Effects of Cholera and Pertussis Toxins on Prolactin Stimulation of Lactose Synthesis and Ornithine Decarboxylase Activity in Mouse Mammary Gland Explants (43618)

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Abstract. **Studies indicate that G proteins are likely involved in the signal transduction pathway for prolactin's stimulation of mitogenesis in Nb_2 cells. In the mammary gland, little is known about the possible role of G proteins in the prolactin (PRL) stimulation of milk product synthesis. Therefore, the effects of cholera and pertussis toxin, enzymes that modify G protein activity, were tested on several actions of prolactin on mouse mammary tissue in culture. At concentrations of 0.1–0.5 μg/ml, cholera toxin stimulated ornithine decarboxylase activity in a dose-response fashion; when tested in concert, cholera toxin and prolactin caused an additive response. Cholera toxin by itself did not affect the rate of lactose synthesis, but at concentrations above 0.5 μg/ml, it attenuated the magnitude of the prolactin stimulation of lactose synthesis. Pertussis toxin (0–0.5 μg/ml), both by itself and in concert with PRL, had no effect on ornithine decarboxylase activity. At concentrations of 25 ng/ml and above, pertussis toxin inhibited the PRL stimulation of lactose synthesis, whereas at 0.2 and 0.5 μg/ml, pertussis toxin abolished the PRL response. These observations suggest that a G protein, but not G_s, may be involved in prolactin's mechanism of signal transduction in the mouse mammary gland.**
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Prolactin (PRL) appears to initiate its actions by interacting with specific receptors located in the plasma membrane of target cells. Subsequent to its binding, PRL orchestrates a series of events that result in cellular differentiation, proliferation, and milk product formation in the mammary gland (1).

Recent studies indicate that G proteins are regulated by PRL and are involved in PRL's stimulation of Nb_2 cell proliferation (2, 3). Furthermore, experimental cross-linking studies by Too *et al.* (4) suggest that the Nb_2 cell lactogen receptor is complexed with G proteins. This implicates G proteins as having a possible role in mediating PRL-stimulated mitogenesis of Nb_2 cells.

The midpregnant mouse mammary gland explant system provides a useful model to elucidate PRL's mechanism of action on differentiative processes (5). In the studies described in this paper, we explore the possible role of G proteins in PRL's signal transduction pathway in cultured mouse mammary gland tissues. Cholera toxin (CT) and pertussis toxin (PT), agents that modify G protein activity, were employed. CT catalyzes the ADP-ribosylation of the α-subunit of G_s and causes the constitutive activation of adenylate cyclase (6). PT causes a similar modification in the α-subunits of certain G proteins classified as G_i, G_o, G_q, and G_t (7). ADP-ribosylation of G proteins by PT can have diverse effects on cells depending on which PT-sensitive G proteins are present in any specific type of cell. In the present studies, we report on the effects of CT and PT on the PRL stimulation of lactose synthesis and ornithine decarboxylase (ODC) activity in cultured mouse mammary tissues. Since lactose is a specific milk product, the rate of lactose synthesis was employed to study the signal transduction pathway involved in the differentiative action of PRL. The effects of CT and PT on the PRL stimulation of ODC activity were determined because the PRL stimulation of this enzyme is one of

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the earliest (30 min) effects expressed in cultured mouse mammary tissues. In addition, ODC is one of the key enzymes involved in the biosynthesis of the polyamines, which are required for PRL to stimulate milk product synthesis (1).

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 the National Institute of Diabetes, Digestive, and Kidney Diseases. Hanks' balanced salt solution and Medium 199-Earle's salts (M-199) were from Gibco Laboratories (Grand Island, NY); bovine insulin, penicillin, and streptomycin were from Eli Lilly Co. (Indianapolis, IN); [I-^{14}C] Ornithine (52.5 mCi/mmol), hyamine hydroxide, and [5,6-^{3}H]glucose (82.9 Ci/mmol) were from New England Nuclear (Boston, MA); cholera toxin and pertussis toxin were from Sigma Chemical Co. (St. Louis, MO); cellulose thin layer chromatography plates (250 μM, nonfluorescent indicator) and spectranalyzed 2-propranol were from Fisher (Watham, MA).

