Temporal Effect of Prolactin on the Activities of Lactose Synthetase, α-Lactalbumin, and Galactosyl Transferase in Mouse Mammary Gland Explants (43278)

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Abstract. **The onset of the prolactin (PRL) stimulation of lactose synthesis is between 4 and 8 hr after adding PRL to cultured mouse mammary tissues. The synthesis of lactose is catalyzed by the enzyme lactose synthetase, which is composed of two parts, α-lactalbumin and galactosyl transferase. In time-sequence studies, it was found that the activity of galactosyl transferase is enhanced by PRL in concert with the onset of the PRL stimulation of lactose synthesis. In contrast, the earliest detectable effect of PRL on α-lactalbumin activity occurred 24 hr after adding PRL to the cultures. It is, therefore, apparent that the rate-limiting component for the PRL stimulation of lactose synthesis in cultured mouse mammary tissues is galactosyl transferase activity.**

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The primary sugar component of milk is lactose. In cultured mammary gland explants derived from mice that are 12–14 days pregnant, a 4–6-hr exposure period is required before prolactin (PRL) stimulates the rate of lactose biosynthesis (1). It is well-known that lactose synthesis is catalyzed by the enzyme lactose synthetase; this enzyme is composed of two proteins: the ubiquitous A protein named galactosyl transferase (GT), and a B protein called α-lactalbumin (2, 3). α-Lactalbumin is the specifier in the lactose biosynthetic process; the α-lactalbumin changes the affinity of galactosyl transferase such that it favors glucose as the substrate (2, 4, 5). Alone, α-lactalbumin has no catalytic activity (6), whereas GT can act alone in forming other complex sugars (5, 7). Thus, it has been surmised by many investigators that the mechanism by which PRL stimulates lactose synthesis in the mammary gland is by enhancing the rate of α-lactalbumin synthesis (4). On the other hand, it has been reported by Bolander and Topper (8) that the activity of lactose synthetase more closely follows the activity of GT than the tissue content of α-lactalbumin.

The purpose of the present studies was to determine whether α-lactalbumin and/or GT activity is rate limiting for the onset of the PRL stimulation of lactose synthetase activity, and hence the synthesis of lactose, in mouse mammary gland explants.

Materials and Methods

Materials. Midpregnant (10–14 days of pregnancy) Swiss-Webster mice were used in all experiments; they were purchased from Harlan Laboratories, Inc. (Indianapolis, IN). Ovine PRL was a gift from National Institute of Arthritis, Metabolism, and Digestive Diseases. Other substances were purchased from the following sources: cortisol from Charles Pfizer and Co. (New York, NY); Hanks' balanced salt solution and medium 199-Earle's salts from Gibco Laboratories (Grand Island, NY); [5,6-^{3}H]glucose (82.9 Ci/mmol) and UDP-[^{14}C]galactose (272.8 mCi/mmol) from New England Nuclear Corp. (Boston, MA); porcine insulin and streptomycin from Eli Lilly Co. (Indianapolis, IN); UDP-galactose, bovine galactosyl transferase (3.0 units/mg solid), bovine α-lactalbumin, *N*-acetylglucosamine, glucose, and crystalline bovine serum albumin (Fraction V) from Sigma Chemical Co. (St. Louis, MO); AG 1 × 8 ion exchange resin from Bio-Rad Laboratories (Richmond, CA); and cellulose thin layer chromatography plates (250-μM nonfluorescent indicator) and spectran-

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alyzed 2-propanol from Fisher Chemical Co. (Waltham, MA).

Organ Culture. Mice were sacrificed by cervical dislocation and the caudal pair of mammary glands were removed and placed in Hanks' balanced salt solution. Explants were then prepared as described previously (9, 10). Briefly, the glands were cut into pieces weighing 3–5 mg, and 24 pieces, four from each of six mice, were placed in a dish on siliconized lens paper floating on 6 ml of medium 199-Earle's salts containing 1.0 μg/ml insulin and 10^{-7} *M* cortisol; tissues were then incubated for 24 hr. Each dish of explant pieces was treated as one observation. All incubations were carried out in 60 × 15 mm petri dishes maintained at 37°C in an atmosphere of 95% air and 5% CO_2. The explants were subsequently exposed to 1 μg/ml PRL for the times indicated in the tables.

