Changes in Peripheral Plasma Progesterone During the Rat 4-Day Estrous Cycle: An Adrenal Diurnal Rhythm[1] (37213)

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Since the pioneering studies of Everett (1) it has been recognized that the sex steroids are intimately involved in the ovulatory surge of the gonadotropins. When administered at appropriate times during the cycle, either estrogen or progesterone will advance ovulation and LH release (1–6). Furthermore, the initiation of the proestrous LH surge can be prevented in rats by the administration of antisera to either estrogen (7–8) or progesterone (7). Such types of studies suggest that a rise in the sex steroids precedes the proestrous LH surge. For example, Shaikha (9) observed that the plasma estrogens peak at approximately 1000–1200 hr proestrus and reach nadir prior to the LH surge. Similar changes have been reported in ovarian secretion rates of 20-α-hydroxypregn-4-en-3-one (10, 11). While Barraclough *et al.* (12) were unable to demonstrate an increased ovarian secretion rate of progesterone prior to the LH surge, significantly elevated peripheral plasma concentrations of this steroid were noted between 1000 and 1400 hr proestrus. Feder *et al.* (13) have shown that the adrenal glands contribute substantially to the peripheral progesterone plasma pool. Twenty-five days following ovariectomy, this steroid was still measurable in plasma and only after adrenalectomy of such animals did plasma progesterone disappear. Seemingly, the adrenal secretion of progesterone is enhanced on the morning of proestrus and acting synergistically with estrogen may play an important role in the subsequent proestrous LH surge.

The present study evaluated the changes which occurred throughout the rat estrous cycle in peripheral plasma concentration of progesterone, the adrenal contribution to the steroid pool and also correlated the observable changes with the proestrous LH surge.

Materials and Methods. Sprague–Dawley female rats were purchased and maintained in a temperature and light controlled room (14 hr light, 10 hr dark) for two weeks prior to experimentation. Only those animals which had demonstrated at least two consecutive 4-day estrous cycles were studied.

Groups of 6–9 animals were Nembutalized (30 mg/kg body wt.) at appropriate times throughout the estrous cycle, and the left ovarian vein was cannulated as described previously (14). After a 10-min ovarian vein blood sample was collected, a peripheral sample was obtained from the abdominal aorta.

Peripheral and ovarian plasma samples were extracted twice with 10 volumes of diethyl ether. A celite column was used for separation of the progesterone according to the procedure of Abraham *et al.* (15) and the radioimmunoassay technique was utilized for quantitation of the steroid (16).

Ovariectomy and/or adrenalectomy was performed under ether anesthesia. Statistical evaluations were performed by the Student *t* test.

Results. In initial studies, ovariectomy and/or adrenalectomy was performed on diestrous day 2 at 0100 hr, and peripheral concentrations and/or ovarian secretion rates of progesterone were measured 24 hr later (0100 hr proestrus). As shown in Fig. 1, ovariectomy alone reduced peripheral plasma progesterone by only 59% in 24 hr, whereas adrenalectomy resulted in a 75% decrease in peripheral plasma concentration without

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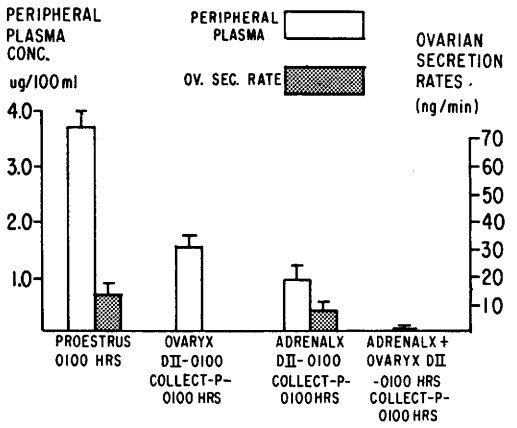

FIG. 1. Peripheral plasma concentrations and ovarian secretion rates of progesterone in rats 24 hr after adrenalectomy (adrenalx) and/or ovariectomy (ovaryx). The surgery was done at 0100 hr diestrous day 2 and the collections made 0100 hr proestrus. Vertical lines represent standard errors. P, proestrus; DII, diestrous day 2.

altering ovarian secretion rates. Removal of both adrenals and ovaries resulted in an almost complete disappearance of progesterone from the peripheral plasma pool.

