PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 216–221 (1982)

Serum Testosterone Concentrations during the 4-Day Estrous Cycle in Normal and Adrenalectomized Rats[1] (41334)

MICHAEL E. RUSH[2] AND CHARLES A. BLAKE[3]

Department of Anatomy, University of Nebraska Medical Center, Omaha, Nebraska 68105

Abstract. We investigated the role of the adrenal glands in the maintenance of serum testosterone concentrations during the 4-day estrous cycle in the rat. Animals were untreated, adrenalectomized (ADX), or sham-ADX. The operations did not alter with the length of the estrous cycle. Three to five estrous cycles after surgery, we decapitated rats for the collection of trunk blood and measured the FSH, LH, and testosterone in the serum by radioimmunoassays. Serum FSH and LH concentrations were similar in the three groups of rats during the estrous cycle except at 1000 hr of estrus when serum FSH levels were lower in the untreated rats than in the other two groups. In untreated rats, serum testosterone concentrations were lowest during estrus and highest after the onset of the preovulatory surges of FSH and LH in serum during the afternoon of proestrus. No significant differences in serum testosterone concentrations were observed between groups except that levels in the ADX rats were lower than levels in the untreated rats at 1000 and 1800 hr of diestrous day 1, and levels in the ADX rats were lower than levels in the sham-ADX rats at 0600 hr of estrus. Serum testosterone levels were undetectable in rats ovariectomized and adrenalectomized for 2 weeks. The results indicate that the adrenal glands contribute little to the maintenance of serum testosterone levels during the rat 4-day estrous cycle and that the rise in serum testosterone concentration from estrus to the afternoon of proestrus is likely the result of ovarian secretions.

Steroids play a major role in the control of anterior pituitary gland luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion. Whereas various studies have investigated the effects of estrogen and progesterone on gonadotropin secretion in the cyclic female rat (1–3), few studies have examined the effects of testosterone on cyclic gonadotropin release. Testosterone has been implicated as playing a role in FSH release during estrus (4).

There are a few studies in which serum testosterone levels have been measured in cyclic female rats (4–7). These studies indicate that serum testosterone concentrations are higher on proestrus than on other days of the estrous cycle. Although serum testosterone levels were measured at multiple times during the estrous cycle in a mixture of 4- and 5-day cyclic rats (4), a critical evaluation of serum testosterone concentrations during the periovulatory period in the 4-day cyclic rat is lacking. Moreover, the site of testosterone secretion which results in the cyclic variations in serum testosterone levels is not known. There is evidence that both the ovaries and, to a lesser extent, the adrenal glands may contribute significantly to the total testosterone production (6).

The purposes of this study were to characterize serum testosterone concentrations during the rat 4-day estrous cycle and to determine to what extent these testosterone levels represent adrenal gland secretions.

Materials and Methods. Female Sprague–Dawley rats (Simonsen Laboratories, Inc., Gilroy, Calif.) were housed in a temperature-controlled (24–26°) room with the lights on from 0500 to 1900 hr daily and provided free access to Wayne Laboratory rat chow and water. Starting 2 weeks after the rats were first housed in our vivarium,

[1] Supported by grants from the NIH (HD11011 and HD07097).

[2] Present address: Department of Anatomy, University of Kentucky Medical Center, Lexington, Ky. 40536.

[3] To whom requests for reprints should be sent.

0037-9727/82/020216-06$01.00/0

we prepared vaginal smears daily by saline lavage. Rats which exhibited two consecutive 4-day estrous cycle were used (body weight range was 201 to 316 g).

We bilaterally adrenalectomized (ADX) rats between 0900 and 1300 hr on the day of estrus under brief anesthesia according to the method of Zarrow *et al.* (8) using a single, dorsal midline skin incision. Controls consisted of untreated rats and sham-operated animals (Sham). The sham operation consisted of exposing but not touching the adrenals. The ADX rats received 0.9% saline to drink. Vaginal smears were prepared daily to monitor the stages of the estrous cycle. The Sham and ADX rats which continued displaying consecutive 4-day estrous cycles were decapitated during the third to fifth estrous cycle after surgery. Untreated controls which also demonstrated a total of five to seven consecutive 4-day estrous cycles were decapitated at the same times as were the Sham and ADX rats. Trunk blood was collected and the sera was stored at $-20°$ for subsequent measurement of FSH, LH, corticosterone, and testosterone. After decapitation, a search was made for remnants of adrenal tissue in the ADX rats.

