PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 229-231 (1976)

Effect of Nonsuckling Interval in the Ether-Induced Release of Immunoprecipitable Prolactin into the Plasma of the Lactating Rat (39367)

C. E. GROSVENOR AND N. S. WHITWORTH

Department of Physiology and Biophysics, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163

The concentration of prolactin in the plasma quickly rises following the onset of ether inhalation in the nonlactating rat (1–8, though see 9). The plasma concentration, however, seldom exceeds 50 ng/ml and is sustained for only a few minutes. Prolonged ether anaesthesia has no effect upon plasma prolactin levels (6). In the lactating rat, the plasma prolactin concentration following ether inhalation exhibits a pattern similar to that obtained in nonlactating rats, but contrasts markedly with the 200–300 ng/ml which appear in the plasma after 10–12 min of suckling (10–15) and which can be maintained for periods up to 90 min by continued suckling (14–15).

Since the release of prolactin by suckling can be modified by a variety of factors (16) it was of interest to determine which factors might modify the release of prolactin in response to stress in the lactating rat. In the present study, we have analyzed the influence of the nonsuckling interval.

Materials and methods. Primiparous lactating rats of the Holtzman strain were housed in individual cages in a room maintained at 23–25° and with alternating 14-hr light:10-hr dark periods. They were given a starch–casein–sucrose diet to which 5% cellulose was added for fecal bulk; tap water was available *ad libitum*. Litters were adjusted to eight pups on Days 1–2 postpartum and to six pups on Day 4 postpartum. The mothers were handled periodically during the lactation period.

Two experimental manipulations of the nonsuckling interval were made prior to application of an ether stress. (i) The pups were removed on the morning of Day 14 and the mothers were subjected to ether stress either 8 or 12 hr later. (ii) The pups were removed the morning of Day 13. During the next 12 hr, they were returned to their mothers either for a single 10-min period of suckling (at the end of the twelfth hour), for two 10-min periods of suckling (at the sixth and twelfth hours), or for three 10-min periods of suckling (at the fourth, eighth, and twelfth hours). The pups were discarded at the completion of the twelfth hour suckling and, after a subsequent 12-hr period allowed to standardize pituitary prolactin levels, the mothers in each groups were subjected to ether stress.

Ether stress. Ether (anesthesia grade) was placed in a wide-mouth glass jar (22 × 25 cm) which was covered and placed on a lab stool close to the cage of the mother to be tested. Approximately 5 min later (the 5 min to allow for the atmosphere in the jar to become heavily saturated with ether fumes) the rat was gently removed from her cage and placed into the jar. The animals were etherized in this manner in groups of three to four rats at a time. Once the rats became unconscious (usually within 1 min) they were removed from the jar, quickly guillotined and the trunk blood collected in cooled test tubes. The plasma was separated and kept frozen until later assayed for prolactin. Control mothers (litters removed 6 hr earlier) were gently removed from their cages and swiftly guillotined.

Radioimmunoassay of prolactin. Prolactin in plasma was determined in duplicate 25-μl samples by the NIAMDD radioimmunoassay system (See 14, for details). Mean differences in plasma prolactin concentration between groups were judged significantly different (using Student's t test) if the probability was 5% or less.

Results. In the first experiment (Fig. 1A), the concentration of prolactin in the plasma of lactating rats averaged 74 ng/ml (46–160 ng/ml range) following application of acute ether stress after an 8-hr period of nonsuckling. When ether stress was applied after a 12-hr period of nonsuckling, the plasma level of prolactin was significantly lower and averaged 24 ng/ml (18–40 ng/ml range).

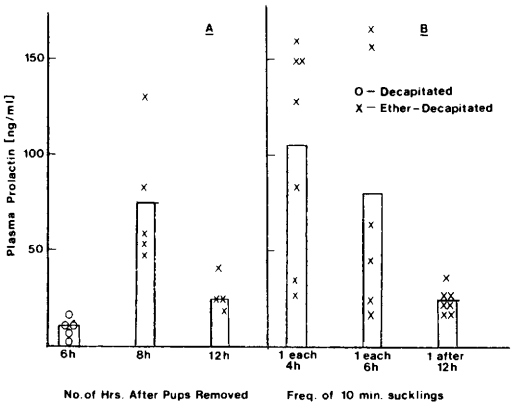

FIG. 1. Prolactin concentration in plasma of lactating rats following acute exposure to ether before decapitation. (A) Rats nonsuckled for either 8 or 12 hr prior to ether exposure. (B) Rats suckled, one, two, or three times, each time for 10 min during an initial 12-hr nonsuckling period; all rats then were exposed to ether 12 hr after the last suckling. Bars represent mean values; each symbol represents a single rat.

In the second experiment (Fig. 1B), the plasma prolactin concentration following acute ether stress increased significantly from 24 ng/ml (17–36 ng/ml range) to 104 ng/ml (26–160 ng/ml range) as the frequency of 10-min sucklings was experimentally altered during a 12-hr period from once at the end of the 12-hr period to once every 4 hr. The intermediate frequency of suckling once every 6 hr resulted in an average plasma prolactin concentration of 79 ng/ml. The range of values in this group, however, overlapped with those of the other two groups and thus the mean concentration was not significantly different from that of either of the other two groups. All groups exposed to ether had mean plasma prolactin concentrations which were significantly elevated over that of rats isolated for 6 hr and similarly killed but without being exposed to ether (Fig. 1).