In Fig. 2 are illustrated the temporal changes which exist in peripheral plasma concentrations and ovarian secretion rates of progesterone during the estrous cycle. Peripheral plasma concentrations of progesterone increased significantly ($p < 0.01$) between 1400 hr diestrous day 2 (2.13 ± 0.34 μg/100 ml) and 0300 hr proestrus (3.78 ± 0.24), even though ovarian secretion rates of the steroid remained unchanged during that period. Plasma concentrations of progesterone then fell to a nadir by 1000 hr. Significantly, however, by 1300 hr proestrous values were again elevated ($p < 0.05$). In our colony the critical period extends from 1300 to 1500 hr so that the changes observed in plasma progesterone occurred before LH release. Ovarian secretion rates during the same interval were not altered.

Associated with the period from 1300 to 1700 hr proestrus we observed a twofold rise in peripheral plasma levels of progesterone ($p < 0.01$) while ovarian secretion rates were enhanced tenfold ($p < 0.01$). This time period corresponds to maximum increase in plasma LH in our animals.

During estrus, peripheral plasma concentrations of progesterone remained elevated between 0100 and 0500 hr and then fell to a reduced level by 1000 hr ($p < 0.025$). On the other hand, ovarian secretion rates dropped from a high of 113.5 ng/min at 1700 hr proestrus to near basal levels of 20.7 ng/min at 0100 hr estrus.

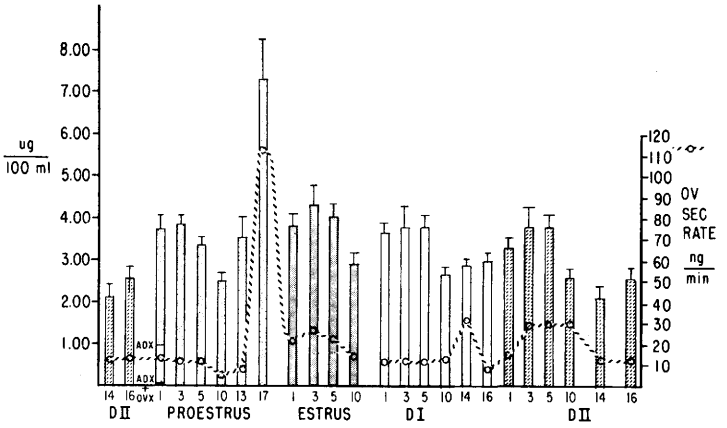

FIG. 2. Peripheral plasma concentrations (μg/100 ml) and ovarian secretion rates (ng/min) of progesterone during the 4-day estrous cycle of rats. At 0100 hr proestrus a comparison is made of intact, adrenalectomized (adx), and adrenalectomized–ovariectomized (ovx) animals (solid portion of bar). Bar graphs indicate peripheral plasma concentrations, and ovarian secretion rates are designated by the striped line. Standard errors are represented by vertical lines. DI, diestrous day 1; DII, diestrous day 2.

The changes observed in the peripheral plasma concentration of progesterone during proestrus and estrus was also present during the two days of diestrus. Plasma values rose from a low (2.89 ± 0.32 μg/100 ml) at 1000 hr estrus to a peak (3.81 ± 0.49 μg/100 ml) at 0300 hr diestrous day 1 ($p < 0.1$). The rhythm was the same the following day rising from a reduced concentration at 1000 hr diestrous day 1 to an elevated level early (0300 hr) on diestrous day 2 ($p < 0.01$) and then decreasing to minimum by 1400 hr that same day. Although ovarian secretion rates remained essentially unchanged during this period, a small increase in the ovarian secretion rate was observed during the afternoon of diestrous day 1 ($p < 0.01$) and during the morning of diestrous day 2 ($p < 0.05$) when compared to the 1000 hr diestrous day 1 value.

Discussion. In confirmation of earlier observations by Feder *et al.* (13) the adrenal glands are a significant source of circulating progesterone and only after removal of both gonads and adrenal glands was plasma progesterone reduced to insignificant levels.

