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Characteristics of the Early Effect of Prolactin on Lactose Biosynthesis in Mouse Mammary Gland Explants[1] (42744)

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Abstract. These studies were carried out to characterize the early effect of prolactin (PRL) on lactose biosynthesis in cultured mammary gland explants derived from 12- to 14-day pregnant mice. The rate of lactose biosynthesis was assessed by the rate of radiolabeled glucose incorporation into lactose. For the rapid isolation of lactose, a new method which involves the use of thin-layer chromatography on cellulose-impregnated plastic sheets was employed. The onset of the PRL stimulation of [^{3}H]glucose incorporation into lactose occurred 6–8 hr after exposing the explants to PRL. The response to PRL was essentially all or none with maximum responses occurring with PRL concentrations above 25 ng/ml. The lowest stimulatory concentration of PRL was 10 ng/ml. The action of PRL on lactose biosynthesis requires both ongoing RNA and protein synthesis since puromycin, cyclohexamide, and actinomycin D abolished the PRL effect.

Lactose is a disaccharide which is unique to the mammary gland and is the primary sugar component of milk. The biosynthesis of lactose is catalyzed by the enzyme lactose synthetase (1); this enzyme is composed of two components, galactosyl transferase (GT) and α-lactalbumin (1, 2). α-Lactalbumin is the specifier in the biosynthetic process, changing the affinity of galactosyl transferase to favor glucose as a substrate (2–4). Alone, α-lactalbumin has no catalytic activity (5), while GT can act alone in forming complex sugars other than lactose (4, 6). Prolactin (PRL) stimulates the rate of lactose biosynthesis in the mammary gland, apparently by enhancing the rate of α-lactalbumin synthesis (3).

In earlier studies (3, 7) PRL has been shown to stimulate lactose biosynthesis and the accumulation of α-lactalbumin by 24 hr in cultured rabbit and mouse mammary tissues. In addition, these relatively long-term actions of PRL require ongoing RNA and protein synthesis.

The purpose of the present studies was to characterize the early effects of PRL on lactose biosynthesis in cultured mouse mammary tissues. To accomplish this, we employed a new method for rapidly isolating lactose.

Materials and Methods. 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 NIAMDD. Other substances were purchased from the following sources: cortisol from Charles Pfizer and Co. (New York, NY); Hank's balanced salt solution and medium 199–Earle's salts, from K. C. Biologicals, Inc. (Lenexa, KS); [5,6-^{3}H]glucose (82.9 Ci/mmol) and [1-^{14}C]lactose (57.0 mCi/mmol) from New England Nuclear Corp. (Boston, MA); procine insulin and streptomycin from Eli Lilly Co. (Indianapolis, IN); actinomycin D, puromycin, and cycloheximide from Sigma Chemical Co. (St. Louis, MO); and cellulose TLC plates (nonfluorescent indicator) and spectranalyzed 2-propanol from Fisher Chemical Co. (Waltham, MA).

Mice were killed by cervical dislocation, and the caudal pair of mammary glands were removed and placed in Hank's balanced salt solution. Explants were then prepared as described previously (8, 9). Briefly, the glands were cut into pieces weighing 3–5mg, and 16 pieces, four from each of 4 mice, were placed in each dish on siliconized lens paper floating on 8 ml medium 199–Earle's salts containing

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1.0 mg/ml insulin and 10^{-7} *M* cortisol for 24–36 hr. Each dish of explant pieces was treated as one observation. All incubations of these explants were carried out in 60 × 15-mm petri dishes maintained at 37°C in an atmosphere of 95% air–5% Co_2. The explants were then exposed to PRL and/or the metabolic inhibitors for varying times. In all cases, they were exposed to 1 μCi/ml [5,6-^{3}H]glucose for the last 1 hr of incubation for the pulse labeling of lactose.

