Effect of Insulin on Iodide Uptake in Mouse Mammary Gland Explants

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Studies were carried out primarily to assess the role of insulin in regulating iodide uptake in the mammary gland. Using cultured mammary gland explants from virgin and pregnant mice (12–14 days into gestation), insulin (1 µg/ml) was shown to stimulate iodide uptake after a 2-day exposure period. The effect of insulin was manifested by itself, as well as in the presence of cortisol and prolactin. Optimal iodide uptake was observed when tissues were treated with all three lactogenic hormones (insulin, cortisol, and prolactin). In a time-course experiment, the effect of insulin alone was initially observed after a 10-hr treatment; the effect was maintained for 30 hr. In dose-response studies, 1 ng/ml insulin elicited a significant effect after 24 hr in culture; a maximal effect was achieved with 50–100 ng/ml insulin. The optimal cortisol concentration for a maximum stimulation of iodide uptake was 10^{-7}M. In a quantitative Western blot analysis employing an antibody to the sodium-iodide symporter, insulin stimulated an upregulation of the transporter protein after a 4-, 8-, or 20-hr treatment with insulin. Perchlorate and thiocyanate abolished the insulin effect on iodide uptake, further suggesting that the insulin response occurs via a stimulation of the sodium-iodide symporter. Clearly, insulin is an important and essential hormone in the lactogenic hormone complex for regulating iodide uptake in the mammary gland, but maximal expression of iodide uptake is only expressed when all three lactogenic hormones are present.

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In recent studies, we have reported the characteristics of the prolactin regulation of iodide uptake and incorporation into protein in the mammary gland (1–5). In these studies, a specific effect of prolactin on the expression of a sodium-iodide symporter was reported; this transporter is the same as that which is in thyroid follicular cells, as well as other cell types (6). Iodide is an important nutrient in milk, in that it is an essential element for the synthesis of the thyroid hormones. The thyroid hormones are required for normal growth and development of the newborn.

Milk product synthesis in the mammary gland requires a complement of three hormones: insulin, a glucocorticoid, and a lactogenic hormone (7). The assembly of milk products can be further enhanced by the thyroid hormones. In studies referenced above, prolactin effects on iodide uptake were determined in a hormonal milieu containing cortisol and insulin. The present studies were carried out to examine the specific characteristics of the insulin effect on iodide uptake in mouse mammary gland explants. The importance of insulin for normal lactation is apparent from the fact that diabetic mothers exhibit impaired milk production.

Materials and Methods

Virgin and midpregnant (10–14 days of pregnancy) Swiss-Webster mice were used in all experiments; they were purchased from Harlan Laboratories (Indianapolis, IN). Ovine prolactin (PRL; National Institutes of Health PS-19) was a gift from the National Institutes of Health. The sodium-iodide symporter (NIS) antibody was provided by Dr. Sissy M. Jhiang (Ohio State University). 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); ^{125}I from Amersham Life Sciences (Arlington Heights, IL); and [^{3}H]OH and [^{14}C]-inulin from New England Nuclear (Boston, MA).

Explants of mouse mammary tissues were prepared and cultured as described earlier (8, 9); typically, explants were pooled and randomly distributed for each experiment. The explants were cultured on siliconized lens paper floating on 6 ml of medium 199-Earle's salts; 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 hormones on iodide transport were to be de-

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termined, the tissues were cultured for 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 of HBSS; incubations were carried out in a rotary water bath at 37°C (120 cycles/min). The tissues were then weighed, and radioactivity was determined by scintillation spectrometry. 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 (1). For these calculations, the total water content (51.5%) and extracellular space (24.6%) were determined by the volume of distribution of [^{3}H]OH and [^{14}C]inulin (1 mM), respectively. In time course studies, equilibration was achieved with [^{3}H]OH and [^{14}C]-inulin (1 mM) by 15 min after their addition. 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. 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. Statistical comparisons were made using analysis of variance (ANOVA) followed by Dunnett's test. All data are expressed as the mean ± SE, and means were deemed significantly different with P values of less than 0.05 unless specified otherwise.