All samples were assayed for FSH and LH in duplicate by the radioimmunoassay method of Niswender *et al.* (9) as described previously (10). Serum corticosterone concentrations were measured as described previously (11). Corticosterone was determined in the serum of all 30 sham rats and of 34 of the 39 ADX rats killed between 1400 hr of proestrus and 1000 hr of estrus (serum samples from one rat killed at 1400 hr of proestrus, two rats killed at 0200 hr of estrus, and two rats killed at 1000 hr estrus were not measured).

Serum testosterone concentrations were determined in 178 of the 189 samples by radioimmunoassay methods described by Abraham *et al.* (12) using an antiserum (No. 250) supplied by Dr. Gordon D. Niswender, Colorado State University. In all cases, we extracted the testosterone from 0.5 ml of serum twice with 2.0 ml of freshly opened diethyl ether. Serum testosterone ("total androgen") concentrations were measured without chromatography. The anti-testosterone sera used has been shown to exhibit a 69% cross-reaction with 5α-dihydrotestosterone and a low cross-reactivity with a large number of other steroids (13). Serum testosterone concentration in pooled blood collected from three rats which were ovariectomized and adrenalectomized for a total of 2 weeks was 7 pg/ml. This serum blank was not different from our water blank of 6 pg/ml. Values were determined by subtracting the average value of water blanks (6 pg/ml) and by correcting for recovery (all recoveries were $\geq$ 72%). All samples were measured in a single testosterone assay. The within-assay coefficient of variation was 9%.

Statistical comparisons of the data were performed by two-way analysis of variance. One-way analysis of variance was used when the treatment caused a significant difference among the three groups at any time interval. Post hoc Newman–Keuls tests were performed and P values < 0.05 were considered statistically significant.

Results. Neither operation had a significant effect on the occurrence of consecutive 4-day estrous cycles. Over 81% of untreated control, 89% of Sham, and 83% of ADX rats displayed consecutive 4-day estrous cycles throughout the duration of the experiment. The remainder of the rats had one or more 5-day estrous cycles and were not used.

We considered our rats to be completely adrenalectomized for two reasons. First, we were unable to detect remnants of adrenal tissue. Second, the ADX rats had low serum corticosterone levels (<8 ng/100 ml). These low levels are comparable to the nonspecific fluorescence reported by others (14). Sham rats had serum corticosterone levels >40 ng/100 ml.

Serum FSH (Fig. 1) and LH (Fig. 2) concentrations during the estrous cycle were similar to those reported in previous studies (15, 16). In brief, serum FSH concentrations rose ($P < 0.01$) in all groups of rats between 1400 and 1800 hr of proestrus and remained significantly elevated ($P < 0.05$) through 1000 hr of estrus when compared to the presurge FSH levels observed at 1400 hr of proestrus. No differences were observed between groups except at 1000 h of

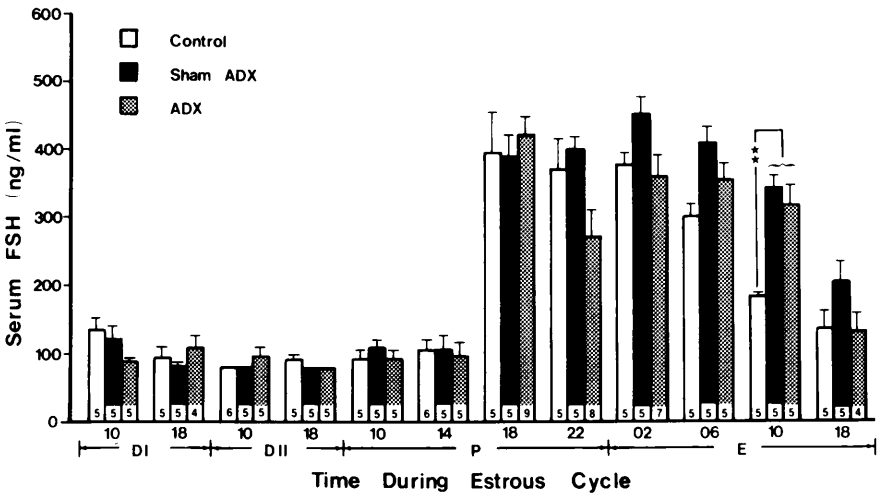

FIG. 1. Serum FSH concentrations (mean ± SE) in untreated (Control), sham adrenalectomized (Sham ADX), and adrenalectomized (ADX) rats are plotted during the 4-day estrous cycle. The Sham ADX and ADX rats were decapitated 2–3 weeks after surgery. The numbers within the bars indicate the number of rats in each group. D1= diestrous day 1; D11 = diestrous day 2; P = proestrus; E = estrus. The times encompass the interval from 1000 hr (10) of D1 to 1800 hr (18) of E. ★★ = $P < 0.01$.