After pulse labeling, the explants were removed, blotted, and weighed. They were then homogenized in 1:50 (wt/vol) 15% TCA, and centrifuged at 2000*g* for 10 min. Labeled lactose was separated from the labeled glucose on TLC plates. Aliquots (50 μl) of the supernatant were spotted on the cellulose TLC sheets 2.5 cm from the bottom, in columns 2.0 cm apart. Standard solutions of glucose and lactose (10 μl from a 10 mg/ml stock) were also spotted for each column. The first column on every sheet contained only standards for visualization. Separation of the sugars was carried out by developing the plates in a 2-propanol:distilled water (160:40) system for 3–4 hr. Visualization of the standard columns was accomplished with a benzidine reagent (1 g benzidine in 40 ml glacial acetic acid added to a premixed solution of 30 g TCA in 40 ml distilled water; just before use this mix is diluted 1:9 with acetone) (10). After spraying the plates with the benzidine reagent, the sugar spots were visualized by heating in an oven at 100°C for 3–5 min. The R_f values for glucose and lactose were 0.44 and 0.18, respectively. The lactose and glucose areas on the plates were identified and the cellulose from the appropriate areas was scraped into scintillation vials; the sugars were then eluted from the cellulose with 1 ml of 1 *N* HCl. Ten milliliters Triton X-100 scintillation fluid [52.5 mg 1,4-bis-(5-phenyloxazol-2-yl)benzene (POPOP), 4.2 g 2,5-diphenyloxyzol (POP), 900 ml toluene, and 300 ml Triton X-100] was then added and the radioactivity was determined in a liquid scintillation spectrophotometer. The rate of [5,6 ^{3}H]glucose incorporation into lactose was expressed as disintegrations pre minute per milligram wet tissue weight and 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 90-min period (data not shown). The efficiency of extraction of lactose and glucose from the chromatography plates was determined using [^{14}C]lactose and [^{3}H]glucose as standards. The recovery of lactose was found to be greater than 93% and that of glucose 99–100%.

Statistical comparisons were made with Student's *t* test or an analysis of variance followed by Duncan's test.

Results. The time course for the early action of PRL on the rate of [^{3}H]glucose incorporation into lactose is shown in Fig. 1. The onset of the response to PRL is between 6 and 8 hr after adding PRL to explants that had been precultured for 24 hr with insulin and cortisol. The magnitude of the PRL response continued to increase through 16 hr. In these same experiments the amount of [^{3}H]glucose that accumulated in the tissues was shown to be unaffected by PRL treatment (data not presented). It would therefore appear unlikely that an altered specific activity of ^{3}H in the intracellular glucose pool contributes to the enhanced rate of [^{3}H]glucose incorporation into lactose following PRL treatment.

In further studies, the effect of PRL concentration on the rate of lactose biosynthesis

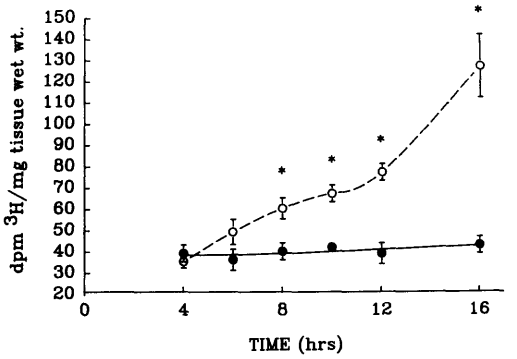

FIG. 1. Time course of PRL stimulation of lactose synthesis. Explants were incubated 24 hr with insulin (1 μg/ml) plus cortisol (10^{-7} *M*). Control groups (—) and groups containing PRL at 1 μg/ml (---) were then incubated for the times indicated. [^{3}H]Glucose (1 μCi/ml) was added for the final 1 hr of incubation. Values represent the means ± SE of six observations. *Significantly greater than control ($P < 0.01$).

was determined after a 16-hr culture period (Fig. 2). The PRL response is essentially "all or none" with an initial response occurring with 10 ng/ml PRL and a maximum response with PRL at concentrations of 25 ng/ml and above.

The requirement for ongoing RNA and protein synthesis for the early effect of PRL on lactose biosynthesis was assessed by culturing tissues with the RNA synthesis inhibitor actinomycin D and the protein synthesis inhibitors cyclohexamide and puromycin. As shown in the data of Table I, actinomycin D abolished the effect of PRL on stimulation of the rate of [^{3}H]glucose incorporation into lactose during a 16-hr culture; actinomycin D, by itself had no effect on the basal rate of [^{3}H]glucose incorporation. Puromycin and cyclohexamide similarly abolished the PRL stimulation of lactose synthesis, but each of these antibiotics also lowered the basal rate of [^{3}H]glucose incorporation into lactose.

