliterated the afternoon surge of plasma LH in estrogen-treated Ovx rats (Fig. 5). Plasma concentrations of prolactin were less severely affected. Although there was a temporal shift in the onset of the peak, a plasma prolactin surge ($P < 0.01$) was observed.

Pituitary hormone regulation of adrenal steroid secretion. Dexamethasone administration significantly reduced ($P < 0.001$) plasma levels of prolactin, and blocked any rise in the secretion of this hormone that accompanies bleeding stress (Fig. 1).

Bleeding stress did not significantly elevate B in animals not receiving dexamethasone (Fig. 6). However, there was a tendency for B to rise with bleeding stress. Pretreatment with dexamethasone reduced initial concentrations and blocked the effects of bleeding on plasma levels of this steroid. ACTH administration but not prolactin stimulated a significant rise ($P <$

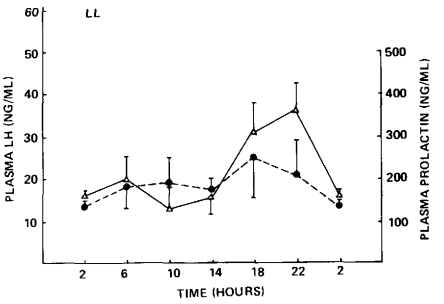

FIG. 5. Plasma LH (broken line) and prolactin (solid line) concentration in Ovx rats bearing E_2 implants and maintained in LL. Each point represents the mean plasma concentration from six decapitated rats. Vertical lines above points represent standard error of mean.

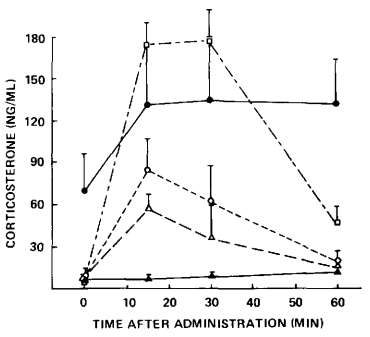

FIG. 6. Effect of administration (iv) of pituitary hormones on plasma corticosterone concentrations in dexamethasone-treated Ovx rats. Each point represents the mean value of at least six rats. Vertical lines above points represent standard error of mean. Solid circles = control rats receiving neither dexamethasone nor any pituitary hormones; solid triangles = dexamethasone-treated rats which received no pituitary hormones; open triangles = dexamethasone-treated rats which received prolactin; open circles = dexamethasone-treated rats which received ACTH; open squares = dexamethasone-treated rats which received both prolactin and ACTH.

0.05) in plasma B in dexamethasone-treated rats. B concentrations were elevated within 15 min of ACTH administration, but returned to near baseline values within 60 min. The simultaneous injection of ACTH and prolactin was far more effective than ACTH alone in stimulating B secretion. Peak concentrations attained were more than twofold greater ($P < 0.05$, by Student's t test) following administration of both hormones than in animals receiving only ACTH (173.9 ± 26.4 versus 84.3 ± 24.2 ng/ml). Neither FSH nor LH had any significant effect on plasma corticosterone levels.

Adrenal progesterone secretion was enhanced by bleeding stress ($P < 0.05$), but only in animals which were not treated earlier with dexamethasone (Fig. 7). The iv administration of prolactin stimulated a significant ($P < 0.001$) increase in adrenal progesterone secretion within 15 min in dexamethasone-treated animals. Conversely, ACTH did not significantly alter adrenal secretion of this hormone. This failure most likely resulted from the great degree of variability observed in this group of animals. The combination of ACTH and prolactin was highly effective ($P < 0.001$)

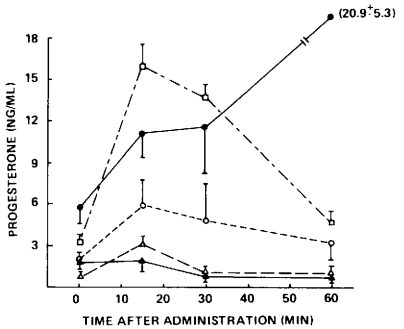

FIG. 7. Effect of administration (iv) of pituitary hormones on plasma progesterone concentrations in dexamethasone-treated rats. Each point represents the mean value of at least six rats. Vertical lines above points represent standard error of mean. Solid circles = control rats receiving neither dexamethasone nor any pituitary hormones; solid triangles = dexamethasone-treated rats which received no pituitary hormones; open triangles = dexamethasone-treated rats which received prolactin; open circles = dexamethasone-treated rats which received ACTH; open squares = dexamethasone-treated rats which received both prolactin and ACTH.

in inducing progesterone secretion. These two hormones synergized with one another to enhance plasma levels of P fivefold within 15 min of administration. Moreover, P concentrations were still elevated 30 min following their injection. LH and FSH had no effect on progesterone levels.