Methods employed for the Western blot studies were as follows. Following incubations with hormones, the tissues were weighed and disrupted in 1:2 (w/v) 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 (100,000g) for 30 min at 4°C. The resulting supernatants, containing more than 95% of extractable NIS protein (4), were separated by SDS-polyacrylamide gel electrophoresis (PAGE; 8%–20% linear gradient) under reducing conditions and were transferred to PVDF membranes (Schleicher & Schuell, Keene, NH). Membranes were probed with 1:2500 rat anti-NIS for 2 hr followed by treatment with anti-rabbit IgG HRP conjugate (Amersham NA934; 25 ml at 1:3000 dilution for 1.5 hr). Detection was accomplished by incubation with enhanced chemiluminescence reagents (Amersham) and exposure to photographic film. The bands (molecular mass of 70 kDa) were quantitated via laser densitometry. Results are expressed on the basis of the relative density of the bands.

Results

Figure 1 shows the effects of three lactogenic hormones on iodide uptake in mammary explants derived from 12- to 14-day-pregnant mice; the explants were taken from the animals and cultured for 2 days with the hormones after which iodide uptake was assessed. Insulin, by itself or in the presence of cortisol or prolactin, significantly enhanced iodide uptake. Prolactin and cortisol, by themselves or in the presence of one of the other lactogenic hormones, significantly reduced iodide uptake after the 2-day treatment. Only when all three hormones were added concurrently was there a 2- to 4-fold increase in iodide accumulation. When mammary tissues from virgin mice were cultured for 2 days with the lactogenic hormones (Fig. 2), insulin again stimulated iodide uptake both by itself and in the presence of the other lactogenic hormones. Cortisol was without effect, whereas prolactin impaired iodide uptake by itself and with all combinations of the other lactogenic hormones. Cortisol was employed at a concentration of 10^{-7}M in the studies reported in Figures 1 and 2, as this cortisol concentration was found to elicit a maximum iodide uptake when combined with insulin and prolactin (Fig. 3).

The time course for the insulin stimulation of iodide uptake in mammary tissues taken from pregnant mice is shown in Figure 4. The insulin effect is initially detected after a 10-hr treatment, and a maximum response is ex-

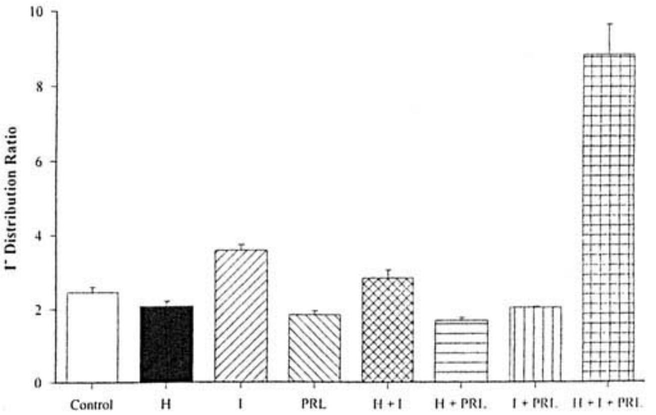

Figure 1. Effect of lactogenic hormones on iodide uptake in mammary tissues from 12- to 14-day-pregnant mice. Explants from pregnant mice were cultured for 2 days with combinations of 10^{-7}M cortisol, 1 μg/ml insulin, and 1 μg/ml prolactin. Tissues were pulse labeled with I^{125} (0.25 μCi/ml, 0.3 ng/ml) for the final 2 hr of culture. Differences ($P < 0.05$) among the treatment combinations as presented in the text. $n = 6$.

