PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 78–82 (1981)

The Effects of Various Methods of Cervical Stimulation on Continuation of Prolactin Surges in Rats[1] (41128)

WILLIAM C. GOROSPE AND MARC E. FREEMAN[2]

Department of Biological Science, Florida State University, Tallahassee, Florida 32306

Abstract. In rats, the induction of pregnancy or pseudopregnancy is dependent upon the number of penile intromissions as well as the interintromission interval. The mating-induced stimulation of the uterine cervix is "stored" within the brain and repetitively expressed as two daily surges of prolactin for at least 10 days. It has been reported that the prolactin surges persist for only 6 days if the uterine cervices of ovariectomized (OVX) rats are stimulated artificially (CS). The present study demonstrates the effects of different methods of CS on prolongation of nocturnal (*N*) and diurnal (*D*) surges of prolactin secretion through Day 10 in OVX rats. Female rats were permitted to mate with males from proestrus through estrus, or received a mechanical (glass rod) or electrical stimulus of the uterine cervix at 1700 hr proestrus. All animals were OVX on the morning of estrus and blood was sampled by decapitation 10 days later. Both *N* and *D* surges of prolactin were present on Day 10 in mated rats or rats receiving a mechanical CS on proestrus. However, rats stimulated electrically on proestrus had neither *N* nor *D* surges of prolactin on Day 10. On the other hand, a second electrical stimulus on the morning of estrus prior to OVX induced prolactin surges which persisted through Day 10. Similar results were obtained in rats whose uterine cervices were artificially stimulated 14 days after OVX. However, *N* surges, when significantly elevated, were smaller than those of acutely OVX rats and *D* surges were present in only a small proportion of the animals sampled on Day 10. These results suggest that the stimulus provided by penile intromission can account for the induction and persistence of daily prolactin surges independently of ovarian support and that this physiological stimulus can be simulated by electrical or mechanical stimulation of the uterine cervix at appropriate intervals.

The successful induction of pregnancy (1, 2) or pseudopregnancy (3, 4) is dependent upon the number of penile intromissions as well as the interintromission interval (5, 6). The intromission-generated impulses arising from the uterine cervix are transmitted via neural pathways to the brain (7, 8) where they initiate two daily surges of prolactin secretion (9). The prolactin surges, in turn, transform the inactive corpora lutea of the estrous cycle into active progesterone-secreting structures (10). If the number of intromissions is inadequate, prolactin surges are not initiated (11), and consequently, the corpus luteum (8) and pregnancy (2) are not maintained. Artificial stimulation of the uterine cervix induces a similar semicircadian pattern of prolactin release (12) and consequent progesterone secretion (13). The prolactin secretory response does not require ovarian steroids since both surges can be initiated by artificial stimulation of the cervices of ovariectomized rats (12, 14, 15). Both surges persist for at least 10 days after *ad libitum* mating (16) or artificial stimulation of the cervices in intact rats (12). On the other hand, in acutely ovariectomized rats, the surges persist for only 6 days after a single brief artificial stimulation of the cervices (12), while progesterone replacement will prolong the surges beyond this time (14). These observations led to the suggestion that the copulatory stimulus is "stored" in the brain and expressed repetitively for only 6 days (12). Thereafter, ovarian steroids insure the prolonged expression of the neuroendocrine reflex (14). Using a more vigorous method of cervical stimulation, we have recently found that prolactin surges in ovariectomized rats will continue beyond the length of a normal pseudopreg-

[1] Supported by a grant from the NIH, HD-11669.

[2] Recipient of a Research Career Development Award from the NIH, HD-00231.

0037-9727/81/050078-05$01.00/0

nancy (15). Moreover, Everett showed earlier that the multiple stimulations provided by a copulatory male were more effectively "stored" for induction of a delayed pseudopregnancy compared to a single brief stimulus provided artificially to the cervix (17). Such observations suggest that the length of the prolactin secretory response to cervical stimulation is closely linked to the method of stimulation. The purpose of the present study was to determine if physiological or various artificial mating stimuli influence the persistence of prolactin surges independently of ovarian steroids.

Materials and Methods. *Animal care and surgery.* Adult Sprague–Dawley rats (Harlan, Madison, Wisc.) were housed under controlled illumination (lights on 0600–1800 hr) and temperature (25°) with continuous access to food and water. The estrous cycle was monitored by daily vaginal smears. Ovaries were removed through a mid-ventral incision while the rats were anesthetized with ether. Day 0 was designated the day of estrus in intact rats or the 15th day after surgery in long-term ovariectomized rats.

