Prolactin Stimulation of Iodide Uptake and Incorporation into Protein Is Polyamine-Dependent in Mouse Mammary Gland Explants (44512)

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Abstract. **Previous studies have demonstrated that the prolactin stimulation of most lactational processes (casein, lactose, and triglyceride synthesis) requires an earlier stimulating effect of prolactin on the synthesis of the polyamines. Spermidine appears to be the specific polyamine required for prolactin to enhance milk product synthesis. Inorganic iodide is present in milk at more than an order of magnitude higher concentration than that of the maternal plasma. Since prolactin stimulates iodide accumulation in milk, the goal of these studies was to determine the role of the polyamines in this hormone response. Two drugs were employed in these studies: DFMO (difluoromethylornithine), which inhibits ornithine decarboxylase, and MGBG [methylglyoxal bis(guanyl-hydrazone)], which inhibits S-adenosyl methionine decarboxylase. In mammary gland explants from midpregnant (10–14 days of pregnancy) mice, MGBG at 100 μ*M* abolished the prolactin stimulation of iodide uptake and incorporation into milk proteins, whereas DFMO caused a concentration-dependent inhibition of the PRL response. Selected sensitivity of the MGBG and DFMO inhibitions was validated by a reversal of the drug inhibitions with the addition of 1 m*M* spermidine to the culture medium. These data suggest that the polyamine signaling pathway is involved in the prolactin stimulation of iodide uptake into milk.** [P.S.E.B.M. 2000, Vol 224:41–44]

The assembly of milk constituents in the mammary gland involves the prolactin (PRL) stimulation of the uptake of an array of substances (1–4), including iodide (5), from the maternal plasma. We have recently reported that iodide uptake occurs *via* a sodium-iodide transporter that is inhibited by perchlorate and thiocyanate (6–8) like the iodide transporter in the thyroid gland. Recent cloning studies have reported that the iodide transporter is identical in thyroid cells and a variety of extra thyroidal tissues including the mammary gland (9). The sodium-iodide symporter can support an intracellular:extracellular iodide concentration gradient of more than 25:1 (5). This is consistent with the iodide concentration in milk, which is about 50-fold higher than that found in the maternal plasma. In mouse milk, about half the iodide is present as inorganic iodide whereas the other half is incorporated into tyrosyl residues of milk proteins, primarily casein (5).

Several signaling pathways have been shown to be involved in the PRL stimulation of milk product synthesis (10). One of these is the polyamine pathway in which spermidine appears to be the specific molecule required to enhance the synthesis of milk substances. The present studies were designed to assess the role of the polyamines in the PRL regulation of iodide uptake and incorporation into protein in the mammary gland.

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 prolactin (NIH PS-17) was a gift from National Institute of Diabetes and Digestive and Kidney Diseases (Dr. A. F. Parlow, Director, Torrence, CA). Other substances

This work was supported by funds from the Wayne State University.
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Received Received June 30, 1999. [P.S.E.B.M. 2000, Vol 224]
Accepted Accepted January 11, 2000.

0037-9727/00/2241-0041$15.00/0

were purchased from the following sources: cortisol from Charles Pfizer and Co. (New York, NY); Hank's balanced salt solution (HBSS) and medium 199-Earle's salts from Gibco Laboratories (Grand Island, NY); [^{3}H]OH and [^{14}C]-inulin from New England Nuclear Corp. (Boston, MA); porcine insulin, penicillin, and streptomycin from Eli Lilly Co. (Indianapolis, IN); ^{125}I from Amersham Life Sciences, Inc. (Arlington Heights, IL); methylglyoxal bis(guanyl hydrazone) (MGBG) from Aldrich (Milwaukee, WI); and α-difluoromethylornithine (DFMO) was a gift from Merrell Dow Pharmaceuticals (Cincinnati, OH).