Mice were sacrificed by cervical dislocation, and the caudal pair of mammary glands were removed and placed in M-199 Earle's salts. Explants were then prepared as described in detail earlier (5). The glands were cut into pieces weighing about 3 mg each and placed on siliconized lens paper floating on 6 ml of M-199.

When the effect of CT, PT, and/or PRL on ODC activity was to be determined, explants were placed on siliconized lens paper floating on 2 ml of M-199 Earle's salts containing 1 μg/ml of insulin and 10^{-7} M cortisol. Explants from six to eight animals were randomly distributed among treatment combinations. All incubations were carried out in polypropylene vials maintained at 37°C in an atmosphere of 95% O_2 and 5% CO_2. After a 1-day culture, the tissues were preincubated with CT for 1 hr, after which PRL (1 μg/ml) was added. Incubation was continued for an additional 2 hr. In the case of PT, the tissues were preincubated with PT for 12 hr, after which PRL (1 μg/ml) was added and incubation was continued for 2 hr. The tissues were homogenized 1:10 (w/v) in 50 mM Tris buffer (pH 7.4) and ODC activity was determined using a modification of the methods described by Janne and Williams-Ashman (8). ODC activity was expressed as dpm/10 mg wet tissue wt.

When the effect of CT or PT on the PRL stimulation of lactose synthesis was to be determined, explants were initially cultured for 24 hr with M-199 containing 1 μg/ml of insulin and 10^{-7} M cortisol. Optimal conditions were established in preliminary experiments for the culture regimen with the toxins. For the PT experiment, PT was also present during the initial 24-hr culture period. For the CT experiment, CT was present for 6 to 7 hr after the initial 24-hr culture. For both experiments, 1 μg/ml of PRL was added after the preculture periods and incubation continued for 14 hr. The tissues were pulse-labeled with 0.5 μCi/ml of [^{3}H] glucose for the final 2 hr of incubation, after which the rate of lactose synthesis was determined by methods outlined earlier (9). Results are expressed as dpm/mg wet tissue wt.

Statistical comparisons were made using analysis of variance followed by Scheffe's test.

Results

Figure 1 shows the effects of CT (0–500 ng/ml) and/or PRL (1 μg/ml) on ODC activity. At concentrations of 0.1–0.5 μg/ml, CT stimulated ODC activity in a dose-response fashion; when tested in concert, CT and PRL caused an additive response. Figure 2 shows the effect of CT on the PRL stimulation of lactose synthesis. Cholera toxin, by itself, had no effect on the basal rate of [^{3}H]glucose incorporation into lactose. However, CT, when tested in concert with PRL (1 μg/ml) in the concentration range 0.1–0.5 μg/ml, caused a significant decrease in the rate of lactose synthesis.

The effect of PT (0–0.5 μg/ml) on the PRL stimulation of ODC activity is shown in Figure 3. PT, both by itself and when combined with PRL, had no significant effect on ODC activity. Figure 4 shows the effect of PT on the PRL stimulation of lactose synthesis. A dose-response inhibition of the PRL response was observed with PT concentrations between 10 and 200 ng/ml. At 0.2 and 0.5 μg/ml, PT abolished the PRL effect on lactose synthesis.

