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Plasma Prolactin and Progesterone during the Estrous Cycle in the Mouse[1] (39522)

SANDRA D. MICHAEL

Department of Biological Sciences, State University of New York, Binghamton, New York 13901

The estrous cycle of laboratory mice is influenced by the presence or absence of a male (1-3). Absence of a male results in irregular cycles when the females are individually caged, prolonged diestrus and pseudopregnancies when they are caged in small groups, and anestrus when they are caged in large groups. Females caged in the olfactory presence of a male exhibit shorter and more regular cycles and can be made to cycle synchronously by previous grouping in large numbers.

In all species thus far studied, including mice (3, 4), ovulation is preceded and presumably caused by a sharp rise in circulating LH and FSH. The ultimate regulation of ovulation, however, resides in an interaction between the pituitary and gonadal hormones (5). The interactions between pituitary and gonadal hormones during the estrous cycle have been extensively studied in rats (5-8). Hormone patterns during the estrous cycle in laboratory mice have not been as thoroughly investigated, despite the importance of mice in cancer and genetic studies. In the present study, we report the simultaneous measurement of both plasma progesterone and prolactin in the same strain of mice in which we previously characterized LH and FSH (3).

Materials and methods. Animals. Mice of a random-bred stock derived from a four-way cross, Line C (9), were maintained on a light schedule of 14 hr light (on between 0500 and 1900 hr). Nulliparous females, 60-70 days of age were synchronized (10) by being grouped 15 to a cage for 2 weeks then examined for vaginal smear pattern. Females showing evidence of cycling, i.e., a nucleated and/or cornified cell pattern, were eliminated from the study. Male and female animals were housed in separate cages (7 × 8 × 10.5 in.) which were composed of wire mesh on three sides. The cages were placed so that a common mesh side of one cage was in contact with the other to allow olfactory and visual stimuli. Three females were placed in one cage and a mature male in the other. Vaginal smears were taken daily by saline lavage and examined in unstained wet preparations. The following criteria were used for identification of cycle stages: proestrus, nucleated or nucleated and cornified cells; estrus, cornified cells; metestrus, leukocytes and cornified cells; diestrus, leukocytes or leukocytes and nucleated cells. Only those females exhibiting two consecutive, 4-day cycles were used in the study. Females were sacrified at intervals throughout their third or subsequent cycle within 10 sec after removal from their cages. The animals were killed by decapitation, their blood collected through heparinized funnels, and the plasma obtained following centrifugation stored frozen until assay.

Assays. Materials and protocol for the radioimmunoassay of prolactin were supplied by Dr. Y. N. Sinha (11). The prolactin concentrations are expressed as nanograms of the standard per milliliter of plasma. The biological potency of the mouse prolactin standard is 25 IU/mg. All prolactin samples were run in one assay. Plasma progesterone concentrations were determined by radioimmunoassay using an antiserum (provided by Dr. G. D. Niswender) produced in rabbits against progesterone conjugated to bovine serum albumin at the 6 position. The specificity of this antiserum is such that progesterone measurements can be made without prior chromatography (12). Water blanks were always undetectable and recoveries were between 60 and 85%. Low blood yield per animal necessitated the assay of progesterone at each time interval from a single plasma pool. To assure that each animal made an equal contribution to the pool, pools were made up of 10 μl from each

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animal (8 to 12 animals/pool). All progesterone samples were included in one assay. Within-assay coefficients of variation were 6.8% for prolactin and 7.4% for progesterone. The lowest detectable concentration was 5 ng/ml for prolactin and 0.8 ng/ml for progesterone. Times are based upon a 24-hr clock.

Statistical comparisons of peak concentrations to baseline levels (sum of all baseline values) were determined by a confidence interval of the mean.

Results. The concentrations of prolactin and progesterone in plasma of Line C females during different stages of the estrous cycle are shown in Fig. 1. Concentrations of prolactin were around 50 ng/ml throughout the estrous cycle except during proestrus. At 1100 hr on the morning of proestrus, slightly elevated levels were found (93 ± 32 ng/ml). During the afternoon of proestrus, prolactin rose to a peak concentration of 558 ± 64 ng/ml at 1900 hr. This increase was significant ($P < 0.01$) over baseline values. After 1900 hr, the prolactin levels began to decrease and by 0900 hr on the day of estrus, the concentrations were back to around 50 ng/ml.

The pattern of progesterone secretion during the cycle consisted of two major surges, one during the late afternoon of proestrus and the other on the morning of metestrus. Progesterone values remained low (around 5 ng/ml) until the afternoon of proestrus when a surge occurred, reaching a peak concentration of 64 ng/ml at 2100 hr. After this time, a precipitious drop in progesterone levels occurred, falling to around 5 ng/ml by 0900 hr on the morning of estrus. The second surge occurred during metestrus reaching a peak level of 27 ng/ml at 2100 hr. Both surges were significant changes from baseline concentrations ($P < 0.01$). Elevated levels were followed by a decline to baseline values of around 5 ng/ml by 0900 hr on diestrus.