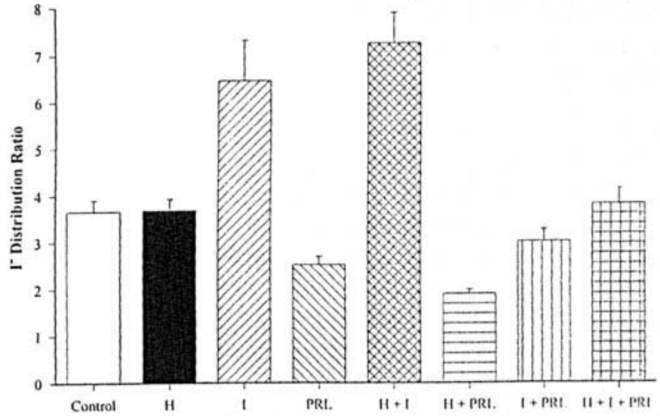

Figure 2. Effect of lactogenic hormones on iodide uptake in mammary tissues taken from virgin mice. (See legend to Fig. 1 for details).

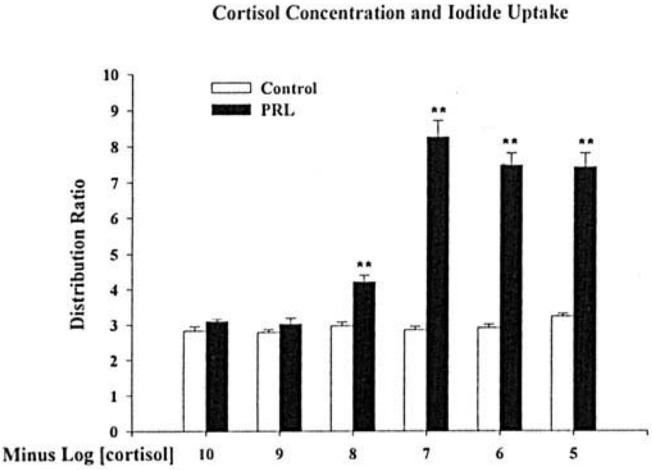

Figure 3. Dose-response effect of cortisol on iodide uptake. Tissues were cultured for 48 hr with 1 μg/ml insulin, cortisol at the concentrations specified, and 1 μg/ml PRL where indicated. Tissues were pulse-labeled for the final 2 hr with I^{125} (0.25 μCi/ml, 0.3 ng/ml). An asterisk indicates a significant increase ($P < 0.05$, $n = 7$).

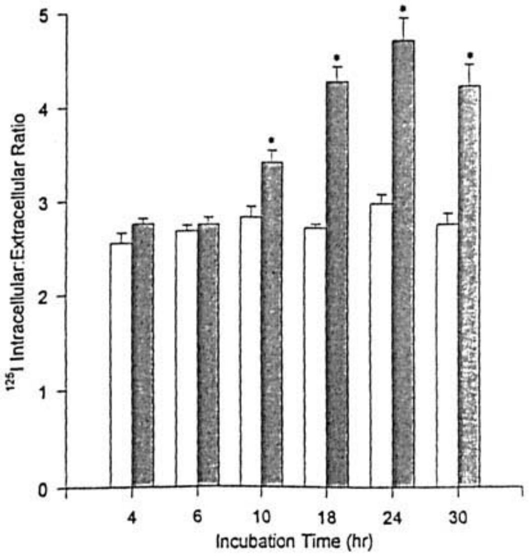

Figure 4. Time course of insulin stimulation of iodide uptake in mammary tissues from pregnant mice. Tissues were cultured for the times indicated and were pulse-labeled for the final 2 hr with I^{125} (0.25 μCi/ml, 0.3 ng/ml). An asterisk indicates a significant increase ($P < 0.01$, $n = 6$).