Method of cervical stimulation. The prolactin secretory response to natural mating and two types of artificial mating stimuli was evaluated.

a. Natural mating: Intact female rats were subjected to physiological stimulation of the uterine cervix by *ad libitum* mating when placed with sexually experienced males from 1400 hr on proestrus until 0800 hr on estrus. The presence of sperm in the vaginal smear and >5 vaginal plugs beneath the cage the following morning was indicative of successful stimulation of the cervix with a high number of intromissions.

b. Mechanical stimulation: The uterine cervix was stimulated by tapping vigorously with the blunted end of a glass rod for 1 min.

c. Electrical stimulation: Rectangular 1-msec pulses of 25 V and 200 Hz were applied to the cervix in three consecutive 10-sec trains. The electrode consisted of two platinum wires placed 2 mm apart and protruding 2 mm from a Teflon rod.

Blood collection and assays. All samples were collected by rapid decapitation within 5 sec of removal from the home cage. Samples were collected at 0500 hr to detect "noctural" surge levels, 1200 hr to detect basal levels and 1900 hr to detect "diurnal" surge levels (14). The concentrations of prolactin in these serum samples were estimated with the NIAMDD radioimmunoassay kit supplied by Dr. Albert Parlow with methods described previously (14). The intra- and interassay coefficients of variation were 7.6 and 11.1% respectively, while the sensitivity was 3 ng/ml.

Statistical analyses. Analysis of variance and least significant difference tests were performed on logarithmically transformed data to evaluate the significance of means. Serum prolactin concentrations obtained at 0500 and 1900 hr were considered "surge" levels if they were significantly greater than those of samples collected at 1200 hr.

Experiments. I. The first experiment was designed to determine the effects of various methods of cervical stimulation on maintenance of prolactin surges in acutely ovariectomized rats. The cervices of proestrous rats were stimulated (a) by natural mating, (b) mechanically at 1700 hr on proestrus only, (c) mechanically at 1700 hr on proestrus and again at 0800 hr on estrus, (d) electrically at 1700 hr on proestrus only, or (e) electrically at 1700 hr on proestrus and 0800 hr on estrus. All animals were ovariectomized between 0900 and 1000 hr on estrus Day 0 and blood samples were collected by decapitation at 0500, 1200, and 1900 hr on Day 10.

II. The next experiment was designed to determine if the method of cervical stimulation influences the maintenance of prolactin surges in long-term ovariectomized rats. On the 14th day after ovariectomy groups of rats were stimulated (a) mechanically at 1700 hr. (b) mechanically at 1700 hr and again at 0800 hr the following morning, (c) electrically at 1700 hr, and (d) electrically at 1700 hr and again at 0800 hr the following morning. Blood samples were collected on Day 10 by decapitation at 0500, 1200, and 1900 hr.

Results. Figure 1 illustrates the serum concentration of prolactin on Day 10 in rats acutely ovariectomized on estrus after nat-

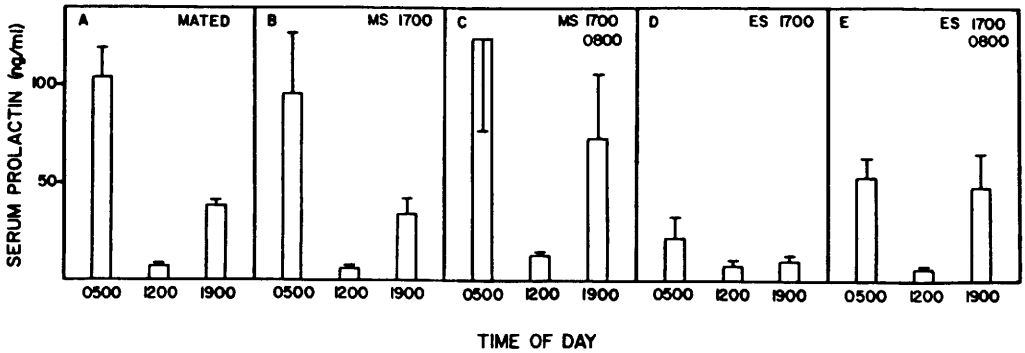

FIG. 1. The serum concentration of prolactin (ng/ml; mean ± SE) at 0500, 1200, and 1900 hr in rats on the 10th day after (A) mating on proestrus and ovariectomy on estrus, (B) mechanical stimulation of their cervices on proestrus at 1700 hr and ovariectomy on estrus, (C) mechanical stimulation of their cervices on proestrus at 1700 hr and again on estrus at 0800 hr followed by ovariectomy, (D) electrical stimulation of their cervices on proestrus at 1700 hr and ovariectomy on estrus, and (E) electrical stimulation of their cervices on proestrus at 1700 hr and again on estrus at 0800 hr followed by ovariectomy. Each bar represents the mean of five rats.