Discussion. The pattern of progesterone secretion during the estrous cycle described here for mice is similar to that of the rat (7, 8). Both species show two major increases, during metestrus and on the afternoon of proestrus, and progesterone levels peak

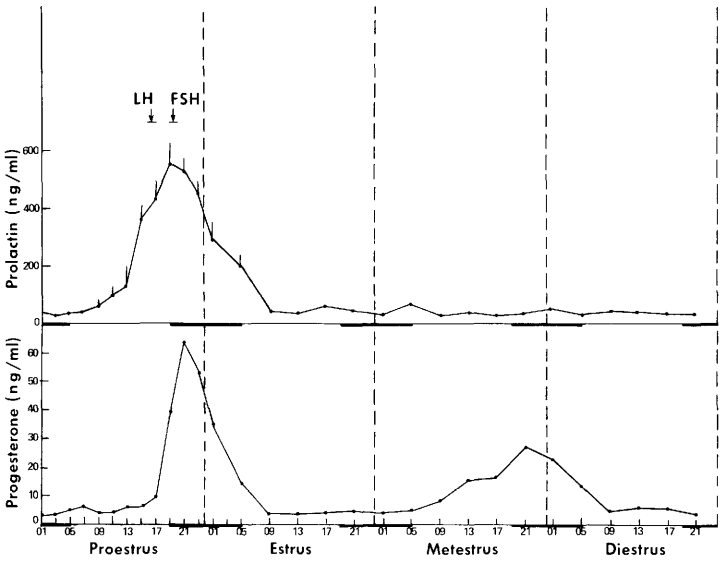

FIG. 1. The pattern of prolactin and progesterone during the mouse estrous cycle. Eight to 12 animals were decapitated at each time interval and the plasma subjected to the two hormone analyses. Prolactin was determined in individual animals. Standard errors are not shown on some prolactin means because they are smaller than the width of the dot on the graph. At ech time interval, progesterone was determined in a single plasma pool, therefore no standard errors are indicated. The numbers along the abscissa represent the time of day (24-hr clock). The black bars represent the dark period (1900–0500 hr) and the dashed lines denote midnight. The proestrous surges of LH and FSH secretion are indicated by arrows (↓) (data taken from ref. (3)).

after initiation of the proestrous surge of prolactin. Some investigators (13–15) report that in rats serum progesterone levels display a circadian rhythm during the estrous cycle. If mice have a similar diurnal rhythmicity of plasma progesterone levels, it could have been missed since samples were collected only at 4-hr intervals at stages other than proestrus.

In an earlier study (16) of plasma progesterone levels during pregnancy and parturition in Line C mice, we found that levels of progesterone were as low as baseline levels of the estrous cycle (around 5 ng/ml) only on the day of parturition. From Days 2 through 9 of pregnancy, concentrations of progesterone were between 41 and 54 ng/ml. These values are about midway between the peak of 64 ng/ml found during proestrus and the peak of 27 ng/ml found during metestrus. The highest level of progesterone (113 ng/ml) was found on Day 15 of pregnancy.

The pattern of prolactin secretion during the estrous cycle of mice has been reported only in two preliminary studies, Yanai and Nagasawa (17) and Sinha *et al.* (18). In the former study, mice were maintained on the same light schedule as those in the present study, but they were sacrificed at only a few time intervals. Yanai and Nagasawa found that the highest level of plasma prolactin (around 120 ng/ml) occurred on the late afternoon of diestrus. At all other stages of the cycle, prolactin levels were around 50 ng/ml, which is the same baseline level we found using the same RIA (11). Their failure to find elevated prolactin concentrations during the afternoon of proestrus was probably due to either sacrificing animals at only one time interval (1700–1730 hr) or due to a strain difference (they used strain C3H/He). The elevated levels of prolactin which they found on diestrus might possibly be due to bleeding the animals under ether anesthesia (19), although it is not clear why this stage of the cycle should be more susceptible to the stressful method of blood collection used by these investigators.

In the studies of Sinha *et al.* (18), the animal quarters were lighted from 0600 to 2000 hr daily. In the two strains of mice which they studied (C3H/St and C57BL/St), levels of serum prolactin were higher during the afternoon of proestrus than at any other stage of the cycle with concentrations of 111 and 153 ng/ml at 1400 hr on proestrus. Since these investigators sampled animals at only one time interval during the afternoon of proestrus, these levels are probably not the peak concentrations.

Linkie and Niswender (20) found in the rat that, except for Day 0 of pregnancy (day of the vaginal plug) and the day prior to parturition, circulating prolactin levels were equivalent to or less than concentrations found during the diestrous phase of the cycle. In mice, however, the baseline values of around 50 ng/ml found throughout all stages of the cycle except for proestrus are generally lower than those which we previously reported in pregnant animals of Line C (21).

