Effects of Prolactin on Galactosyl Transferase and α-Lactalbumin mRNA Accumulation in Mouse Mammary Gland Explants (43913)

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Abstract. **Studies were carried out to determine the possible mechanism(s) by which prolactin (PRL) stimulates α-lactalbumin and galactosyl transferase activity in cultured mouse mammary tissues. In earlier studies it was shown that the onset of the PRL stimulation of galactosyl transferase activity occurs between 4 and 8 hr after adding prolactin to cultured mouse mammary gland explants, and a maximum effect is evoked by 24 hr. In contrast, an effect of prolactin on α-lactalbumin activity occurred 24 hr after adding PRL to mammary gland cultures, but not at earlier times. In the present studies, it is shown that prolactin effects an increased tissue accumulation of mRNAs for both α-lactalbumin and galactosyl transferase after a 4 to 6 hr culture with prolactin. The lowest concentration of prolactin that stimulates the accumulation of both galactosyl transferase and α-lactalbumin mRNA is approximately 10 ng/ml, and a maximum response is achieved with 200 ng/ml. Thus, it is likely that the effect of PRL on the activity of galactosyl transferase is causally associated with the effect of prolactin on the accumulation of its mRNAs. However, the PRL stimulation of α-lactalbumin activity is not temporally associated with the tissue accumulation of the mRNA for α-lactalbumin.** [P.S.E.B.M. 1995, Vol 209]

Lactose is secreted in copious quantities in milk. The synthesis of lactose is catalyzed by the enzyme lactose synthetase employing the substrates UDP-galactose and glucose. Lactose synthetase is composed of two components: the A protein commonly known as galactosyl transferase (GT) and the B protein termed α-lactalbumin (αLA) (1, 2). GT is widely distributed among several cell types, whereas αLA is localized in mammary epithelial cells (2). GT alone cannot catalyze the synthesis of lactose. Instead, it catalyzes the attachment of galactose to N-acetylglucosamine units on glycoproteins (3, 4). αLA alters the specificity of GT such that galactose is transferred to glucose rather than to N-acetylglucosamine (1, 3, 5). Thus, the induction of αLA indicates overt differentiation of mammary cells.

Jagoda and Rillema showed several years ago that prolactin stimulates αLA activity following a 24-hr culture period of mouse mammary gland explants with PRL (6). Additionally, the onset of the PRL stimulation of GT activity occurs between 4 and 8 hr after PRL treatment (6). These results indicate that the activity of GT is rate limiting for the onset of the PRL stimulation of lactose synthesis. The purpose of the present studies is to determine whether the stimulatory effect of PRL on αLA and GT activity occurs in concert with an increase in the tissue accumulation of αLA and/or GT mRNAs.

Materials and Methods

Midpregnant (10–14 days of pregnancy) Swiss-Webster mice were purchased from Harlan Laborato-

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ries, Inc. (Indianapolis, IN). Ovine prolactin (NIDDK-oPRL-19) was a gift from the NIH. Other materials were purchased from the following sources: cortisol from Charles Pfizer and Co. (New York, NY): medium 199 Earle's salts, restriction enzymes, and DNA random labeling kits from Gibco Laboratories (Grand Island, NY); porcine insulin, penicillin, and streptomycin from Ell Lilly Co. (Indianapolis, IN); [α-^{32}P]CTP from Dupont Corp. (Wilmington, DE); Tri reagent from Molecular Research Center, Inc. (Cincinnati, OH); all other reagents and chemicals were from Sigma Chemical Co. (St. Louis, MO). The plasmids containing the mouse cDNA probes were received as followings: α-lactalbumin was a gift from Dr. Pradman Qsba of the National Institutes of Health, β-1,4-galactosyl transferase from Dr. Joel H. Shaper of the Johns Hopkins School of Medicine, and mouse β-actin from Dr. David Smith of the Wayne State University.

Mice were sacrificed by cervical dislocation, and the caudal pair of mammary glands were removed and placed in medium 199 Earle's salts. Explants (3–5 mg each) were prepared as described earlier (7). Explants (8–12) from 6–8 animals were randomly placed on siliconized lens paper floating on 6 ml medium 199 Earle's salts containing 1 μg/ml insulin plus 10^{-7} M cortisol in 60 × 15 mm plastic petri dishes. The tissues were initially cultured for 24 hr at 37°C in a culture oven gassed with 5% CO_2:95% air. After the initial 24-hr culture, 1 μg/ml oPRL was added to selected plates and incubation continued for times up to 24 hr.