estrus when serum FSH concentrations were higher ($P < 0.01$) in the Sham and ADX groups than in the untreated controls. Serum LH concentrations increased ($P < 0.01$) dramatically from 1400 to 1800 hr of proestrus in all groups. They then fell to approach presurge LH levels by 2200 hr of proestrus. No differences in LH concentrations were observed between groups at any of the time periods.

In untreated controls, serum testosterone concentrations rose from low values on the day of estrus to peak values on the afternoon of proestrus (Fig. 3). A two-fold rise ($P < 0.05$) in serum testosterone levels occurred between 1800 hr of estrus and 1000

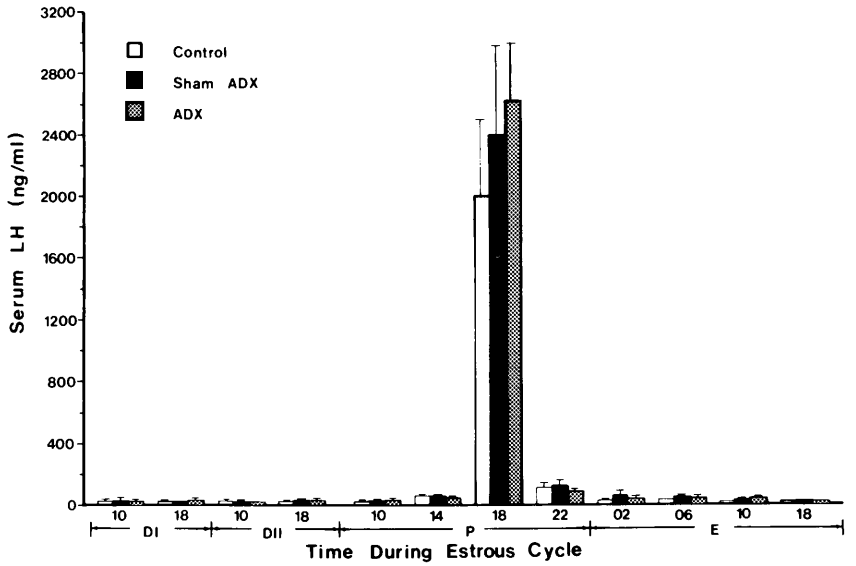

FIG. 2. Serum LH concentrations (mean ± SE) in untreated (Control), sham adrenalectomized (Sham ADX), and adrenalectomized (ADX) rats are plotted during the 4-day estrous cycle. See Fig. 1 for number of rats per group and see legend to Fig. 1 for details.

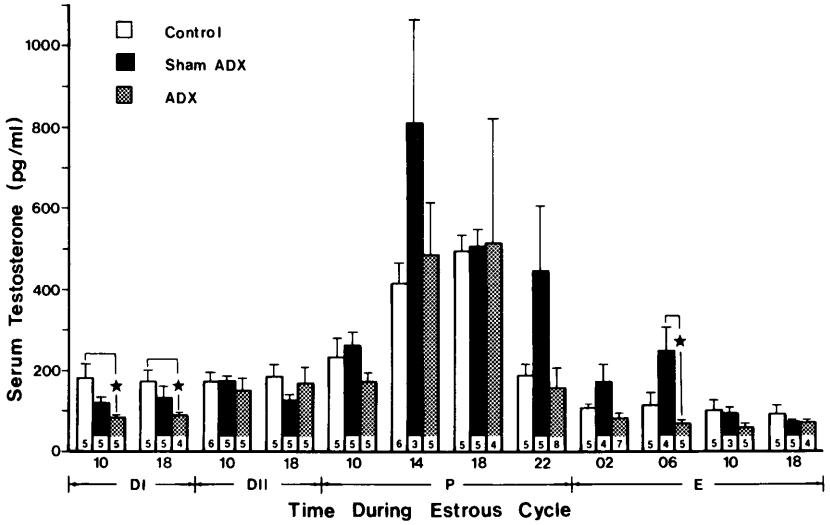

FIG. 3. Serum testosterone concentrations (mean ± SE) in untreated (Control), sham adrenalectomized (Sham ADX), and adrenalectomized (ADX) rats are plotted during the 4-day estrous cycle. ★ = $P < 0.05$. See legend to Fig. 1 for details.