To determine the rate of lactose synthesis, the tissues were pulse-labeled with 1 μCi/ml [5,6-^{3}H]glucose for the last 2 hr of incubation. After pulse-labeling, the explants were removed, blotted, and weighed. They were then homogenized in 1/100 (w/v) 15% trichloroacetic acid and centrifuged at 2000*g* for 10 min. Labeled lactose was separated from the labeled glucose on the thin layer chromatography plates using 30-μl aliquots of the supernatant; the plates were developed in the 2-propanol to water ratio system as described previously (1). Visualization of standards was accomplished with a benzidine reagent (11). Labeled lactose areas on the cellulose plates were scraped into scintillation vials and radioactivity was determined via liquid scintillation counting. The rate of [5,6-^{3}H]glucose incorporation into lactose was expressed as disintegrations per minute (dPM) per milligram of wet tissue weight and was used as an index of the rate of lactose biosynthesis. In preliminary studies, the incorporation of [5,6-^{3}H)glucose into lactose was found to be linear with time over a 120-min period (data not shown).

α-Lactalbumin/GT Activity. For these studies, tissues were taken at the end of incubation, blotted, weighed, and processed by the methods of Ono and Oka (12) and Bhattacharjee and Vonderhaar (13), as modified from the methods of Fitzgerald *et al.* (5). Briefly, the tissues were homogenized (ground glass) quickly in 1/100 (w/v) ice-cold Buffer A solution containing 20 m*M* Tris/HCl (pH 7.7, at 25°C), 10 m*M* $MgCl_2$, 100 m*M* KCL, 2% Triton X-100, and 0.1% bovine serum albumin (w/v). The homogenate was centrifuged at 12,000*g* for 20 min at 4°C. The fat-free supernatant was removed, placed in clean tubes, and maintained at 0°C. In some experiments, the supernatant was frozen and used at a later time to determine α-lactalbumin activity; in preliminary studies, it was found that α-lactalbumin activity was maintained in samples that were stored frozen. For each time point tested, six plates of tissues were employed (three control and three PRL treated). Each supernatant sample was tested in quintuplet. The GT assay was carried out immediately after preparation of the supernatant, since it was observed in preliminary studies that freezing reduced or destroyed endogeneous GT activity.

The activity of α-lactalbumin in the lactose synthesis reaction was measured in the presence of excess exogenous UDP-galactosyl transferase by a modification of the methods of Ono and Oka (12) and Bhattacharjee and Vonderhaar (13) using bovine α-lactalbumin as a standard. Briefly, 10 μl of prepared supernatant were combined with 50 μl of 0.04 *M* glucose acceptor solution (360.37 μg/50 μl Buffer A) and 40 μl of the enzyme solution, which was composed of Buffer A containing 0.34 μmol $MnCl_2$, 0.1 μmol ATP, 60 nmol UDP-galactose (containing 40,000 cPM of UDP-[^{14}C] galactose), and 0.02 units of purified galactosyl transferase. The enzyme reaction was carried out for 30 min at 37°C in a shaker bath. At 30 min the rate of product formation was linear with time and proportional to α-lactalbumin concentration. After the 30-min incubation, the reaction was stopped by placing the tubes on ice and adding 100 μl of 10^{-3} *M* lactose at 4°C. The total mixture was immediately passed through a column (0.5 × 6 cm) of Bio-Rad AG 1 × 8 anion exchange resin pre-equilibrated with a 10^{-3} *M* lactose solution at 4°C. The column was rinsed three times with 400 μl of a 10^{-3} *M* lactose solution at 4°C and the total eluate was collected in a scintillation vial. 1N HCl (600 μl) was added to the vials and radioactivity was determined by liquid scintillation techniques. Results are expressed as dPM/30 min/0.1 mg tissue. Ono and Oka (12) have reported a 98% recovery of tissue α-lactalbumin when employing the methods described.

To determine endogenous GT activity in the sample supernatants, the same procedure as employed for the α-lactalbumin assay was used, except that the glucose acceptor solution was replaced by 50 μl of a 0.04 *M* *N*-acetylglucosamine acceptor solution (442.40 μg/50 μl Buffer A) and the purified galactosyl transferase was omitted from the enzyme mixture. To correct for nonspecific hydrolysis of the UDP-[^{14}C]galactose in both assays, the acceptor solutions were replaced with 50 μl of Buffer A only. A 30-min reaction period was employed since the rate of product formation is linear with time through 30 min and proportional to substrate concentrations. Results are expressed as dpm/30 min/0.1 mg tissue. Optimal conditions for extraction of GT activity as originally set forth by Fitzgerald *et al.* (5) were employed in our studies. Although the exact recovery of GT from the tissues is not known, we have assumed that the percentage of recovery of GT from the control and prolactin-treated tissues does not differ.

Statistical analysis was carried out using Student's *t* test for the comparison of two population means. Each time point represents a unique experiment with

tissues pooled from six to nine animals. This accounts for the variance in control values.

Results

The temporal actions of PRL on α-lactalbumin activity and GT activity are shown in Tables I and II, respectively. As was pointed out above, we have reported earlier that the onset of the PRL stimulation of lactose biosynthesis is between 4 and 8 hr after PRL addition to the explants (1); the magnitude of the PRL effect on lactose synthesis is maximal by 12 hr and is maintained at 24 hr after PRL addition. The results of these time-course studies were confirmed with the same tissue preparations that were employed for the GT and α-lactalbumin activity determinations (data not included).

