

Anterior Pituitary and Plasma Prolactin in Rats After 2 to 90 Minutes of Suckling^{1,2} (38892)

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The importance of the suckling stimulus in the maintenance of lactation has been demonstrated (1-3) and the stimulus decreases anterior pituitary (AP) prolactin (PRL) content (4-6) and increases plasma PRL (7-9). However, no systematic study has been reported comparing the pattern of PRL release in response to different lengths of suckling, after a standard period of litter separation, in rats in early and in late lactation.

Materials and Methods. Hooded Norway lactating rats were housed in individual cages in a room maintained at $74 \pm 2^\circ \text{F}$ with 14 hr light and 10 hr darkness. Rats received Purina Laboratory Chow and water ad lib. Litter size was adjusted to eight pups on day 2 of lactation and dams were sacrificed in either early (4-8 days) or late (20-22 days) lactation. In all groups, prior to sacrifice, pups were removed for a 6-hr separation period (8 AM to 2 PM) at which time control rats were sacrificed and the experimental rats were allowed to suckle their litters for either 2, 30, 60, or 90 min before sacrifice. Dams were sacrificed by guillotine within 10 sec of handling. Blood from the trunk was collected into heparinized ice-cold tubes, centrifuged at 3090g for 15 min, plasma separated and stored at -20° until assayed. APs were collected, weighed, sonified in phosphate buffer (pH 7.6), diluted 1:1000, and aliquots stored at -20° until assayed.

The PRL content in plasma and APs was estimated by the double-antibody radioimmunoassay (RIA) method as described by the National Institute of Arthritis and Meta-

bolic Diseases (NIAMD), National Institutes of Health, Rat Pituitary Hormone Distribution Program. All samples were assayed in duplicate and values averaged. PRL is expressed in terms of NIAMD-Rat Prolactin-RP-1 which had a potency of 11 IU per mg.³ The data were subjected to statistical analysis for significance of difference and differences among groups were estimated by the least significant difference method (10).

Results. Early stage of lactation (4-8). The plasma PRL level, 6 hr after litter separation, was 48 ± 5.8 ng per ml. Two minutes of suckling after the separation of litter for 6 hr resulted in a significant increase in plasma PRL to 294 ± 36.9 ng per ml ($P < 0.01$). The other three durations of suckling used, viz., 30, 60, and 90 min, also resulted in increases of plasma PRL levels over the non-suckled controls ($P < 0.01$). The differences in the levels of plasma PRL reached after 2 min of suckling and 60 min of suckling, and 2 min of suckling and 90 min of suckling were significantly different ($P < 0.05$). The differences in plasma PRL level of all other group comparisons (2 vs 30; 30 vs 60; 30 vs 90; and 60 vs 90) were not significant.

Litter separation for 6 hr resulted in an AP PRL content of 53.6 ± 5.3 μg and 6.1 ± 0.6 μg per mg AP. Stimuli of all four durations of suckling decreased PRL concentration ($P < 0.01$). The differences in AP PRL concentration among the suckled groups were not significant (Table I).

Regression analysis of the plasma PRL levels and AP PRL concentration after litter removal and different durations of suckling showed a highly significant correlation ($P < 0.01$) between the fall in AP PRL

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TABLE I. EFFECTS OF SUCKLING FOR 2, 30, 60, AND 90 MIN AFTER 6-HR LITTER SEPARATION ON PLASMA AND AP PROLACTIN LEVELS IN EARLY LACTATION (4-8 DAYS).^a

Treatment	Plasma prolactin ^b (ng per ml)	Total AP prolactin (μ g)	Prolactin per mg AP ^c (μ g)
Nonsuckled	48 $\pm$ 5.8 ^d	53.6 $\pm$ 5.3	6.1 $\pm$ 0.6 ^d
2-min suckling	294 $\pm$ 36.9 ^e	39.5 $\pm$ 4.5	3.8 $\pm$ 0.4 ^f
30-min suckling	425 $\pm$ 25.4 ^f	25.5 $\pm$ 2.3	2.8 $\pm$ 0.2 ^f
60-min suckling	477 $\pm$ 68.5 ^e	26.3 $\pm$ 4.1	2.7 $\pm$ 0.5 ^f
90-min suckling	454 $\pm$ 60.5 ^e	27.8 $\pm$ 6.3	3.1 $\pm$ 0.6 ^f

^a Values are mean $\pm$ SEM of five observations.^b LSD (.01): 183.5, LSD (.05): 134.5.^c LSD (.01): 1.8, LSD (.05): 1.4.^d Significantly different from the other four groups ($P < 0.01$).^e Significantly different from nonsuckled ($P < 0.01$), 60-min suckling ($P < 0.05$) and 90-min suckling ($P < 0.05$).^f Significantly different from nonsuckled ($P < 0.01$).^g Significantly different from nonsuckled ($P < 0.01$) and 2-min suckling ($P < 0.05$).TABLE II. EFFECTS OF SUCKLING FOR 2, 30, 60, AND 90 MIN AFTER 6-HR LITTER SEPARATION ON PLASMA AND AP PROLACTIN LEVELS IN LATE LACTATION (20-22 DAYS).^a