Explants of mouse mammary tissues were prepared and cultured as described earlier (11, 12). The explants were cultured on siliconized lens paper floating on 6 ml medium 199-Earle's salts containing 1 μg/ml insulin plus 10^{-7} *M* cortisol; all incubations were carried out in 60 × 15-mm Petri dishes maintained at 37°C in an atmosphere of 95% O_2–5% CO_2. In experiments in which the effects of PRL on iodide transport were to be determined, the tissues were initially cultured for 24 hr in the absence of PRL. PRL(1 μg/ml) was then added, and incubations continued for the times specified for each experiment. For the final 2 hr of culture, the tissues were transferred to vessels containing ^{125}I(0.25 μCi/ml; 0.3 ng/ml iodide) in 4 ml HBSS; incubations were carried out in a rotary water bath at 37°C (120 cycles/min). The tissues were then weighed and homogenized in 2 ml 5% trichloroacetic acid (TCA) containing 0.1 m*M* NaI. After a determination of total radioactivity, the samples were centrifuged at 2000*g* for 10 min. After the pellet was washed with an additional 5 ml 5% TCA, radioactivity in the TCA-insoluble fraction was determined. The intracellular accumulation of radiolabeled iodide was calculated by subtracting the amount of radiolabel in the extracellular space from the total radioactivity in the tissue homogenates (5). For these calculations, the total water content (51.5%) and extracellular space (32.5%) were determined by the volume of distribution of [^{3}H]OH and [^{14}C]inulin (1 m*M*), respectively. In time course studies, equilibration was achieved with [^{3}H]OH and [^{14}C]inulin (1 m*M*) by 15 min after their addition. PRL had no effect on the volumes of distribution of these substances under the conditions employed in these experiments. Results of the iodide uptake studies are expressed as a distribution ratio that represents the ratio of the intracellular specific activity divided by the extracellular specific activity of the radiolabeled iodide. The results of the incorporation studies are expressed as counts/min/mg wet tissue weight.

Statistical comparisons were made with Student's *t* test, when two means were compared, and with an analysis of variance followed by Dunnett's test for multiple comparisons. All experimental results are the mean ± standard error of six observations.

Results

Methylglyoxal bis(guanyl hydrazone) (MGBG) is a reversible inhibitor of S-adenosylmethionine decarboxylase (SAMD) and hence inhibits putrescine conversion to spermidine (SPD). Figure 1 shows that at a concentration of 100 μ*M*, MGBG abolishes the PRL stimulation of iodide accumulation after a 24-hr incubation with the drug. MGBG also reduces the basal extent of iodide uptake by about 50%. The addition of 1 m*M* SPD to the culture medium restored both the basal rate of iodide uptake as well as the PRL effect on iodide accumulation. Similar results were generated when the extent of iodide incorporation into the TCA-precipitable fraction was determined (Fig. 2). The PRL enhancement in this fraction was abolished by simultaneous treatment with 100 μ*M* MGBG, and the PRL response was restored with the addition of spermidine to the culture medium. A 24-hr treatment with MGBG also significantly reduced the incorporation of I^{125}, whereas the addition of SPD prevented this inhibition. The selected sensitivity for the MGBG inhibition of the polyamine pathway is thus indicated by these studies.

Difluoromethylornithine (DFMO) is another drug that interferes with the synthesis of the polyamines. DFMO is an irreversible inhibitor of ornithine decarboxylase (ODC) and thus inhibits the conversion of ornithine to putrescine. At concentrations between 1 and 20 m*M*, DFMO caused a concentration dependent inhibition of the PRL stimulation of iodide uptake (Fig. 3) and its incorporation into protein (Fig. 4). At concentrations up to 20 m*M*, DFMO by itself had no effect on the total uptake or incorporation of I^{125} (Figs. 3 & 4). When 1 m*M* SPD was added with 20 m*M* DFMO to the culture medium (Fig. 5), the inhibition of the PRL stimulation of iodide uptake was prevented. Similar results were generated when the TCA-insoluble fraction was analyzed (unpublished data). The selected sensitivity of the DFMO inhibition of polyamine biosynthesis is thus indicated by this observation.

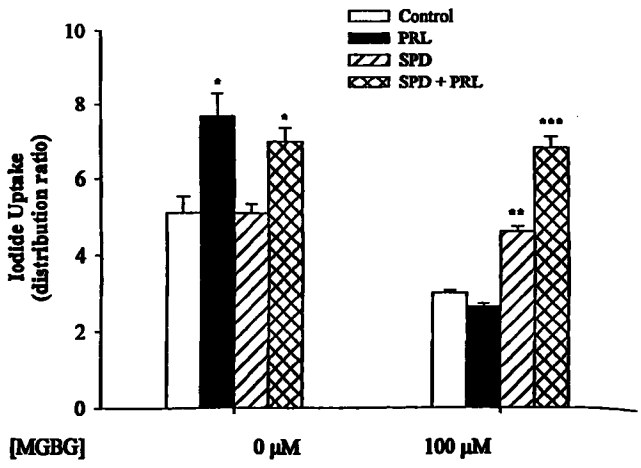

Figure 1. The effect of MGBG on PRL stimulation of iodide transport. Mammary explants were cultured for 24 hr in cortisol (10^{-7} *M*) and insulin (1 μg/ml). The explants were then cultured for an additional 24 hr in 0 or 100 μ*M* MGBG in the presence or absence of 1 μg/ml PRL. The tissues were pulsed with ^{125}I(0.3 ng/ml) during the last 2 hr of incubation. *Significantly greater than control and significantly greater than SPD with $P < 0.05$; **significantly greater than control and significantly greater than PRL with $P < 0.05$; ***significantly greater than control, SPD, and PRL with $P < 0.05$.