Table I shows the time course for the PRL stimulation of α-lactalbumin activity. Clearly, an effect of PRL on α-lactalbumin activity is not apparent until 24 hr after PRL treatment.

As is indicated by the data in Table II, GT activity increases in concert with the PRL stimulation of lactose biosynthesis. The onset of the PRL stimulation of GT activity occurs between 4 and 8 hr after PRL addition.

Table I. Time Course of PRL Stimulation of α-Lactalbumin Activity

Time (hr)	Treatment[a]		Percentage of increase
	Control (dpm)	PRL (dpm)	
4	2244 ± 51[b]	2163 ± 51	NS[c]
8	3316 ± 77	3497 ± 60	NS
12	3352 ± 235	3318 ± 85	NS
24	3833 ± 73	4582 ± 91	20

[a] Explants were incubated for 24 hr with insulin (1 μg/ml) and cortisol (10^{-7} *M*). Control groups and groups treated with PRL (1 μg/ml) were incubated for the times indicated. Explants were then processed and assayed for radioactivity as indicated in Materials and Methods.
[b] Values represent the mean ± SE of 15 observations.
[c] NS, not significant.

Table II. Time Course of PRL Stimulation of Galactosyl Transferase Activity

Time (hr)	Treatment[a]		Percentage of increase
	Control (dpm)	PRL (dpm)	
4	1719 ± 92[b]	1839 ± 105	NS[c]
8	853 ± 95	1092 ± 69	28
12	1227 ± 123	2325 ± 100	89
24	487 ± 36	1880 ± 70	286

[a] Explants were incubated for 24 hr with insulin (1 μg/ml) and cortisol (10^{-7} *M*). Control groups and groups treated with PRL (1 μg/ml) were incubated for the times indicated. Explants were then processed and assayed for radioactivity as indicated in Materials and Methods.
[b] Values represent the mean ± SE of 15 observation.
[c] NS, not significant.

The magnitude of this response continues to increase during a 24-hr culture period with PRL; at 24 hr, PRL elicits about a 3-fold increase in GT activity.

Discussion

Since lactose is the primary sugar component of milk, it is important to understand the regulation of its synthesis by PRL. Lactose is the final product of a biochemical pathway that is catalyzed by the enzyme complex lactose synthetase; this complex is composed of two molecular components, α-lactalbumin and galactosyl transferase. The GT protein is widely distributed among several cell types, whereas α-lactalbumin is localized in mammary alveolar epithelial cells. Accordingly, it has been assumed by most researchers that the α-lactalbumin content of mammary tissues is rate limiting for the hormonal regulation of lactose synthetase activity, and hence the rate of lactose biosynthesis (4). Other studies, however, indicate that GT activity is rate limiting for the rate of lactose formation in the mammary glands of lactating rats (14) and mature virgin mice (8). In addition, another study (15) suggests that during lactation, the availability of substrate may be a rate-limiting step for the synthesis of lactose.

The results of our studies support the conclusion that GT activity is rate limiting for the onset of the PRL stimulation of lactose synthesis in cultured mouse mammary tissues. This conclusion is based on the observations that the temporal effects of PRL on enhancing GT activity and the rate of lactose biosynthesis occurs in concert. In contrast, the onset of the PRL stimulation of α-lactalbumin activity occurs many hours subsequent to the initial PRL effects on GT activity and lactose synthesis. Since the biosynthesis of lactose requires the presence of α-lactalbumin, it is thus apparent that the cultured tissues must contain an adequate "basal" amount of α-lactalbumin to allow the initial expression of the PRL stimulation of lactose synthesis (between 8 and 24 hr).

When considering the physiological regulation of lactose production in a lactating subject, our results, as well as those discussed above (8, 14), clearly suggest that the regulation of GT activity by PRL may be an important component when considering the mechanism of regulation of lactose biosynthesis. Whether GT activity is necessarily more important than α-lactalbumin activity for the PRL regulation of the rate of lactose synthesis during lactation cannot be determined unequivocally from our studies; however, it can be inferred that the regulation of GT activity is likely an integral part of the mechanism by which the rate of lactose synthesis is regulated by PRL. Clearly, α-lactalbumin content of mammary tissues may not be rate limiting under all conditions for lactose synthesis. In addition, the PRL stimulation of GT activity may be permissive for the mechanism by which progesterone withdrawal,

at the time of parturition, causes an enhanced rate of lactose synthesis consequent to a greatly elevated rate of α-lactalbumin synthesis; GT activity is clearly elevated in the mammary gland after midgestation (16, 17).

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