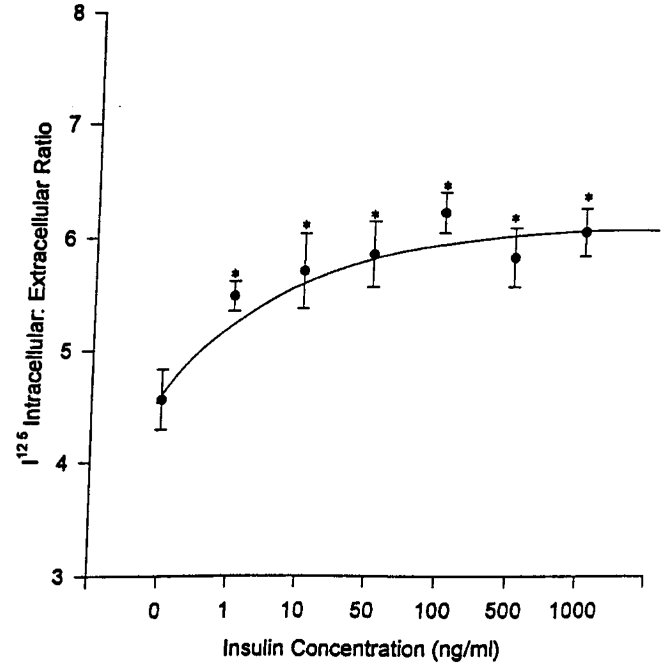

Figure 5. Dose-response effect of insulin on iodide uptake. Tissues were cultured for 24 hr with insulin at the concentrations indicated. I^{125} (0.25 μCi/ml, 0.2 ng/ml) was present during the final 2 hr of culture. An asterisk indicates a significant increase ($P < 0.01$, $n = 6$).

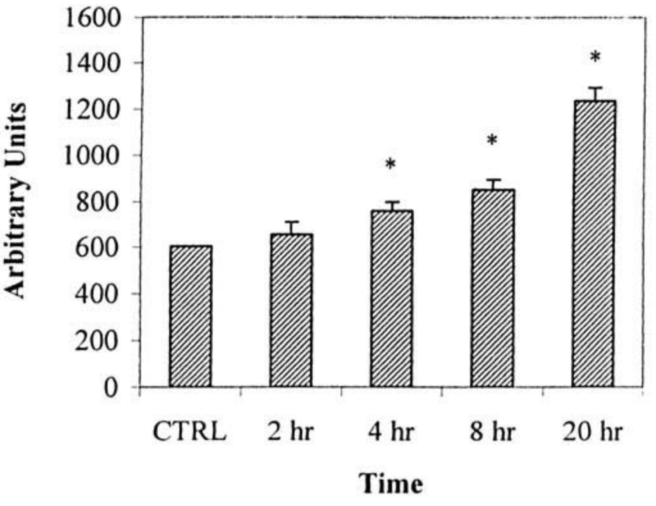

Figure 6. Time course of insulin stimulation of NIS expression in mammary tissues from pregnant mice. Tissues were cultured for the times indicated after which NIS was quantitated as specified in the text. Significant increases were noted ($P < 0.05$) after 4, 8, and 20 hr ($n = 3$).

pressed after 18 hr. The dose-response effect of insulin on iodide uptake is presented in Figure 5. A significant insulin response is expressed at 1 ng/ml and a maximum effect is elicited with concentrations above 10 ng/ml.

Figure 6 presents a time course for the insulin stimulation of sodium-iodide transporter expression in mammary explants from pregnant mice. A significant stimulation was first noted after a 4-hr treatment, and the NIS expression was about 2-fold after a 20-hr insulin treatment. Pictures of the blots are the same as presented earlier (4). The data in Figure 7 further support the involvement of the NIS transporter in the insulin stimulation of iodide uptake. Perchlorate and thiocyanate have been used in experiments for decades to inhibit the NIS transporter in thyroid cells. Both of these inhibitors abolish the insulin stimulation of iodide uptake in mammary cells (Fig. 7). Thiocyanate also reduces by about 4-fold the extent of iodide uptake in the control tissues; this may be caused by additional effects of thiocyanate beyond its inhibition of the NIS transporter. In studies not presented, the effect of insulin on iodide uptake was also abolished when iodide uptake was determined with a sodium-free medium (choline substituted for so-