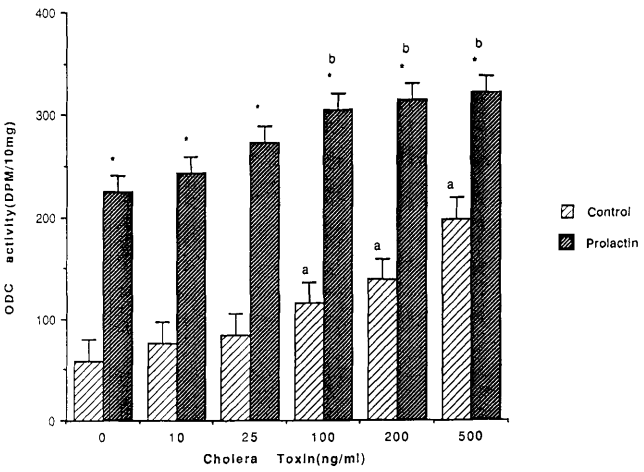

Figure 1. The effect of CT on PRL stimulation of ODC activity. Explants were cultured for 24 hr in the presence of 1 μg/ml of insulin and 10^{-7} M cortisol. After preincubation with CT for 1 hr, 1 μg/ml of PRL was added and incubation continued for 2 hr. ODC activity was determined as described in the text. Values are mean ± SE of six observations. *Greater than control; a, significantly greater than control without CT; b, significantly greater than PRL alone ($P < 0.05$).

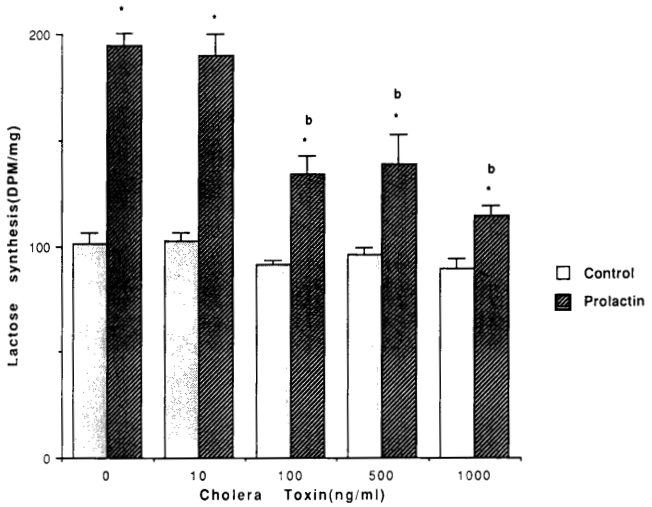

Figure 2. The effect of CT on PRL stimulation of lactose synthesis. Explants were cultured for 24 hr in the presence of 1 μg/ml of insulin and 10^{-7} *M* cortisol. After preincubation with CT for 6 to 7 hr, 1 μg/ml of PRL was added and incubation continued for 16 hr. Lactose synthesis was then determined as described in the text. Values are mean ± SE of six observations. *Significantly greater than control; b, less than PRL alone ($P < 0.05$).

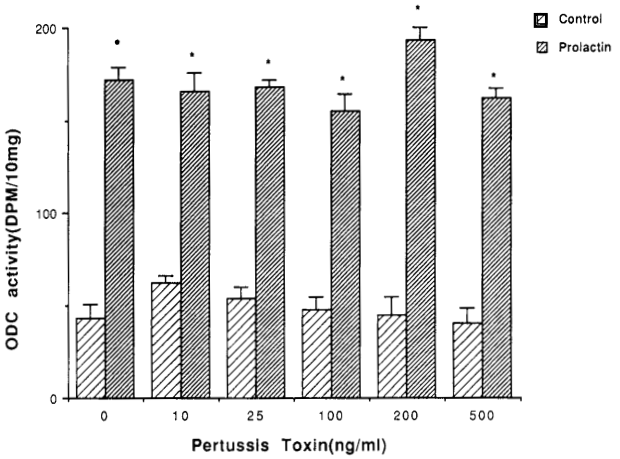

Figure 3. The effect of PT on PRL stimulation of ODC activity. Explants were cultured for 24 hr in the presence of 1 μg/ml of insulin and 10^{-7} *M* cortisol. After preincubation with PT for 12 hr, 1 μg/ml of PRL was added and incubation continued for 2 hr. ODC activity was determined as described in the text. Values are mean ± SE of six observations. *Significantly greater than control ($P < 0.05$).