Discussion. Employing a newly devised method for isolating lactose, we have characterized the early effect of PRL on stimulating the rate of [^{3}H]glucose incorporation into lactose in mouse mammary gland explants. The earliest detectable PRL effect was observed 8 hr after addition of PRL. *In vivo* studies in rats correlate with this observation in that there is an 8- to 16-hr time delay from the suckling-induced release of PRL until PRL manifests a maximum stimulation of milk product formation (11). The delayed effect of PRL on lactose synthesis is similar to that elicited with the PRL stimulation of the synthesis of other primary milk components; the earliest detectable effect of PRL on triglyceride synthesis is 6 hr (12) and the earliest effect on phosphoprotein (casein) synthesis is 10 hr (9). It is thus clear that the "turn on" of the synthesis of the primary milk components occurs in concert in response to PRL in cultured mammary tissues. It is possible that there is a common sequence of metabolic events by which PRL activates the synthesis of the primary milk components. This sequence of events apparently involves a RNA–DNA dependent mechanism since inhibitors of RNA and protein synthesis have been shown to abolish the effects of PRL on lactose synthesis (Table I (13)), lipid synthesis, and casein synthesis (12). The identification of these molecular events remains to be clearly established.

The early action of PRL on lactose synthesis was shown to be essentially "all or none"

TABLE I. EFFECT OF METABOLIC INHIBITORS ON RATE OF [^{3}]GLUCOSE INCORPORATION INTO LACTOSE

Addition	dpm ^{3}H incorporation into lactose (dpm/mg wet tissue wt)	P
Control	30 ± 1	
PRL (1 μg/ml)	59 ± 3	<0.01[a]
Cyclohexamide (200 μg/ml)	14 ± 1	<0.01[b]
Cyclohexamide + PRL	13 ± 2	<0.01[c]
Puromycin (50 μg/ml)	13 ± 1	<0.01[b]
Puromycin + PRL	14 ± 2	<0.01[c]
Actinomycin D (1 μg/ml)	26 ± 1	
Actinomycin D + PRL	26 ± 3	<0.01[c]

Note. Explants were incubated for 24 hr with medium 199 containing Earle's salts, insulin (1 μg/ml), and cortisol (10^{-7} *M*). They were subsequently cultured for 16 hr with the substances listed above. [^{3}H]Glucose (1 μCi/ml) was added for the terminal 1 hr of incubation. Values represent the means ± SE of six observations.

[a] Values significantly greater than control value.

[b] Values significantly less than control value.

[c] Values significantly less than PRL value.

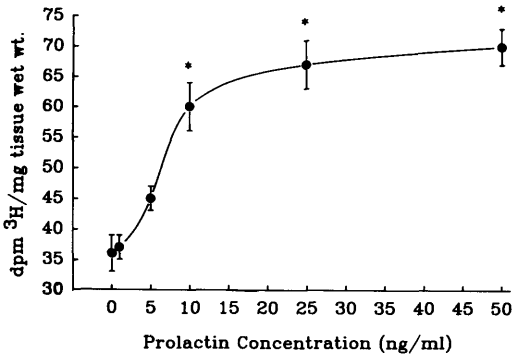

FIG. 2. Effect of PRL concentration on rate of [^{3}H]glucose incorporation into lactose. Explants were incubated for 24 hr with medium containing insulin (1 μg/ml) and cortisol (10^{-7} *M*). PRL was then added in the concentrations indicated, and incubation was continued for 16 hr; [^{3}H]glucose was added to each flask (1 μCi/ml) for the terminal 1 hr of incubation. Values represent the means ± SE for six observations. *Significant increase as determined by Duncan's test ($P < 0.01$).

type response. The lowest concentration of PRL that elicited a response was 10 ng/ml and a maximum response occurred with concentrations of 25 ng/ml and above. A similar concentration-response curve was earlier shown to exist for the PRL stimulation of casein synthesis (9) and lipid synthesis (12). These concentration-response characteristics are consistent with *in vivo* studies in which the amount of PRL required to stimulate milk secretion *in vivo* was shown to be quite small compared to the total amount of PRL released due to the suckling stimuli in rats (14). These studies therefore clearly show that the characteristics of the PRL stimulation of the synthesis of the three primary milk components are similar in several ways, both *in vivo* and *in vitro*.

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