hr of diestrous day 1. The serum testosterone concentration rose ($P < 0.05$) further between 1000 and 1400 hr of proestrus and reached peak values at 1800 hr of proestrus. The levels then decreased ($P < 0.01$) by 2200 h of proestrus and from 2200 h of proestrus to 0200 hr of estrus ($P < 0.01$). Serum testosterone concentrations were lower ($P < 0.05$) in ADX rats than in untreated controls at 1000 and 1800 hr of diestrous day 1. However, testosterone levels in the Sham rats at these two times were not different from those observed in the ADX or untreated rats. Serum testosterone levels in Sham rats were higher ($P < 0.05$) than those of ADX rats at 0600 hr of estrus. No other statistically significant differences were observed between any of the groups throughout the estrous cycle.

Discussion. To our knowledge, this is the first study to report serum testosterone concentrations at several time periods during the 4-day estrous cycle in unanesthetized rats. Serum testosterone levels were observed to increase gradually from low levels on the day of estrus to peak levels on the afternoon of proestrus. In general, these results are similar to those of previous reports on serum testosterone levels at various times during the 4- and/or 5-day estrous cycle. In 4-day cyclic rats in which blood samples were collected during exposure to ether, plasma testosterone concentrations were not different at 1000 hr of estrus, diestrous day 1, and diestrous day 2 nor were they different at 1600 hr of diestrous day 2, 1000 hr of proestrus and 1600 hr of proestrus, but they did rise significantly from 1000 hr of diestrous day 2 to 1600 hr of proestrus (7). In 5-day cyclic rats in which blood samples were collected during exposure to ether, plasma testosterone concentrations were low on the day of estrus and on the day of diestrus and a significant rise was observed between the day of diestrus and the day of proestrus (6). In a mixture of 4- and 5-day cyclic rats killed by decapitation, serum testosterone concentration rose gradually from low levels on the day of estrus to peak levels at 1800 hr on the day of proestrus (4). We observed statistically significant rises in serum testosterone levels in untreated controls between 1800 hr of estrus and 1000 hr of diestrous day 1 and between 1000 and 1400 hr of proestrus (Fig. 3). The rise which occurred on proestrus preceded the onset of the LH surge and the associated increase in serum FSH concentration.

There are similarities between serum testosterone and estrogen levels during the 4-day estrous cycle ((17–19) and present

study). The concentration of both hormones is lowest on the day of estrus and highest on the day of proestrus. The adrenal glands secrete substantial amounts of estrogen and may play a major role in the maintenance of serum estrogen levels during the rat estrous cycle (19). Also, estradiol-17β concentrations in the adrenal venous and peripheral plasma appear to parallel each other during the estrous cycle (19). However, adrenal gland testosterone secretion can not be considered to be primarily responsible for the changes in serum testosterone levels observed during the estrous cycle.

Despite the fact that the adrenal glands do secrete androgens (20), it appears clear that they contribute little to the maintenance of the levels of testosterone observed in serum during the 4-day estrous cycle and that they are not responsible for the increase in serum testosterone levels observed during proestrus. Although the adrenal glands do appear to play a role in increasing serum testosterone levels from 1800 hr of estrus to 1000 hr of diestrous day 1 when one compares levels in ADX and in untreated controls, this adrenal contribution was not observed when levels in ADX rats were compared with levels in Sham controls. In any event, any such adrenal contribution to serum testosterone levels appears to disappear at other times during the estrous cycle.

The absence of testosterone or any other secretion by the adrenal glands did not interfere appreciably with the periovulatory increases in LH and FSH concentrations. This is in agreement with previous reports (11, 21). As serum testosterone levels were undetectable in ovariectomized–ADX rats and not appreciably affected by adrenalectomy alone, it appears that any effect of serum testosterone levels on pituitary gland gonadotropin secretions during the rat estrous cycle must be mediated by changes in ovarian testosterone secretion.

We thank Drs. Albert F. Parlow, Leo Reichert, Jr., and Gordon D. Niswender as well as the Rat Pituitary Hormone Program of NIAMDD for their generous gifts of materials used for the radioimmunoassays.