Discussion. Various factors can influence the degree to which the concentration of prolactin increases in the plasma following stress. Neil (1) found that a consistently higher plasma prolactin concentration occurred each day throughout the estrous cycle following ether–laparotomy stress, in comparison with that found in plasma of decapitated rats, except on the afternoon of proestrous when ether–laparotomy failed to increase the already elevated levels. Reir *et al.* (7) demonstrated that in rats treated for 6 days with estradiol the serum prolactin level resulting from ether–laparotomy stress was raised, whereas, following treatment with progesterone it was lowered. Progesterone also reduced the estrogen-stimulated elevation in prolactin levels provoked by ether–laparotomy, an effect previously shown by Chen and Meites (17). Serum prolactin levels resulting from ether inhalation were shown to increase twofold in male and female rats when they were handled twice daily the previous 4 days (2). It is possible, however, that handling in this instance constituted an additional stress.

In the present investigation the plasma prolactin concentration in the lactating rat was significantly more elevated when ether was inhaled after 8 hr than after 12 hr of nonsuckling. When the suckling frequency during a 12-hr period was increased in groups of rats from one 10-min suckling at the end of the twelfth hour to a 10-min suckling once every 4 hr and the rats then exposed to ether 12 hr after the last suckling, we observed the same relationship between length of the nonsuckling interval and the rise in plasma prolactin in response to ether, i.e., the shorter nonsuckling interval resulted in the more elevated prolactin concentration. Prolactin concentration in the anterior pituitary is approximately the same after nonsuckling intervals of 4, 8, and 12 hr (16) so a difference in available prolactin would not appear to be involved in the explanation of these differences. Rather it would appear that more frequent activation of the prolactin releasing mechanism by suckling in some way facilitates the subsequent release of prolactin in response to ether. A central serotinergic mechanism has been implicated in both the suckling-induced (13) and ether-induced (18) release of prolactin. The mechanism of release of prolactin by suckling also contains a hypothalamic dopaminergic system (19, 20) which appears not to be implicated in the ether-induced release (7, 18). Thus, the fact that the frequency of suckling in some way facilitates the release of prolactin in response to ether as well as that in response to suckling (16) suggests the serotinergic link may be common to both mechanisms.

Summary. The concentration of prolactin

in the plasma of lactating rats increased from 24 to 74 ng/ml following acute ether stress as the length of the preceding period of nonsuckling diminished from 12 to 8 hr. In a second experiment the prolactin concentration increased from 24 to 79 to 104 ng/ml as the frequency of 10-min sucklings during a 12-hr period was altered from once at the end of the twelfth hr to once every 6 hr to once every 4 hr and the mothers then exposed to ether 12 hr after the last suckling. These results indicate that more frequent activation of the prolactin-releasing mechanism facilitates the subsequent release of prolactin in response to ether stress.

We are grateful to the NIAMDD Rat Pituitary Hormone Distribution Program for the rat prolactin radioimmunoassay kit, and to Mrs. Tanya McGee for technical assistance.

1. Neil, J. D., Endocrinology **87,** 1192 (1970).
2. Wakabayashi, I., Arimura, A., and Schally, A. V., Proc. Soc. Exp. Biol. Med. **137,** 1189 (1971).
3. Ajika, K., Kalra, S. P., Fawcett, C. P., Krulich, L., and McCann, S. M., Endocrinology **90,** 707 (1972).
4. Watson, J. T., Krulich, L., and McCann, S. M., Endocrinology **89,** 1412 (1971).
5. Valverde-R, C., Chieffo, V., and Reichlin, S., Life Sci. **12,** 329 (1973).
6. Krulich, L., Hefco, E., Illner, P., and Read, C. B., Neuroendocrinology **16,** 293 (1974).
7. Reier, P. J., Morishige, W. K., and Rothchild, I., Neuroendocrinology **16,** 43 (1974).
8. Euker, J. S., Meites, J., and Riegle, G. D., Endocrinology **96,** 85 (1975).
9. Wuttke, W., and Meites, J., Proc. Soc. Exp. Biol. Med. **135,** 648 (1970).
10. Blake, C. A., Endocrinology **94,** 503 (1974).
11. Blake, C. A., and Sawyer, C. H., Science **177,** 619 (1972).
12. Terkel, J., Blake, C. A., and Sawyer, C. H., Endocrinology **91,** 49 (1972).
13. Kordon, C., Blake, C. A., Terkel, J., and Sawyer, C. H., Neuroendocrinology **13,** 213 (1972).
14. Grosvenor, C. E., and Whitworth, N. S., J. Dairy Sci. **57,** 900 (1974).
15. Subramanian, M. G., and Reece, R. P., Proc. Soc. Exp. Biol. Med. **149,** 754 (1975).
16. Grosvenor, C. E., and Mena, F., Bi. Sym. Anim. Reprod. **32,** 115 (1971).
17. Chen, C. L., and Meites, J., Endocrinology **86,** 503 (1970).
18. Marchlewska-Koj, A., and Krulich, L., Fed. Proc. **34,** 252 (1975).
19. Chen, H. J., Mueller, G. P., and Meites, J., Endocrine Res. Commun. **1,** 238 (1974).
20. Voogt, J. L., and Carr, L. A., Endocrinology **97,** 891 (1975).

Received December 1, 1975. P. S. E. B. M. 1976. Vol. 152.