Treatment	Plasma prolactin ^b (ng per ml)	Total AP prolactin (μ g)	Prolactin per mg AP ^c (μ g)
Nonsuckled	45 $\pm$ 5.7 ^d	56.4 $\pm$ 4.8	6.3 $\pm$ 0.5 ^e
2-min suckling	78 $\pm$ 6.4 ^e	27.4 $\pm$ 4.7	3.1 $\pm$ 0.4 ^h
30-min suckling	106 $\pm$ 21.4 ^f	31.5 $\pm$ 8.8	3.9 $\pm$ 0.9 ^f
60-min suckling	92 $\pm$ 9.6 ^e	25.9 $\pm$ 6.5	3.2 $\pm$ 0.7 ^h
90-min suckling	97 $\pm$ 33.2 ^e	25.6 $\pm$ 7.7	3.1 $\pm$ 0.9 ^h

^a Values are mean $\pm$ SEM of five observations.^b LSD (.01): 74.7, LSD (.05): 55.3.^c LSD (.01): 2.9, LSD (.05): 2.1.^d Significantly different from the 30-min suckling ($P < 0.05$).^e Not significantly different from other groups.^f Significantly different from nonsuckled ($P < 0.05$).^g Significantly different from 2-, 60-, and 90-min suckling ($P < 0.01$). and 30 min suckling ($P < 0.05$).^h Significantly different from nonsuckled ($P < 0.01$).

concentrations and the increase in plasma PRL levels ($r = .83$).

Late stage of lactation (20-22 days). The plasma PRL level after litter separation was 45 ± 5.7 ng per ml. Two, 30, 60, or 90 min of suckling after litter separation for 6 hr did not result in an increase in plasma PRL levels as observed in the early stage of lactation. A significant difference in the plasma PRL level was seen only between the nonsuckled group and the 30-min-suckled group ($P < 0.05$).

The AP PRL concentration of 6.3 ± 0.5

μ g per mg AP in the nonsuckled groups was significantly higher than that of the four suckled groups (Table II). Regression analysis of the fall in AP PRL concentrations and rise in plasma PRL levels in response to 2, 30, 60, and 90 min of suckling showed a low correlation ($r = .16$).

Discussion. It has been reported that 30 min of suckling decreased AP PRL level up to 95% (6) and that 2 min of suckling by six pups, after 8 hr of nonsuckling, induced a decrease in AP PRL concentration equivalent to that of 30 min of suckling (11). Our

results agree with these reports and furthermore in early as well as late stages of lactation all four durations of suckling resulted in highly significant decreases in AP PRL concentration compared with those in non-suckled rats. It has also been reported that plasma PRL levels were not significantly elevated after 4–5 min of vigorous suckling (12) whereas in our study 2 min of suckling resulted in highly significant increases in plasma PRL in rats in early lactation.

Since the decrease in AP PRL contents and concentrations of suckled rats in late lactation were similar to those of rats in early lactation, but the plasma PRL levels were much lower, it is suggested that PRL is removed more rapidly from the blood during late lactation than it is in early lactation. It has been shown that PRL transfer into milk is dependent on a concentration gradient (13) and the synthetic activity of the mammary gland of the hooded Norway rat is greater in late lactation than it is in early lactation as indicated by the RNA/DNA ratios, 2.95 vs 1.55 (14).

Summary. Separation of litter for 6 hr resulted in plasma PRL levels of 48 ± 5.8 and 45 ± 5.7 ng per ml and AP PRL concentrations of 6.1 ± 0.6 and 6.3 ± 0.5 μ g per mg AP in early (4–8 days) and late (20–22 days) lactation, respectively. After a 6-hr separation period, suckling for 2, 30, 60, and 90 min resulted in highly significant increases in the plasma PRL level in early

lactation. Increases in plasma PRL levels were not seen in late lactation except in the 30-min suckled group. In both stages of lactation the AP PRL concentrations in the suckled groups were significantly lower than in the nonsuckled groups.

1. Selye, H., *Amer. J. Physiol.* **107**, 535 (1934).
2. Selye, H., Collip, J. B., and Thompson, D. L., *Endocrinology* **18**, 237 (1934).
3. Ingelbrecht, P., *C. R. Soc. Biol.* **120**, 1369 (1935).
4. Reece, R. P., and Turner, C. W., *Proc. Soc. Exp. Biol. Med.* **35**, 367 (1936).
5. Reece, R. P., and Turner, C. W., *Proc. Soc. Exp. Biol. Med.* **35**, 621 (1937).
6. Grosvenor, C. E., and Turner, C. W., *Endocrinology* **63**, 535 (1958).
7. Amenomori, Y., Chen, C. L., and Meites, J., *Endocrinology* **86**, 506 (1970).
8. Yanai, R., and Nagasawa, H., *Horm. Res.* **4**, 169 (1973).
9. Shiino, M., Williams, G., and Rennels, E. G., *Endocrinology* **90**, 176 (1972).
10. Steel, R. G. D., Torrie, J. H., "Principles and Procedures in Statistics," McGraw-Hill, New York (1960).
11. Grosvenor, C. E., Mena, F., and Schaeffgen, D. A., *Endocrinology* **81**, 449 (1967).
12. Terkel, J., Blake, C. A., and Sawyer, C. H., *Endocrinology* **91**, 49 (1973).
13. McMurtry, J. P., and Malven, P. V. *Endocrinology* **95**, 559 (1974).
14. Tucker, H. A., and Reece, R. P., *Proc. Soc. Exp. Biol. Med.* **112**, 409 (1963).

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