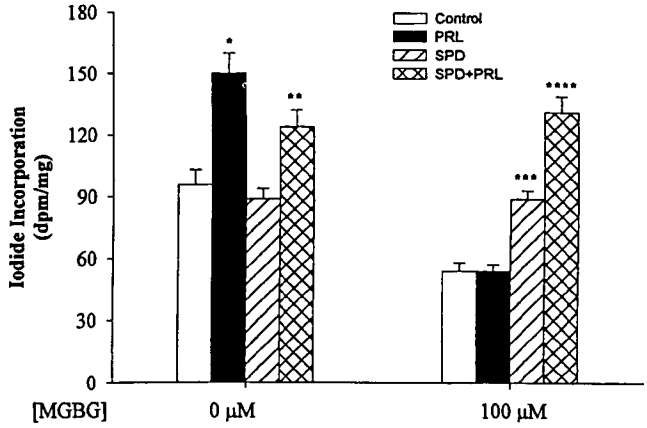

Figure 2. The effect of MGBG on PRL stimulation of iodide incorporation. Mammary explants were cultured as in Figure 1. After I^{125} treatment, the tissues were homogenized in TCA (5%) and centrifuged. The TCA was then decanted, and I^{125} was quantitated in the samples. *Significantly greater than control, SPD, and SPD + PRL with $P < 0.05$; **significantly greater than control and significantly greater than SPD with $P < 0.05$; ***significantly greater than control and PRL with $P < 0.05$; ****significantly greater than control, PRL, and SPD with $P < 0.05$.

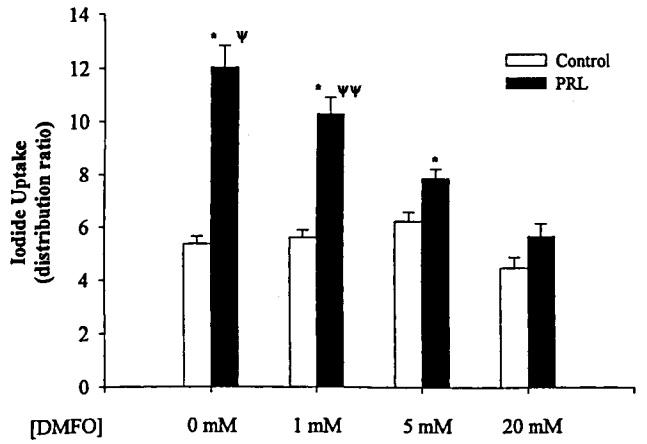

Figure 3. The effect of DMFO on PRL stimulation of iodide uptake in 24-hr treatment. Mammary explants were cultured for 24 hr in cortisol (10^{-7} *M*) and insulin (1 μg/m*L*). The explants were then cultured for an additional 24 hr in 0–20 m*M* DFMO in the presence or absence of 1 μg/ml PRL. The tissues were pulsed with ^{125}I(0.3 ng/ml) during the last 2 hr of incubation. *Significantly greater than control with $P < 0.05$; ψsignificantly greater than PRL in 1 m*M* DFMO and PRL with 5 m*M* DFMO with $P < 0.05$; and ψψsignificantly greater than PRL in 5 m*M* DFMO with $P < 0.05$.

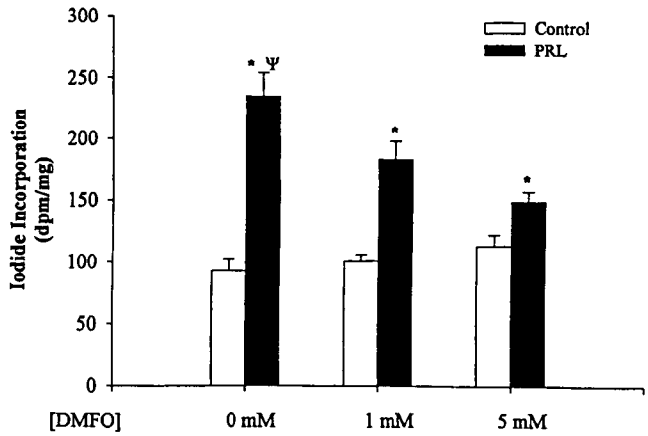

Figure 4. The effect of DFMO on PRL stimulation of iodide incorporation in 24-hr treatment. Mammary explants were cultured as in Figure 3. After I^{125} treatment the tissues were homogenized in TCA (5%) and centrifuged. The TCA was then decanted, and I^{125} in the samples was quantitated. *Significantly greater than control with $P < 0.05$; ψsignificantly greater than PRL in 1 m*M* DFMO and PRL with 5 m*M* DFMO with $P < 0.05$.