Quantitation of mRNAs was carried out as follows. Total RNA from cultured mammary tissues was extracted by a single-step procedure employing the guanidium thiocyanate-phenol-chloroform method described by *Chomcyzynski and Sacchi* (8). Tissue samples (150–200 mg) were homogenized in 2 ml TRI reagent (a monobasic mixture of guanidium thiocyanate and phenol) in a polytron homogenizer. The phases were separated after addition of 400 μl chloroform:isoamyl alcohol (49:1). The aqueous-guanidinium thiocyanate phase was removed and the RNA precipitated by addition of isopropanol. The pellet was washed with 75% ethanol and then dissolved in diethyl pyrocarbonate-treated double distilled water. RNA concentration was spectrophotometrically determined by absorbance at a wavelength of 260 nm. At 10 μg/lane the RNA was subjected to electrophoresis in a 1.2% agarose, 10.65 *M* formaldehyde gel. The RNA was then transferred to a nylon membrane via capillary elution. The immobilized RNA was hybridized overnight at 42°C with [^{32}P]-labeled cDNA probes for β-actin, galactosyl transferase or α-lactalbumin. Labeling of the probes was accomplished with a kit purchased from Gibco BRL Life Technologies, Inc. After hybridization, the membranes were exposed to film for appropriate times at −80°C. The films were then developed and the bands quantitated via laser densitometry. Results are expressed as a ratio of the densities of the galactosyl transferase and α-lactalbumin band to that of the β-actin band. All experiments presented in the results section are selected from at least three experiments from which similar results were obtained. Statistical differences were determined by Student's t test when two means were compared, or with an analysis of variance followed by Dunnet's test for multiple comparisons. Standard errors were all less than 10% of the means and are not included on the data chart.

Results

Figure 1 shows the result of an experiment in which the time course of the effect of prolactin on the accumulation of β-1,4 GT mRNA was determined. The upper panel illustrates the autoradiography banding patterns that were generated and subsequently quantitated using laser densitometry. The resulting ratio of GT: actin mRNA is presented in the lower panel. The onset of the PRL stimulation of β-1,4 GT mRNA accumulation occurs 8 hr after PRL addition, and the magnitude of the response reaches a maximum after

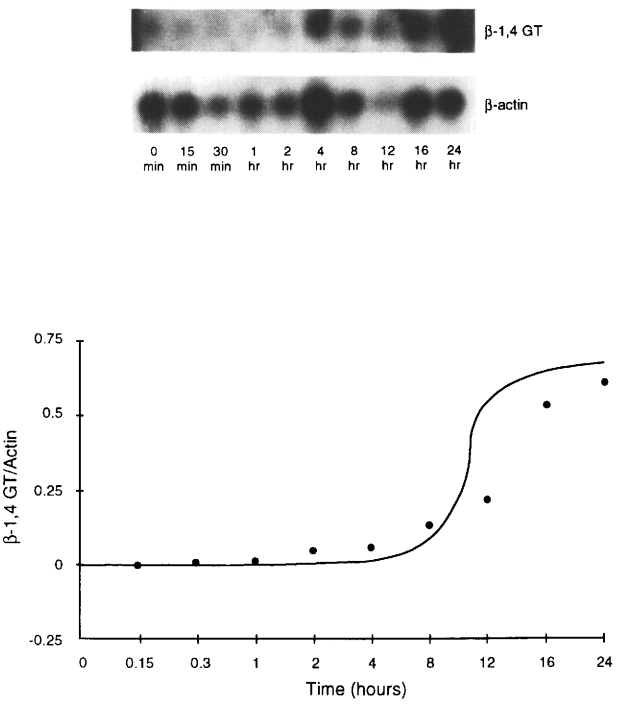


Figure 1. Time course of prolactin stimulation of β1,4 GT mRNA accumulation. Mammary gland explants were cultured ±1 μg/ml prolactin for the times indicated. Total RNA was then extracted, subjected to agarose electrophoresis and blotted to nylon filters. The filters were then hybridized to [^{32}P]-labeled cDNAs for β-actin and β1,4 GT (upper panel). After autoradiography the bands were quantitated by laser densitometry and the ratio of β1,4 GT to β-actin determined (lower panel).

