PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 680–683 (1974)

Maintenance of Hyperprolactinemia by Gonadal Steroids in Androgen-Sterilized and Spontaneously Constant-Estrus Rats[1] (38171)

ALBERT RATNER AND GLENN T. PEAKE
(Introduced by S. Solomon)
Departments of Physiology and Medicine, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

Prolactin production by the lactotropes of the adenohypophysis is influenced by a complex interaction of gonadal hormones and hypothalamic neural control mediated by a prolactin inhibiting factor and perhaps a prolactin stimulating factor (1). Present experimental evidence indicates that the major gonadal hormone which facilitates prolactin secretion is estrogen. Administration of exogenous estrogen derivatives to normal and gonadectomized animals results in increased plasma prolactin concentration (2, 3). Plasma prolactin increases postpubertally (4), and during proestrus (5–8) in rats when ovarian estrogen production is increased. Rats exhibiting constant-estrus have been shown to have enhanced ovarian estrogen production (9, 10). In animals in which constant-estrus was produced by early androgenization (11), lesioning of the hypothalamic suprachiasmatic region (12), or by anterior deafferentiation of the basal hypothalamus (13), serum prolactin levels were found to be considerably higher than in normal cyclic estrus rats. Direct evidence that ovarian hormones supported the enhanced prolactin secretion in constant-estrus rats was obtained by Bishop *et al.* (12) in rats with lesions in the suprachiasmatic region. They observed that ovariectomy resulted in a substantial fall in serum prolactin levels in these constant-estrus rats. It appears reasonable to assume that enhanced estrogen production in these animals acts on the hypothalamic-pituitary axis to stimulate prolactin secretion.

In these studies we have investigated the role of ovarian hormones on prolactin secretion by extending the observations of Bishop *et al.* (12) to two other models of the constant-estrus rats which do not require surgical disruption of normal hypothalamus-brain connections.

Materials and Methods. The animals used in this study were of the Sprague–Dawley strain (Cheek-Jones, Houston). Although regular 4 day and 5 day estrus cycles predominate in rats of this strain, spontaneous constant-estrus occurs in about 25% of the females. The rats began to exhibit this state between 7 and 9 mo of age. This observation is similar to that reported by Terasawa *et al.* (14) for another Sprague–Dawley strain which also exhibits spontaneous constant-estrus. The adults were housed in individual cages, maintained in temperature- and light-controlled quarters (14 hr light/day) and given food and water *ad libitum*. At 3 days of age, female pups were androgenized by injecting 1.25 mg testosterone proprionate (Oreton, Schering) in 0.05 ml sesame oil; control female litter mates received the same volume of sesame oil. All animals were weaned at 21 days of age. Beginning at 2 mo of age, vaginal smears were taken daily to determine the cyclic pattern. Normal females exhibited at least 3 regular 4 day cycles before they were used. Androgen-sterilized rats and those exhibiting spontaneous constant-estrus showed at least 2 mo of constant-estrus vaginal smear pattern before being used. At

[1] This work was supported by NSF Grant GB 32077 (A.R.) and USPHS-NIH Grant HD 05794 (G.T.P.).

appropriate time intervals animals from each group were bilaterally ovariectomized and studied 1 and 3 wk later.

Blood samples were removed from the external jugular during light ether anesthesia. All animals except control proestrus rats were bled between 0900–1100 hr. Proestrus rats were bled between 1700–1800 hr since peak prolactin levels were found to occur in our rats at this time period. The blood was stored at 4° overnight and centrifuged at 2000*g* for 20 min. The serum was separated and stored at −20° until assayed. Prolactin was assayed using the standard radioimmunoassay technique. The materials for the assays were provided by the NIH-NIAMD: Rat Pituitary Program. Concentrations of prolactin were expressed in ng/ml on the basis of standard supplied (RP-1). In all cases the samples were assayed in duplicate at 2 dilutions. Significance of differences between means was determined by the Student's *t*-test for unpaired samples.