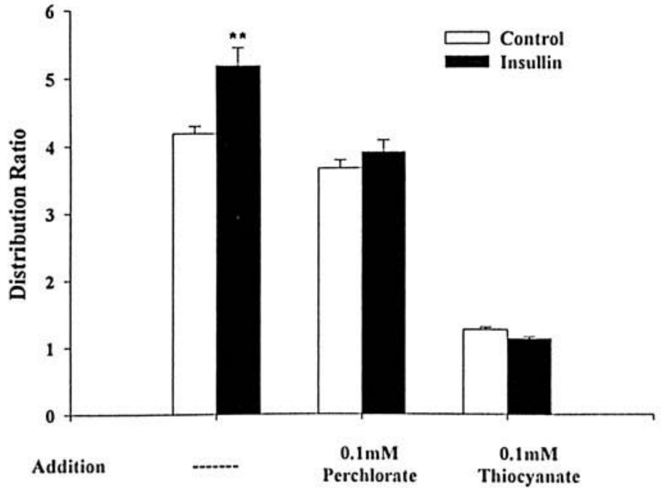

Figure 7. Effect of perchlorate or thiocyanate on insulin stimulation of iodide uptake. Tissues were cultured for 2 days in the presence or absence of 1 μg/ml insulin. Tissues were then pulse-labeled for 2 hr with I^{125} (0.25 μCi/ml, 0.3 ng/ml), and in certain cases with 0.1 mM perchlorate or thiocyanate. An asterisk indicates a significant increase ($P < 0.05$, $n = 6$).

dium). The effect of insulin on iodide uptake thus appears to be expressed primarily through stimulation of the NIS transporter.

Discussion

Insulin, along with cortisol and prolactin, is clearly an important hormone for regulating iodide uptake in the mammary gland. Insulin, by itself at physiological concentrations, elicited stimulatory effects in mammary tissues taken from both pregnant and virgin mice. The Western blot studies with the NIS antibody clearly show that the insulin effect on iodide transport is expressed, at least in part, by an increased tissue content of the NIS transporter. This conclusion is further supported by the relatively similar time courses for the insulin effects on iodide uptake and expression of the NIS transporter protein and finally, inhibition of the insulin stimulation of iodide uptake by perchlorate and thiocyanate further supports the specific effect of insulin on the NIS transporter. In studies not presented, we have observed that 1 μg/ml actinomycin D and 5 μg/ml cyclohexamide abolish the insulin stimulation of iodide uptake in tissues taken from pregnant mice. Therefore, the insulin effect is likely expressed by a stimulation of NIS transporter gene expression.

Although insulin by itself stimulated iodide uptake, by far the greatest extent of iodide uptake was observed when tissues from pregnant mice were cultured with insulin, cortisol, and prolactin. Cortisol appears to primarily have a permissive role in the response to the triple hormone complex, but is required at concentration of 10^{-7}M or above. Although prolactin stimulates NIS expression (4) in tissues that are also exposed to cortisol and insulin, prolactin impaired iodide uptake in tissues from pregnant mice when the tissues were exposed to prolactin alone, or when combined with only one of the other lactogenic hormones. In mammary tissues from virgin mice, prolactin also inhibited iodide uptake with all hormone combinations (Fig. 2). In studies not presented, we have also determined iodide uptake in tissues from pregnant mice that were first cultured for 1 day with insulin and cortisol, and subsequently cultured with all combinations of insulin , cortisol, and prolactin for 1 day. A maximum increase of iodide uptake was observed with insulin plus prolactin or insulin plus cortisol plus prolactin; prolactin was without effect when added alone. Therefore, insulin and prolactin function in a synergistic fashion to stimulate iodide transport in tissues that are pretreated with insulin and cortisol. These studies further indicate the importance of insulin in the regulation of lactogenic processes.

The conclusion from these observations is that the triple lactogenic hormone complex is required for a maximum stimulation of iodide uptake in cultured tissues from pregnant animals. This effect requires differentiation of mammary tissues because the tissues from the virgin mice did not respond to the triple hormone complex. Although insulin increased iodide uptake and NIS expression, these effects may only reflect a doubling of the alveolar epithelial cell number that occurs in response to insulin (7). However, our experimental observations do indicate a critical role for insulin in functioning synergistically with prolactin to stimulate iodide uptake. The fact that prolactin inhibits iodide uptake with all hormone combinations except the triple lactogenic hormone complex was unexpected, and we currently have no reasonable explanation to offer for this response. It may have relevance under certain pathological conditions.

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