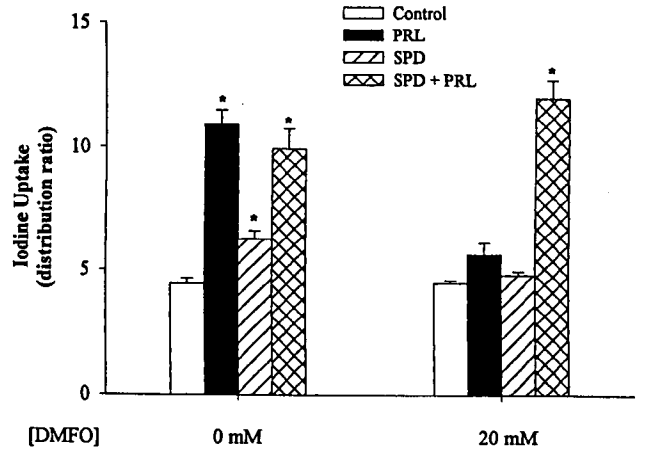

Figure 5. Prevention of DFMO inhibition of PRL stimulation of iodide uptake by spermidine. Mammary explants were cultured for 24 hr with cortisol (10^{-7} *M*) and insulin (1 μg/ml). The explants were then cultured for an additional 24 hr with combinations of 20 m*M* DFMO, 1 m*M* spermidine (SPD), and/or 1 μg/ml PRL. The tissues were then pulse-labeled with 0.25 μCi/ml I^{125}(0.3 μg/ml) for the last 2 hr of incubation. Distribution ratios were then calculated. *Significantly greater than control with $P < 0.05$.

Discussion

These studies clearly show that ongoing polyamine synthesis is essential for PRL to express its effects on iodide uptake and incorporation in the mammary gland. This is supported by the fact that two drugs that inhibit two distinct enzymes in the polyamine biosynthetic pathway attenuate or abolish the PRL effects on iodide uptake and its incorporation into protein. Specificity of the MGBG inhibition was established by the reversal of its inhibition with the addition of spermidine to the culture medium. In earlier studies we reported that ongoing polyamine synthesis is also essential for the PRL stimulation of other lactational processes in the mammary gland including casein synthesis, lactase synthesis, triglyceride synthesis, amino acid uptake, and glucose uptake (1–3, 10). However, two experimental observations indicate that iodide uptake and metabolism in the mammary gland are particularly sensitive to the polyamines. First, 100 μ*M* MGBG reduced the basal rate of iodide uptake and incorporation after a 24-hr exposure. MGBG, even at 200 μ*M*, had no effect on all the other metabolic processes associated with lactation. And second, DFMO inhibited the PRL stimulation of iodide uptake and incorporation in a concentration-response fashion. In contrast, DFMO had no effect on the magnitude of the PRL stimulation of one other lactogenic process. The reason for the selected sensitivity of

the polyamines on iodide metabolism in the mammary gland is not presently known.

The polyamine pathway clearly seems to be one of several important signaling pathways involved in the hormonal regulation of lactogenic processes in the mammary gland. Many years ago, Russell and McVicker (13) reported that spermidine levels of 5 m*M* were maintained in the lactating mammary glands of rats. The three most important lactogenic hormones (insulin, cortisol, and PRL) stimulate the activity of all the enzymes in the polyamine biosynthesis cascade (1). PRL stimulates ODC activity within minutes after the addition of PRL to cultured mouse mammary tissues (14); this effect of PRL is translation-dependent, but not transcription-dependent. Elevated spermidine levels are initially detected 2–4 hr after PRL addition to cultured mouse mammary tissues (15). The onset of the PRL stimulation of iodide uptake occurs immediately after the elevated spermidine levels (i.e., 4 hr after PRL addition to cultured mouse mammary tissues) (5). It thus seems likely that the polyamine pathway is one of the signaling sequences by which PRL expresses its lactogenic effects in the mammary gland. The polyamines are involved in the acceleration of transcriptional and translational processes in a variety of cells, and they likely function in a similar manner in lactating mammary cells.

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