From these studies it also is apparent that a diurnal rhythm exists in the peripheral plasma concentrations of progesterone during the 4-day estrous cycle of rats. Plasma levels of this steroid are at a nadir between 1000 and 1400 hr and then rise to a peak during the early morning hours (0100–0500) on each day of the cycle. Only between proestrus and estrus is this rhythm somewhat masked by the increased ovarian progesterone secretion rate in response to LH release. Except for the changes observed on the afternoon of proestrus, ovarian secretion rates of this steroid remained near a basal level throughout the cycle. Therefore, the conclusion is that the adrenal progesterone contribution was responsible for this rhythm. Critchlow *et al.* (17) demonstrated that a similar diurnal pattern in corticosterone secretion exists in both male and female rats. However, in their studies, plasma corticosterone values were high during the afternoon hours and were reduced in the morning. This reciprocal relationship between progesterone and corticosterone secretion by the adrenal gland may be related to the use of progesterone as a precursor for corticosterone synthesis. More importantly, it may be a mechanism by which increased plasma concentrations of progesterone are made available to synergize with estrogen in altering thresholds of excitability within the CNS prior to the proestrous surge of LH. Resko (18) has demonstrated that adrenal progesterone secretion is under ACTH control. Furthermore, stress-induced ACTH release not only results in increased adrenal corticosterone but in increased progesterone secretion as well (19). Since ACTH secretion also follows a diurnal rhythm which is synchronized by the light–dark cycle (17), a similar pattern of adrenal progesterone might be anticipated.

In a recent investigation (7) estrogen but not progesterone antisera blocked ovulation in female rats when administered at 1000 hr diestrous day 2. However, injection of the same progesterone antisera as late as 1700 hr that day blocked the expected ovulation in 4 of 5 animals. Our study offers an explanation for this observation: The 1000 hr diestrous day 2 treatment may have been too early to neutralize increasing plasma levels of progesterone which occur between 0100 and 0500 hr proestrus, whereas later administration was effective.

Summary. A diurnal rhythm exists in peripheral plasma progesterone concentrations during the 4-day estrous cycle of rats. Peak values in this rhythm were obtained during the early morning hours (0100–0500), whereas in the afternoon plasma concentrations of the steroid were at a nadir. Ovarian progesterone secretion rates within this same period remained unaltered, suggesting that the adrenal glands were responsible for these fluctuations.

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1. Everett, J. W., Endocrinology 43, 389 (1948).
2. Brown-Grant, K., J. Endocrinol. 43, 539 (1969).
3. Brown-Grant, K., J. Endocrinol. 43, 553 (1969).
4. Uchida, K., Kadowaki, M., and Miyake, T., Endocrinol. Japon. 17, 99 (1970).
5. Ying, S. Y., Fang, V. S., and Greep, R. O., Proc. Soc. Exp. Biol. Med. 139, 738 (1972).
6. Ying, S. Y., and Greep, R. O., Proc. Soc. Exp.

Biol. Med. **139**, 741 (1972).

7. Ferin, M., Tempone, A., Zimmering, P. E., and Vande Wiele, R. L., Endocrinology **85**, 1070 (1969).

8. Niell, J. D., Freeman, M. E., and Tillson, S. A., Endocrinology **89**, 1448 (1971).

9. Shaikha, A. A., Biol. Reprod. **5**, 297 (1971).

10. Goldman, B. D., Kamberi, I. A., Siiteri, P. K., and Porter, J. C., Endocrinology **85**, 1137 (1969).

11. Piacsek, B. E., Schneider, T. C., and Gay, V. L., Endocrinology **89**, 39 (1971).

12. Barraclough, C. A., Collu, R., Massa, R., and Martini, L., Endocrinology **88**, 1437 (1971).

13. Feder, H. H., Resko, J. A., and Goy, R. W., J. Endocrinol. **41**, 563 (1968).

14. Siiteri, P. K., Tippit, P., Yates, C., Jr., and Porter, J. C., Endocrinology **82**, 837 (1968).

15. Abraham, G. E., Tulchinsky, D., and Korenman, S. G., Biochem. Med. **3**, 365 (1970).

16. Abraham, G. E., Swerdloff, R., Tulchinsky, D., and Odell, W. D., J. Clin. Endocrinol. Metab. **32**, 619 (1971).

17. Critchlow, V., Liebelt, R. A., Bar-Sela, M., Mountcastle, W., and Lipscomb, H. S., Amer. J. Physiol. **205**, 807 (1963).

18. Resko, J. A., Science **164**, 70 (1968).

19. Fajer, A. B., Holzbauer, M., and Newport, H. M., J. Physiol. **214**, 115 (1971).

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