Effect of prolactin on sodium iodide symporter expression in mouse mammary gland explants

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Effect of prolactin on sodium iodide symporter expression in mouse mammary gland explants. Am J Physiol Endocrinol Metab 279: E769–E772, 2000.—Iodide accumulates in milk at a concentration that is more than an order of magnitude higher than the iodide concentration in maternal plasma. In earlier studies from our laboratory, we have shown that prolactin (PRL) enhances iodide accumulation by two- to threefold in cultured mammary tissues taken from pregnant mice. In the present studies, we demonstrate via Western blotting techniques that prolactin elevates the quantity of the sodium iodide symporter (NIS) in cultured mouse mammary tissues. In time-course studies, the onset of the PRL effect on NIS accumulation was found to be between 4 and 16 h after addition of PRL to the explants. The lowest PRL concentration that elicited a significant response was 1 ng/ml, and a maximum effect was elicited with PRL concentrations >100 ng/ml. Actinomycin D, cycloheximide, and thiocyanate abolished the PRL effect on NIS accumulation, whereas perchlorate was without effect. These studies suggest that the PRL stimulation of iodide accumulation in milk is mediated, at least in part, by the PRL stimulation of NIS accumulation in mammary gland tissues. These studies further demonstrate that the PRL effect on NIS accumulation occurs via an RNA protein synthesis-dependent mechanism.

MATERIALS AND METHODS
Midpregnant (10–14 days of pregnancy) Swiss-Webster mice were used in all experiments; they were purchased from Harlan Laboratories (Indianapolis, IN). Ovine prolactin (National Institutes of Health, PS-19) was a gift from the National Institutes of Health. Other substances were purchased from the following sources: cortisol from Charles Pfizer (New York, NY), Hanks’ balanced salt solution (HBSS) and Medium 199-Earle’s salts from GIBCO Laboratories (Grand Island, NY), porcine insulin, penicillin, and streptomycin from Eli Lilly (Indianapolis, IN), and sodium perchlorate and potassium thiocyanate from Sigma Chemical (St. Louis, MO).

Mammary gland explants were prepared, as previously described (6), from mice that were pregnant 12–14 days. Mice were killed by cervical dislocation, and mammary glands were removed and placed in HBSS. Explants (3–6 mg each) from each of 8–10 animals were prepared and placed on siliconized lens paper in 60-mm petri dishes containing 6 ml of Medium 199-Earle’s salts with 1 mM insulin and 10−7 M cortisol. Next, tissues were incubated for 24 h at 37°C under humidified 95% air-5% CO2 atmosphere before experiments. All studies involving the preparation of mouse mammary gland explants were performed in compliance with the regulations of the Animal Care and Use Committee of Wayne State University.

After incubations with PRL and/or drugs, the tissues were weighed and disrupted in 1:2 (wt/vol) lysis buffer with a ground-glass homogenizer; the lysis buffer contained 2% NP40, 10 mM Tris, 50 mM NaCl, 30 mM sodium pyrophosphate, 2.5 mM EDTA, 1 mM orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin, at pH 7.6. After 30 min on a rocking platform, lysates were centrifuged (100,000 g) for 30 min at 4°C. The resulting supernatants, containing >95% of extractable NIS protein, were separated by SDS-PAGE (8–20% linear gradient) under reducing conditions and transferred to polyvinylidene difluoride.
idine fluoride (PVDF) membranes (Schleicher and Schuell). Membranes were probed with 1:2,500 rat anti-NIS (prepared by S. M. Jhiang) for 2 h, followed by treatment with anti-rabbit IgG HRP conjugate (Amersham NA934; 25 ml at 1:3,000 dilution for 1.5 h). Detection was accomplished by incubation with enhanced chemiluminescence reagents (Amersham) and exposure to photographic film. The bands (molecular mass 70 kDa) were quantitated by laser densitometry. Results are expressed on the basis of the relative density of the bands. Statistical comparisons were made with Student's t-test. All values represent the means ± SE of three or more experimental observations.

The rNIS peptide and rNIS polyclonal antibody were prepared as follows. A peptide, AETHPLYLGHDVETNL, corresponding to the 16 amino acids of the rNIS COOH-terminal segment, was synthesized by a solid-phase procedure with the use of an automated peptide synthesizer (Sigma-Genosys, The Woodlands, TX). The peptide was conjugated to the keyhole limpet hemocyanin with glutaraldehyde as a coupling reagent. The peptide was then purified by reverse phase chromatography using a C18 column. Antisera were raised from two New Zealand White rabbits by an initial injection of 200 μg of the conjugate in complete Freund’s Adjuvant, followed by multipoint booster injections (after 2, 4, 6, 8, and 10 wk) of 100 μg of the conjugate in incomplete Freund’s Adjuvant. Blood was collected 1 wk after the last injection. Two immune sera, rNISAb 1317 and rNISAb 1318, were then affinity-purified by use of a Sepharose 4B column coupled with the 16-aa rNIS peptide. Antisera and purified antibody were tested for their antibody titer against the peptide by ELISA. Antisera rNISAb 1318 were used for Western blot analysis.