ural mating or various methods of artificial stimulation of the uterine cervix. Both noctural and diurnal surges of prolactin were still present on the 10th day after ovariectomy in mated rats (Fig. 1A), and in rats receiving mechanical simulation on only proestrus (Fig. 1B) or on proestrus and estrus (Fig. 1C). Electrical stimulation of the uterine cervix on proestrus and estrus also induced surges which continued until day 10 (Fig. 1E). However, animals receiving an electrical stimulus of their uterine cervices on proestrus only (Fig. 1D) secreted neither nocturnal nor diurnal surges of prolactin when sampled on Day 10.

Figure 2 illustrates the serum concentration of prolactin in long-term ovariectomized rats on the tenth day after various methods of cervical stimulation. Rats subjected to mechanical stimulation of the cervix at 1700 hr (Fig. 2A) or 1700 and 0800 hr the following morning (Fig. 2B) presented nocturnal ($P < 0.001$) but no diurnal surges of prolactin on Day 10. Neither nocturnal nor diurnal surges were secreted in rats receiving a single electrical stimulus of their

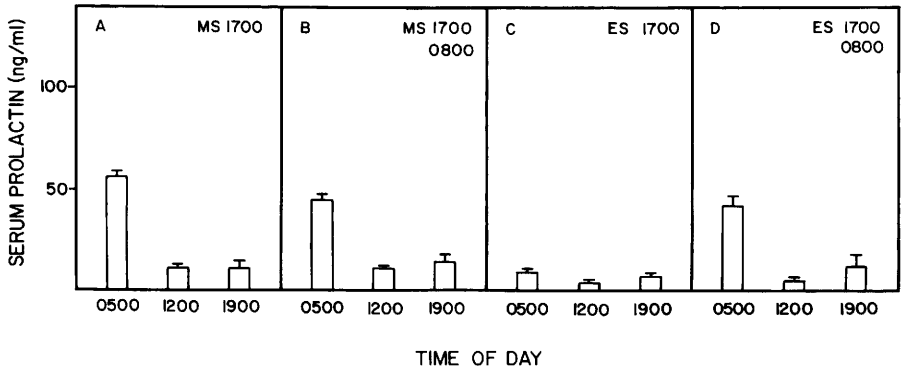

FIG. 2. The serum concentration of prolactin (ng/ml; mean ± SE) in long-term ovariectomized rats at 0500, 1200, and 1900 hr on the 10th day after (A) mechanical stimulation of their cervices at 1700 hr. (B) mechanical stimulation of their cervices at 1700 hr and again at 0800 hr the following morning, (C) electrical stimulation of their cervices at 1700 hr, and (D) electrical stimulation of their cervices at 1700 hr and again at 0800 hr the following morning. Each bar represents the mean of five rats.

cervices 10 days previously (Fig. 2C). Electrical stimulation of the uterine cervix twice (Fig. 2D) resulted in a nocturnal surge of prolactin which persisted until Day 10 ($P < 0.001$). The diurnal surges were not significantly elevated ($P > 0.05$) since only 40% of the rats sampled at this time had elevated prolactin levels (Fig. 2D). In addition, the nocturnal surges of all groups were significantly lower than those of acutely ovariectomized rats. (Compare Figs. 1 and 2.)

Discussion. The initiation of pregnancy or pseudopregnancy is dependent upon the intensity and frequency of the mating stimulus (1–6). Since prolactin provides the initial luteotrophic support which determines the successful maintenance of the pregnancy or pseudopregnancy (18), it follows that the *initiation* of at least the nocturnal surge of prolactin (12, 19) is also dependent upon the intensity of the mating stimulus (11). As clearly shown by the present experiments, the *maintenance* of prolactin surges is also dependent upon the method of cervical stimulation.