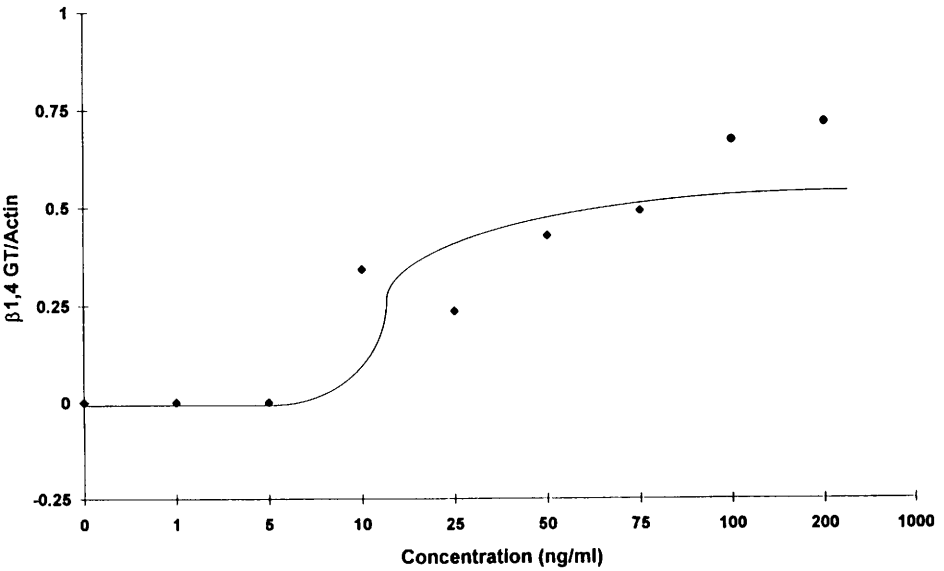


Figure 2. Dose-response of prolactin stimulation of β1,4 GT mRNA accumulation. Mammary gland explants were cultured ±1 μg/ml prolactin for 16 hrs. Total RNA was then extracted, subjected to agarose electrophoresis and blotted to nylon fibers. The filters were then hybridized to [^{32}P-labeled cDNAs for β1,4 GT. After autoradiography, the bands were quantitated by laser densitometry and the ratio of β1,4 GT to β-actin determined.

about 16 hr. These results correlate well with the time course for the PRL stimulation GT activity in cultured mouse mammary tissues (6).

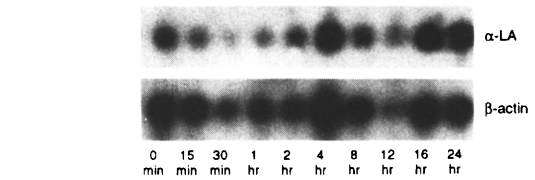
In further studies, the effect of PRL concentration on the accumulation of GT mRNA was determined following a 16-hr culture period (Fig. 2). The lowest PRL concentration that elicited a stimulatory response was 10 ng/ml, and a maximum response occurred with a concentration of 200 ng/ml. Higher concentrations of PRL up to 1 μg/ml produced a magnitude of response similar to that evoked with 200 ng/ml.

The results in Figure 3 show the time course for the effect of PRL on αLA mRNA accumulation in cultured mammary explants. The upper panel shows the results of a selected autoradiogram for αLA and β-actin. The lower panel depicts the ratio of αLA:actin. Clearly, an early effect of PRL on αLA mRNA accumulation occurs 4–8 hr after PRL addition. A maximum effect of PRL on αLA mRNA accruement occurs at 12 hr. Furthermore, this response is maintained throughout the 24 hr culture period with prolactin. These results are similar to those observed for the effect of PRL on GT mRNA accumulation.

Figure 4 represents the average of three experiments in which mammary cells were cultured with increasing concentrations of PRL for 16 hr. The effect of PRL on αLA mRNA levels was apparent at 1 ng/ml and reached a maximum at 75 ng/ml.

Discussion

These studies demonstrate that the temporal effects of prolactin on GT and α-lactalbumin mRNA ac-



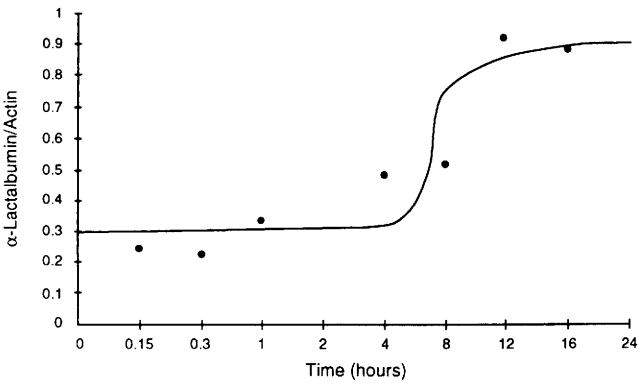


Figure 3. Time course of prolactin stimulation of α-lactalbumin mRNA accumulation. Mammary gland explants were cultured ±1 μg/ml prolactin for the times indicated. Total RNA was then extracted, subjected to agarose electrophoresis and blotted to nylon fibers. The filters were then hybridized to [^{32}P-labeled cDNAs for β-actin and α-lactalbumin (upper panel). After autoradiography the bands were quantitated by laser densitometry and the ratio of α-lactalbumin to β-actin determined (lower panel).