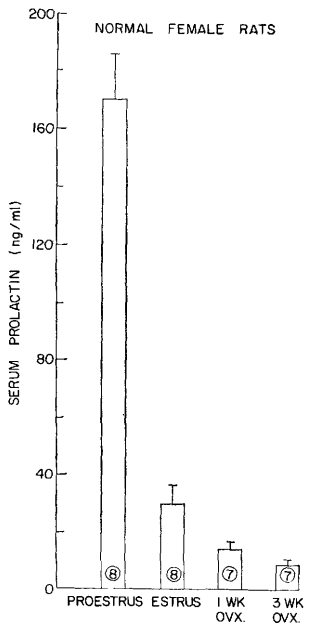

FIG. 1. Serum prolactin levels in intact cycling and ovariectomized (OVX) normal female rats. The bars depict mean values of prolactin; the line extending above the bar represents SEM; the numbers at the bottom of each bar indicate the number of rats used in that group.

Results. 1. Normal Female Rats (Fig. 1): Prolactin levels were considerably elevated on the afternoon of proestrus in rats exhibiting a normal 4 day cycle. One week after ovariectomy, prolactin levels decreased 53% as compared to normal estrus levels. A further decrease in serum prolactin (80%) was noted 3 wk following ovariectomy.

2. Androgen-Sterilized Rats (Fig. 2): Androgenized females had prolactin levels some 700% greater than normal rats on the day of estrus. Following ovariectomy, prolactin levels were considerably reduced. They showed an 80% decrease one week and 88% decrease 3 wk following ovariectomy.

3. Spontaneously constant-estrus rats (Fig. 2): Rats exhibiting spontaneous constant-estrus had prolactin levels some 450% greater than normal cyclic rats on the day of estrus. These rats showed a 62% decrease one week and 74% decrease 3 wk following ovariectomy.

Discussion. These studies support the concept that estrogens facilitate prolactin secretions in constant-estrus rats. Circulating levels of prolactin were found to be very high in rats exhibiting constant-estrus as a result of early androgen treatment and in adult rats which spontaneously developed constant-estrus. Both groups of animals reported here demonstrated continuous vag-

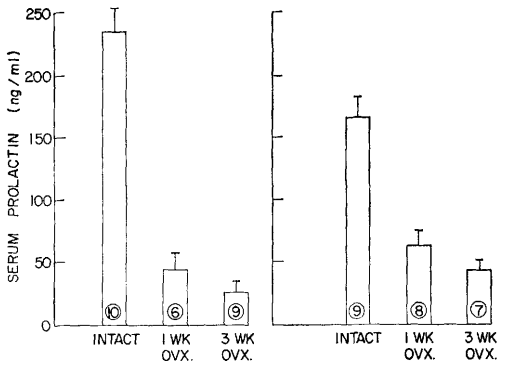

FIG. 2. Serum prolactin levels in intact and ovariectomized (OVX) androgen-sterilized (left) and spontaneously constant-estrus rats (right). The bars depict mean values of prolactin; the line extending above the bar represents SEM; the numbers at the bottom of each bar indicate the number of rats used in that group.

inal cornification, excessive mammary development and increased uterine and pituitary weights, suggesting increased circulating levels of estrogen.

Estradiol has recently been shown to increase prolactin levels in rats made constant-estrus by lesioning of the suprachiasmatic region. Estrogen was ineffective in increasing prolactin levels in rats with median eminence lesions (15). These latter results suggest that the effect of estrogen on prolactin release is mediated principally at an area caudal to the suprachiasmatic region, most probably at the median eminence-arcuate nucleus level. Ratner and Meites (16) showed that injections of estradiol deplete the content of the prolactin inhibiting factor found in this area of the hypothalamus. Implants of estrogen in the median eminence have been shown to stimulate prolactin release (17, 18). Ratner and Adamo (19) reported ultrastructural changes in the vesicles found in nerve terminals ending on cells in the arcuate region of androgenized rats. They suggested that these changes may be due to the feedback effect of persistently high levels of estrogen in these rats.