Discussion. Recent studies implicate a major role for prolactin in the control of adrenal progesterone secretion in the rat. Piva *et al.* (8) found that prolactin as well as ACTH stimulated a significant output of progesterone in dexamethasone-suppressed female castrates. In contrast to ACTH, however, prolactin had no effect on adrenal corticosterone secretion. The effects of a combination of ACTH and prolactin on adrenal steroid secretion were not examined. Acute stress stimulates the release of prolactin as well as ACTH in the rat (14, 15). Moreover, adrenalectomy 2 weeks earlier potentiated ether-induced prolactin secretion, whereas dexamethasone blocked its release (15). These results suggest that there is a strong interaction between adrenal corticoid secretion and pituitary prolactin in the rat. Our data in the present study support this hypothesis. Prolactin stimulated a significant release of adrenal progesterone in female castrates. Furthermore, prolactin synergized with the simultaneously administered ACTH to potentiate the adrenal progesterone response. These results suggest that both of these hormones play important regulatory roles in the control of adrenal progesterone secretion.

In confirmation of earlier results (8), prolactin administration alone did not stimulate significant secretion of corticosterone. Conversely, ACTH and ACTH plus prolactin stimulated significant rises in plasma B levels. The response of the adrenal glands to the combination of hormones was greater than to ACTH alone. These data also suggest a possible synergistic action of prolactin and ACTH in controlling adrenal gland steroidogenesis.

In the cycling rat, adrenal corticosterone secretion has been shown to be greater on proestrus than on other days of the cycle (1). It has been suggested that elevated estrogen levels may enhance adrenal activity by either increasing the secretion of pituitary ACTH or by potentiating adrenal sen-

sitivity to this pituitary hormone (1, 16). Our data suggest another possibility. On proestrus, the plasma prolactin surge which precedes LH release by several hours (6, 17) may be partially responsible for the enhanced secretion of adrenal steroids observed that afternoon.

It has been shown that adrenal B secretion exhibits regular daily fluctuations in the rat with a peak in late afternoon and a trough shortly after lights on (1, 2). However, whether or not adrenal progesterone secretion follows a similar pattern remains unclear. We believe that we have now shown conclusively that adrenal P exhibits a similar daily rhythm. Moreover, the similar effects of constant light on adrenal patterns of B and P secretion indicates that these rhythms are controlled by either the same regulatory mechanism or very similar ones.

As expected, ovariectomized rats bearing Silastic implants of E_2 and maintained in normal lighting showed afternoon surges of plasma LH and prolactin. Constant illumination abolished the LH rise, but not prolactin. However, the prolactin rise occurred approximately 4 hr later in constant light than in a 14 hr light:10 hr dark schedule. It is possible that the prolactin surge occured too late in constant light to influence the diurnal secretion of adrenal steroids and that ACTH alone was maintaining hormone levels. This may possibly explain why the diurnal fluctuations in B and P were less dramatic in constant light than in animals maintained in normal lighting. Further studies, however, are necessary to substantiate this theory.

We reported earlier (18) that the diurnal periodicity in the neurogenic trigger for LH release was dependent on the light–dark cycle for its expression. In the present study, the fact that an LH surge was not observed in constant light-maintained animals appears to bear out our earlier conclusion. Moreover, the presence of a plasma prolactin surge in these animals suggests that prolactin is less dependent on the light–dark cycle for its rhythmicity.

Summary. We have shown that prolactin most likely plays an important regulatory role in the control of adrenal gland steroidogenesis. Both adrenal progesterone and corticosterone secretion were strongly influenced by the administration of this hormone to Ovx rats. Our data also suggest that the proestrous surge of plasma prolactin may be partially responsible for the enhanced secretion of adrenal corticosterone observed on that day of the cycle.

Adrenal progesterone is secreted in a daily rhythm which is in phase with the corticosterone secretion in Ovx rats. Moreover, a comparable temporal shift in both of these rhythms in constant light animals suggests that these rhythms may be under control of the same regulatory system.

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