RESULTS

Figure 1 shows a time course for the PRL stimulation of NIS accumulation in cultured mouse mammary tissues. A significant PRL effect was not observed after 2 or 4 h but was observed at 16 and 24 h. A highly significant (PL-01) PRL effect was expressed. At 24 h, the PRL-treated tissues contained ~20-fold higher content of the NIS than did the control tissues. A representative Western blot is presented with the charted data.

Figure 2 shows the concentration-response effect of PRL on NIS accumulation in mammary explants that were cultured with PRL for 24 h. A significant PRL effect (fourfold increase) was initially observed with PRL at 1 ng/ml, and the magnitude of the response increased progressively with PRL concentrations ≤100–200 ng/ml. Only a small increase was noted with PRL at 1 μg/ml.

The data in Fig. 3 show the results of a study in which mammary tissues were treated for 24 h with PRL and several metabolic inhibitors. Cycloheximide, an inhibitor of protein synthesis, abolished the PRL stimulation of NIS accumulation, whereas actinomycin D, an inhibitor of RNA synthesis, reduced the PRL
aball the PRL stimulation of iodide uptake in cultured mammary tissues (7). Accordingly, the PRL stimulation of iodide accumulation in milk appears to involve an increased number of iodide transporters as well as enzymatic activities that are inhibited by propylthiouracil and aminotriazole. Further experimental work will be required to resolve these issues. Because physiological PRL levels range between 10 and ~300 ng/ml, the different dose-response effects of PRL on iodide uptake and NIS accumulation may be of physiological importance.

Perchlorate and thiocyanate have been used in many earlier studies to inhibit the iodide transporter in thyroid (1, 11, 12) and mammary cells (7). Perchlorate, at a concentration that abolished iodide uptake and the PRL stimulation of iodide uptake (7), had no effect on the PRL stimulation of NIS accumulation. Thiocyanate, in contrast, abolished both the PRL effects on iodide uptake and NIS accumulation; this can be explained by the fact that thiocyanate not only inhibits iodide transporter activity but also functions as a general metabolic inhibitor. In any case, the PRL effect on NIS accumulation occurs, whereas NIS transporter activity is inhibited. The complete inhibition of the PRL effect on NIS accumulation by cycloheximide clearly suggests that the PRL stimulation of NIS accumulation likely involves a translational formation of new NIS proteins. The experiment with actinomycin D suggests that PRL effect also involves the transcriptional formation of more mRNA molecules coded for the transporter protein. Whereas the actinomycin D concentration employed in these studies was shown in earlier studies to completely abolish RNA synthesis in the mammary explant system, it is perhaps important to note that actinomycin D attenuated (a 2-fold increase from a 20-fold increase) the PRL stimulation of iodide transporter accumulation but did not abolish the effect. It is accordingly possible that PRL may also extend the half-life of the NIS mRNA, as has been reported earlier for the message for casein (4).

In summary, these studies clearly show that physiological concentrations of PRL stimulate sodium iodide transporter accumulation in cultured mouse mammary tissues. This likely contributes to the fact that iodide is concentrated by more than an order of magnitude in milk relative to maternal plasma (1, 11, 12).

DISCUSSION

The data in these experiments clearly show that PRL has a profound effect on the accumulation of the NIS protein in cultured mouse mammary tissues. This effect was expressed in a concentration-response fashion, employing a physiological range of PRL levels (1–1,000 ng/ml). In addition, the onset of the PRL effect on NIS accumulation (4–16 h) correlates well with the onset of the PRL stimulation of iodide accumulation that we reported in an earlier study (8). What does not correlate is the magnitude of the PRL stimulation of iodide uptake (two- to threefold increase) and the effect on iodide transporter accumulation (20-fold increase). The difference may be accounted for, at least in part, by the fact that only the transporters inserted into the plasma membrane function to carry iodide into the cells. It is likely that many iodide transporters may be 1) in the process of being assimilated and transported to the cell membrane, 2) stored in vesicles, or 3) in the process of being recycled or metabolized. Also to be considered is the earlier observation that iodoperoxidase inhibitors

FIG. 3. Effect of inhibitors on PRL stimulation of iodide transporter accumulation. Mammary explants were cultured for 24 h with 1 μg/ml PRL and/or 1 μg/ml actinomycin D, 5 μg/ml cycloheximide, 0.5 mM perchlorate, or 0.1 mM thiocyanate (KSCN). Iodide transporter proteins were then quantitated by laser densitometry of Western blots (A). One typical blot from 3 experimental blots is presented (B).

REFERENCES