If increased ovarian estrogen production was involved in maintaining the high levels of circulating prolactin noted in constant-estrus rats, a substantial fall in serum prolactin should occur following ovariectomy. We observed such a decrease in two types of constant-estrus rats with normal hypothalamus-brain connections. These observations extend the studies of Bishop *et al.* (12) who found similar decreases in serum prolactin following ovariectomy in rats made constant-estrus by lesioning the hypothalamic suprachiasmatic region. The pronounced decrease in prolactin following removal of the ovaries strongly indicates that elevated levels of prolactin observed in constant-estrus rats, either induced by early androgenization, surgical procedures or spontaneously developed, are to a great extent a result of estrogen being released from the ovarian follicles. Presumably, the estrogen is feeding back at the hypothalamic levels to stimulate prolactin release.

Summary. Serum prolactin concentrations in constant-estrus rats sterilized by a single injection of testosterone proprionate on the third day of life and in adult female rats, spontaneously constant-estrus, were compared to levels in adult normal cyclic female rats. Prolactin levels were much greater in androgen-sterilized females (235 ± 18 ng/ml) and in spontaneously constant-estrus females (165 ± 17 ng/ml) than in normal cyclic estrus rats (30 ± 7 ng/ml).

In order to demonstrate the role of endogenously produced ovarian estrogen in maintaining the high levels of prolactin, ovariectomy was performed on both groups of constant-estrus animals. Bilateral ovariectomy for one and three weeks resulted in a marked decrease in prolactin levels in both experimental groups. These results show that prolactin levels are elevated in androgen-sterilized and spontaneously constant-estrus rats and that the continuous release of estrogen observed in these animals is acting on the hypothalamic-pituitary axis to promote prolactin release.

The authors acknowledge the expert technical assistance of Ms. Josephine A. Morris, Ms. Lucinda Johnson and Ms. Carolyn R. Duszynski.

1. Meites, J., Lu, K. H., Wuttke, W., Welsch, C. W., Nagasawa, H., and Quadri, S. K., Recent Prog. Horm. Res. **28**, 471 (1972).

2. Chen, C. L., and Meites, J., Endocrinol. **86**, 503 (1970).

3. Kalra, P. S., Fawcett, C. P., Krulich, L., and McCann, S. M., Endocrinology **92**, 1256 (1973).

4. Voogt, J. L., Chen, C. L., and Meites, J., Amer. J. Physiol. **218**, 396 (1970).

5. Niswender, G. D., Chen, C. L., Midgley, A. R., Meites, J., and Ellis, S., Proc. Soc. Exp. Biol. Med. **130**, 793 (1969).

6. Wuttke, W., and Meites, J., Proc. Soc. Exp. Biol. Med. **135**, 648 (1970).

7. Gay, V. L., Midgley, A. R., and Niswender, G. D., Fed. Proc. **29**, 1880 (1970).

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

9. Rosner, J. M., Tramezzani, J. H., Macome, J. C., and Llauro, J. L., Acta Physiol. Latino Amer. **19**, 257 (1969).

10. Blake, C. A., Scaramuzzi, R. J., Hilliard, J., and Sawyer, C. H., Neuroendocrinology **12**, 86 (1973).

11. Mallampati, R. S., and Johnson, D. C., Neuroendocrinology **11**, 46 (1973).

12. Bishop, W., Fawcett, C. P., Krulich, L., and McCann, S. M., Endocrinology **91**, 643 (1972).

13. Blake, C. A., Weiner, R. I., and Sawyer, C. H., Endocrinology **90**, 862 (1972).

14. Terasawa, E., Kawakami, M., and Sawyer, C. H., Proc. Soc. Exp. Biol. Med. **132**, 497 (1969).

15. Bishop, W., Kalra, P. S., Fawcett, C. P., Krulich, L., and McCann, S. M., Endocrinology **91**, 1404 (1972).

16. Ratner, A., and Meites, J., Endocrinology **75**, 377 (1964).

17. Kanematsu, S., and Sawyer, C. H., Endocrinology **72**, 243 (1963).

18. Ramirez, V. D., and McCann, S. M., Endocrinology **75**, 206 (1964).

19. Ratner, A., and Adamo, N. J., Neuroendocrinology **8**, 26 (1971).

Received Feb. 4, 1974. P.S.E.B.M., 1974, Vol. 146.