Earlier experiments suggested that artificial stimulation of the uterine cervix excited a "mnemonic system" which retained the stimulus and expressed it as nocturnal surges of prolactin for up to 6 days (12). It was proposed that after this time ovarian steroids were required for continued maintenance of the surges (12, 14, 20). Such conclusions were based upon the fact that mechanical stimulation of the cervix on proestrus followed by ovariectomy on estrus or shortly thereafter initiated surges of prolactin which recurred until Day 6 (12, 20). Moreover, surges were not maintained until Day 10 in long-term ovariectomized rats whose cervices were electrically stimulated on a single day unless the animals had sufficient circulating levels of progesterone (14). In contrast, we have recently observed that long-term ovariectomized rats respond to electrical stimulation of their cervices at 1700 hr and again at 0800 hr the following morning with prolactin surges which persist for 12 days (15). Thus, under physiological conditions the storage of the stimulus may not be limited to 6 days. Indeed, fully normal nocturnal and diurnal surges of prolactin were present on Day 10 after ovariectomy in rats permitted to mate with males through the night (Fig. 1A). Similar surges were maintained through Day 10 in rats which were ovariectomized after mechanical or electrical stimulation of the cervix on proestrus and estrus (Figs. 1C and 1E). Contrary to previous observations (12, 20) even mechanical stimulation of the cervix on proestrus followed by ovariectomy on estrus led to nocturnal and diurnal surges of prolactin on Day 10 (Fig. 1B). On the other hand, rats receiving electrical stimulation of the cervix on only the day prior to ovariectomy no longer secreted prolactin surges on Day 10 (Fig. 1D). Similar results were obtained when long-term ovariectomized rats were subjected to various methods of cervical stimulation (Figs. 2A–D). However, in contrast to acutely ovariectomized rats, long-term ovariectomized rats had lower nocturnal surges and the majority had no diurnal surges by Day 10 (Figs. 2A, B, D). Thus, the steroid milieu at the time of cervical stimulation determines the magnitude of nocturnal and the presence of diurnal surges of prolactin 10 days later. Taken together these data suggest that the stimulus provided by the male can be effectively simulated by mechanical or electrical stimulations of the cervix separated by a 15-hr interval.

Given the availability of a vigorous male, the greatest physiological stimulus for initiation of prolactin surges in the female is provided by the male's high intromission frequency (11) over the 9–15 hr interval that she is sexually receptive. Thus the optimal combination for "storage" of the stimulus (17) is high intromission frequency and an extended intercopulatory interval achieved under physiological conditions (5, 6). The consequences of such storage is repetition of two daily surges of prolactin for 12 days (15). It is clear that the stimulus is stored through this time and the ovaries are not required for its reinforcement (15). Therefore, the ability of ovarian progesterone to maintain prolactin surges beyond Day 6 (12, 14, 15) can be envisioned as a physiological "fail safe mechanism" which

ensures that prolactin surges will continue when the copulatory stimulus is suboptimal (11).

1. Adler, N. T., Anat. Rec. **160**, 305 (1968).
2. Adler, N. T., J. Comp. Physiol. Psychol. **69**, 613 (1969).
3. Chester, R. V., and Zucker, E., Physiol. Behav. **5**, 35 (1970).
4. Wilson, J. R., Adler, N. T., and LeBoeuf, B., Proc. Nat. Acad. Sci. USA **53**, 1392 (1965).
5. Edmonds, S., Zoloth, S. R., and Adler, N. T., Physiol. Behav. **8**, 161 (1972).
6. Gilman, D. P., Mercer, L. F., and Hitt, J. C., Physiol. Behav. **22**, 675 (1979).
7. Beach, F. A. (ed.), "Sex and Behavior." Wiley New York. (1965).
8. Adler, N. T., Resko, J. A., and Goy, R. W., Physiol. Behav. **5**, 1003 (1970).
9. Butcher, R. L., Fugo, N. W., and Collins, W. E., Endocrinology **90**, 1125 (1972).
10. Smith, M. S., Freeman, M. E., and Neill, J. D., Endocrinology **96**, 219 (1975).
11. Terkel, J., and Sawyer, C. H., Horm. Behav. **11**, 304 (1978).
12. Freeman, M. E., Smith, M. S., Nazian, S. J., and Neill, J. D., Endocrinology **94**, 875 (1974).
13. Welschen, R., Osman, P., Dullart, J., DeGreef, W. J., Uilenbroek, J., and DeJong, F. H., J. Endocrinol. **64**, 37 (1975).
14. Freeman, M. E., and Sterman, J., Endocrinology **102**, 1915 (1978).
15. Gorospe, W. C., and Freeman, M. E., Endocrinology, in press, 1981.
16. Smith, M. S., and Neill, J. D., Endocrinology **98**, 696 (1976).
17. Everett, J. W., Endocrinology **80**, 145 (1967).
18. Smith, M. S., McLean, B. K., and Neill, J. D., Endocrinology **98**, 1370 (1976).
19. Freeman, M. E., and Neill, J. D., Endocrinology **90**, 1292 (1972).
20. Murakami, N., Takahashi, M., and Suzuki, Y., Biol. Reprod. **21**, 263 (1979).

Received November 17, 1980. P.S.E.B.M. 1981, Vol 167.