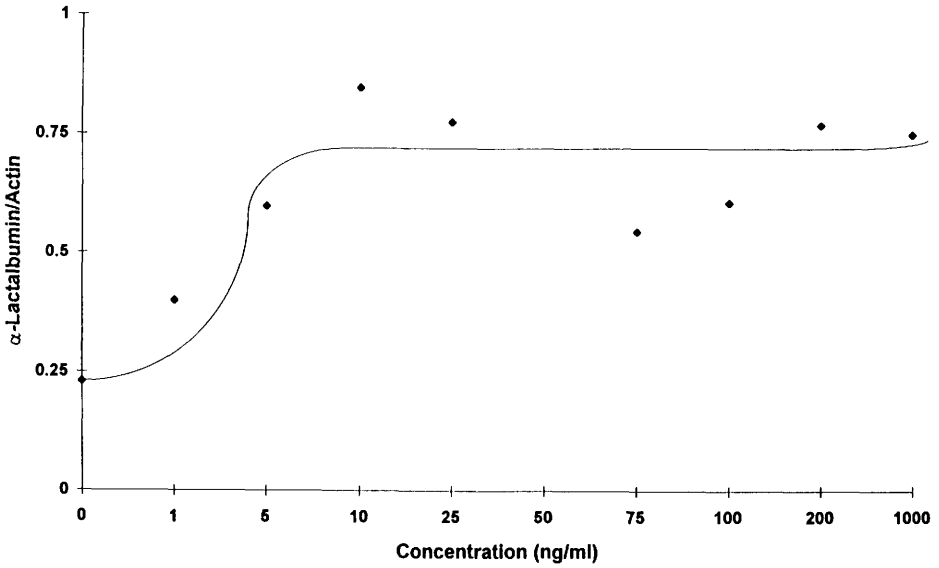


Figure 4. Dose response of prolactin stimulation of α-lactalbumin mRNA accumulation. Mammary gland explants were cultured ±1 μg/ml prolactin for 16 hrs. Total RNA was then extracted, subjected to agarose electrophoresis, and blotted to nylon filters. The filters were then hybridized to [^{32}P-labeled cDNAs for β-actin and α-lactalbumin. After autoradiography the bands were quantitated by laser densitometry and the ratio of α-lactalbumin to β-actin determined.

cumulation in cultured mouse mammary tissues occurs in a synchronous fashion, with the onset of these effects occurring about 6 hr after PRL addition. PRL similarly stimulates the rate of lactose synthesis and GT activity 4 to 8 hr after PRL addition to cultured mammary tissues. However, a significant effect of PRL on α-lactalbumin activity was only apparent after a 24 hr hormone treatment (6). Approximately a 48-fold increase in GT mRNA levels was apparent in tissues treated with PRL for 24 hr, whereas only a 286% increase in the activity of GT was apparent during this same time (6). In contrast, the prolactin effect on the accumulation of α-lactalbumin mRNA resulted in an approximately 1.5-fold increase following a 24-hr culture period, whereas the activity of α-lactalbumin increases only 20% during the same time (6). Interestingly, the time course for the detection of significant effects of prolactin on GT and αLA activity do not occur concurrently. Although the time courses for the PRL accumulation of mRNA for these proteins correspond well with one another, the magnitude of the PRL effect on the accumulation of the mRNAs for GT is several-fold higher than that for the α-lactalbumin mRNA accumulation. The quantitative effects of PRL on the tissue accumulation of the mRNAs for GT and α-lactalbumin clearly appear not directly related to the magnitude of effects of PRL on GT and α-lactalbumin activities after a 24-hr culture. However, the relative increases in GT and α-lactalbumin mRNA accumulation mirror the relative increases in GT and α-lactalbumin activities. The failure to detect an effect of PRL on α-lactalbumin activity at times prior to 24 hr may be due to the relatively small effect of PRL on α-lactalbumin accumulation at earlier times. Further studies are needed to determine if prolactin alters the rate of transcription and/or the intracellular half life of both the α-lactalbumin and GT mRNA transcripts.

Finally, the profile of the dose-response curves for the PRL stimulation of GT and α-lactalbumin mRNA accumulation is compatible with PRL levels found in the plasma (9). The onset of the PRL response occurred with 5 ng/ml, and a maximum response was observed with approximately 100 ng/ml. Circulating levels of PRL in the plasma of a number of species range between 0 and 10 ng/ml; with a suckling stimulus plasma PRL levels rise to concentrations in excess of 100 ng/ml.

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