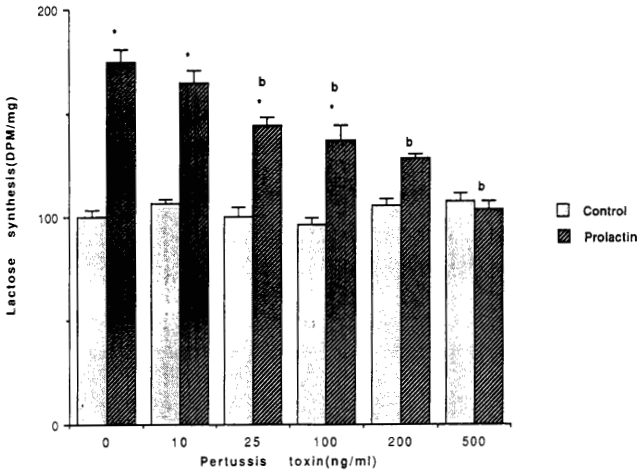

Figure 4. The effect of PT on PRL stimulation of lactose synthesis. Explants were cultured for 24 hr in the presence of 1 μg/ml of insulin, 10^{-7} *M* cortisol, and PT. PRL (1 μg/ml) was then added and incubation continued for 16 hr. Lactose synthesis was then determined as described in the text. Values are mean ± SE of six observations. *Significantly greater than control; b, less than PRL alone ($P < 0.05$).

Discussion

The results of these studies show that CT stimulates ODC activity but inhibits lactose synthesis in cultured mouse mammary gland explants. In previous studies done in our laboratory, it was shown that CT (0.5–1 μg/ml) also inhibits the PRL stimulation of casein and lipid synthesis. These studies are consistent with the results of Perry and Oka (10), in which, with 2-day cultures of mouse mammary tissues, the effects of PRL on casein and α-lactalbumin synthesis were abolished by co-incubation with CT at concentrations above 0.1 μg/ml. The mechanism by which CT elicits these responses in the mammary gland is likely related to the CT stimulation of adenylyl cyclase activity with a consequent elevation of cyclic AMP concentrations. Many studies have shown that dibutyryl cyclic AMP (DBcAMP), as well as several inhibitors of cyclic AMP phosphodiesterase, impairs the rate of milk product formation and suppresses the effects of PRL on lactogenic processes in cultured mammary tissues of several animal species (11–13). In addition, DBcAMP like CT has been shown to stimulate ODC activity in cultured mouse mammary tissues (14). Both the DBcAMP and CT effects on ODC activity, however, were additive to that evoked by PRL. The effects of DBcAMP and CT when compared with the effect of PRL on ODC activity would, therefore, appear to be carried out by different signal transduction mechanisms or on different cell types in the mammary tissues. Thus, the results of the studies with CT indicate that the G_s protein is likely not a part of the signal transduction pathway for PRL in the milk-producing cells of the mammary gland.

PT catalyzes the ADP-ribosylation of the α-subunits of specific subclasses of G_i, G_o, G_t, and G_q in certain cells. PT inhibits agonist-induced GTPase by disjoining the signal from receptors (15). Accordingly, such a modification in specific subclasses of G_q or G_o results in an inhibition of phospholipase, while in G_i it causes an elevation of cAMP levels (16). While eliciting no effect on ODC activity, PT caused a significant decrease in the magnitude of PRL's stimulation of lactose synthesis. In studies not shown here, PT (0.1–0.5 μg/ml) by itself decreased the rate of lipid and casein synthesis; when tested in concert with PRL, PT caused a further reduction in the rate of lipid and casein synthesis. Therefore, our observations would lead us to

speculate that PT is acting on G proteins other than G_i in the mouse mammary gland explant system, since the inhibition of the G_i protein should cause elevated levels of cyclic AMP with a consequent increase in ODC activity; this did not occur. In previous studies, it has been concluded that some of the early actions of PRL in the mammary gland may occur via activation of cellular phospholipases (17, 18). Inhibitors of phospholipases have been shown to abolish the PRL effects on milk product synthesis (1), whereas exogenously added phospholipases cause some lactogenic responses in cultured mouse mammary tissues. Hence, the results of our studies are consistent with the speculation that PRL may stimulate the synthesis of milk products via a PT-sensitive G protein that is associated with membrane-bound phospholipases (G_q or G_o).

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