The times when peak levels of LH and FSH occurred in Line C mice on the same light schedule as in the present study are shown in Fig. 1 (3). In rats, the patterns of LH, FSH, and prolactin secretion are similar during most of the estrous cycle with concentrations remaining low until the afternoon and evening of proestrus (8). All three hormones in rats peaked at about the same time, around 1700 hr on proestrus (1 hr before the dark period). In mice, peak levels of FSH and prolactin occurred at the beginning of the dark period while peak levels of LH were found 2 and 3 hr earlier during the light period. In both rats and mice, prolactin levels began to increase from baseline levels during the morning of proestrus, before LH, FSH, and progesterone began to rise. A brief peak of prolactin on the afternoon of estrus in rats was reported by Butcher *et al.* (7) but was not found in mice or rats by Smith *et al.* (8).

There is some disagreement in the rat studies as to whether the proestrous surge of LH occurs before (22), simultaneous with (7, 8, 23), or after (24) the progesterone surge. The temporal relationship of LH and progesterone cannot be established with certainty in mice since the two hormones were measured in similar groups of animals but not in the same individuals. If the two groups can be considered equivalent then it would appear that in mice the proestrus rise

in plasma LH slightly precedes that of progesterone.

Neill and collaborators (5, 6, 8) have intensively studied and thoroughly discussed the pituitary–ovarian interactions which result in estrous cyclicity in rats. Although LH has been shown to be the stimulus for the proestrous surge of progesterone, the role of the progesterone surge has not as yet been established. The proestrous prolactin surge, insofar as presently known, does not play a major regulatory role in the cycle since a block in the surge does not disrupt cyclicity. The prolactin surge at proestrus does, however, cause lysis of the corpora lutea from the previous cycle. Although the mouse is not exactly identical to the rat in all aspects of hormone secretion, the present research and that which we reported previously (3) indicate a substantial amount of similarity; hence we conclude that the same mechanisms are probably operating.

Summary. The concentrations of prolactin and progesterone in plasma of Line C mice during different stages of the estrous cycle were measured by radioimmunoassay. During the afternoon of proestrus, prolactin rose to a peak concentration at 1900 hr. The pattern of progesterone consisted of two major surges, beginning during the late afternoon of proestrus and the morning of metestrus.

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1. Whitten, W. K., J. Endocrinol. **17,** 307 (1958).
2. Bingel, A. S., Proc. Soc. Exp. Biol. Med. **139,** 515 (1972).
3. Murr, S. M., Geschwind, I. I., and Bradford, G. E., J. Reprod. Fert. **32,** 221 (1973).
4. Kovacic, N., and Parlow, A. F., Endocrinology **91,** 910 (1972).
5. Neill, J. D., and Smith, M. S., in "Current Topics in Experimental Endocrinology" (L. Martini and V. H. T. James, eds.), Vol. 2, p. 73. Academic Press, New York (1974).
6. Freeman, M. E., Reichert, L. E., Jr., and Neill, J. D., Endocrinology **90,** 232 (1972).
7. Butcher, R. L., Collins, W. E., and Fugo, N. W., Endocrinology **94,** 1704 (1974).
8. Smith, M. S., Freeman, M. E., and Neill, J. D., Endocrinology **96,** 219 (1975).
9. Bradford, G. E., Genetics **58,** 283 (1968).
10. Whitten, W. K., in "Advances in Reproductive Physiology" (A. McLaren, ed.), Vol. 1, p. 155. Academic Press, New York (1966).
11. Sinha, Y. N., Selby, F. W., Lewis, U. J., and Vanderlaan, W. P., Endocrinology **91,** 1045 (1972).
12. Niswender, G. D., Steroids **22,** 3 (1973).
13. Mann, D. R., and Barraclough, C. A., Proc. Soc. Exp. Biol. Med. **142,** 1226 (1973).
14. Kalra, S. P., and Kalra, P. S., Endocrinology **95,** 1711 (1974).
15. Shaikh, A. A., and Shaikh, S. A., Endocrinology **96,** 37 (1975).
16. Murr, S. M., Stabenfeldt, G. H., Bradford, G. E., and Geschwind, I. I., Endocrinology **94,** 1209 (1974).
17. Yanai, R., and Nagasawa, H., J. Endocrinol. **62,** 685 (1974).
18. Sinha, Y. N., Salocks, C. B., and Vanderlaan, W. P., Endocrinology **97,** 1112 (1975).
19. Neill, J. D., Endocrinology **87,** 1192 (1970).
20. Linkie, D. M., and Niswender, G. D., Endocrinology **90,** 632 (1972).
21. Murr, S. M., Bradford, G. E., and Geschwind, I. I., Endocrinology **94,** 112 (1974).
22. Piacsek, B. E., Schneider, T. C., and Gay, V. L., Endocrinology **89,** 39 (1971).
23. Mann, D. R., Korowitz, C. D., and Barraclough, C. A., Proc. Soc. Exp. Biol. Med. **150,** 115 (1975).
24. Goldman, B. D., Kamberi, I. A., Siiteri, P. K., and Porter, J. C., Endocrinology **85,** 1137 (1969).

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