1. Davidson JM. Feedback control of gonadotropin secretion. In: Ganong WF, Martini L, eds. Frontiers in Neuroendocrinology. New York, Oxford Univ Press, Vol 1:p343, 1969.
2. Blake CA. Neurohumoral control of cyclic pituitary LH and FSH release. Clin Obstet Gynaecol 5:305–327, 1978.
3. Barraclough CA, Wise PM, Turgeon J, Shander D, DePaolo L, Rance N. Recent studies on the regulation of pituitary LH and FSH secretion. Biol Reprod 20:86–97, 1979.
4. Gay VL, Tomacari RL. Follicle-stimulating hormone secretion in the female rat: Cyclic release is dependent on circulating androgen. Science 184:75–77, 1974.
5. Falvo RE, Kaltenbach CC, Pancoe WL. Determination of testosterone concentration in the plasma of normal and androgen-sterilized female rats, using a competitive protein binding technique. Neuroendocrinology 10:229–234, 1972.
6. Falvo RE, Buhl A, Nalbandov AV. Testosterone concentrations in the peripheral plasma of androgenized female rats and in the estrous cycle of normal female rats. Endocrinology 95:26–29, 1974.
7. Dupon C, Kim MH. Peripheral plasma levels of testosterone, androstenedione and oestradiol during the rat oestrus cycle. J Endocrinol 59:653–654, 1973.
8. Zarrow MX, Yochim JM, McCarthy JL. Experimental Endocrinology, A Source Book of Basic Techniques. New York, Academic Press, p194, 1964.
9. Niswender GD, Midgley Jr AR, Monroe SE, Reichert Jr LE. Radioimmunoassay of rat luteinizing hormone with anti-ovine LH serum and ovine LH-^{131}I. Proc Soc Exp Biol Med 128:807–811, 1968.
10. Elias KA, Blake CA. The effect of prior gonadotrophin injection on the rising phases of simulated preovulatory surges of luteinizing hormone and follicle-stimulating hormone surges in the plasma of phenobarbitone-blocked, pro-oestrous rats. J Endocrinol 83:331–337, 1979.
11. Sridaran R, Blake CA. Effects of long-term adrenalectomy on periovulatory increases in serum gonadotrophins and ovulation in rats. J Endocrinol 84:75–82, 1980.
12. Abraham GE, Manlimos FS, Garza R. Radioimmunoassay of Steroids. In: Abraham GE, ed. Handbook of RIA. New York, Marcel Decker, p591, 1977.
13. Gay VL, Kerlan JT. Serum LH and FSH following passive immunization against circulating testosterone in the intact male rat and in orchidectomized rats bearing subcutaneous silastic implants of testosterone. Arch Andrology 1:257–266, 1978.

14. Fukuda H, Greer MA, Roberts L, Allen CF, Critchlow V, Wilson M. Nyctohemeral and sex-related variations in plasma thyrotropin, thyroxine, and triiodothyronine. Endocrinology **97**:1424–1431, 1975.
15. Ashiru OA, Blake CA. Restoration of the periovulatory follicle-stimulating hormone surges in sera by luteinizing hormone releasing hormone in phenobarbital-blocked rats. Life Sci **23**:1507–1514, 1978.
16. Elias KA, Blake CA. A detailed *in vitro* characterization of the basal follicle-stimulating hormone and luteinizing hormone secretion rates during the rat four-day estrous cycle. Endocrinology **109**:708–713, 1981.
17. Blake CA, Scaramuzzi RJ, Hilliard J, Sawyer CH. Circulating levels of pituitary gonadotrophins and ovarian steroids in rats after hypothalamic deafferentation. Neuroendocrinology **12**:86–97, 1973.
18. Butcher RL, Collins WE, Fugo NW. Plasma concentrations of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat. Endocrinology **94**:1704–1708, 1974.
19. Shaikh AA, Shaikh SA. Adrenal and ovarian steroid secretion in the rat estrous cycle temporarily related to gonadotropins and steroid levels found in peripheral plasma. Endocrinology **96**:37–44, 1975.
20. Maroulis GB, Abraham GE. Concentration of androgens and cortisol in the zones of the adrenal cortex. In: Genazzani AR, Thijssen JHH, Siiteri PK, eds. Adrenal Androgens. New York, Raven Press, p49, 1980.
21. Baldwin DM, Sawyer CH. Light synchronization of the preovulatory LH surge in adrenalectomized rats. Proc Soc Exp Biol Med **161**:295–298, 1979.

Received September 2, 1981. P.S.E.B.M. 1982, Vol. 169.