Characteristics of the Prolactin Stimulation of Iodide Uptake into Mouse Mammary Gland Explants (44145)

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Abstract. **We have recently reported that prolactin (PRL) stimulates iodide uptake into cultured mouse mammary tissues. This effect occurs in both TCA soluble and insoluble tissue fractions. The effect of PRL apparently involves an RNA-DNA–dependent mechanism, since actinomycin D and cyclohexamide abolish the PRL stimulation of iodide uptake and its incorporation into protein. Perchlorate and thiocyanate, inhibitors of the iodide transporter, also abolish the PRL effects on iodide uptake and incorporation. Similarly, propylthiouracil and aminotriazole, inhibitors of peroxidase, abolish both effects of PRL. Finally, the extent of iodide uptake in mammary cells is suppressed by about 50% in sodium-free medium. These studies thus suggest the existence of a sodium-iodide symporter in the mammary gland which has characteristics similar to the iodide transporter in the thyroid gland—that is, it is sodium dependent and is inhibited by perchlorate and thiocyanate. The fact that both iodide transporter inhibitors and peroxidase inhibitors abolish PRL-stimulated iodide uptake and incorporation suggests that there may be a coupled mechanism involving the iodide transporter and the peroxidase enzyme.** [P.S.E.B.M. 1997, vol 215]

It has been known since the 19th century that iodide appears in the milk of mammals (1). The levels of iodide in the milk of a variety of species including humans are 20- to 30-fold higher than that present in the maternal plasma (2–5). Since about 50% of the iodide in milk is incorporated in milk proteins, the mechanisms that drive the accumulation of iodide in milk could include the functioning of an iodide transporter and/or enzymes involved in iodide incorporation into proteins. Many years ago, Grosvenor (6) injected perchlorate (an inhibitor of the iodide transporter) and methimazole (an inhibitor of peroxidase) into lactating rats and observed a decreased ^{131}I accumulation in the milk with either drug. Perchlorate was the most potent in inhibiting total ^{131}I uptake, but methimazole, as expected, primarily inhibited ^{131}I binding to milk proteins. These *in vivo* studies suggest that both an iodide transporter and a peroxidase enzyme are involved in the accumulation of iodide in milk during lactation.

Studies in the literature on the hormonal regulation of iodide transport in the mammary gland are quite sparse. Grosvenor (6) in his studies reported that thyroid-stimulating hormone (TSH) or thyroxin, injected into lactating rats, had no effect on ^{131}I secretion into milk. In addition, Maqsood and Reineke (7) reported in 1960 that TSH, prolactin (PRL), growth hormone (GH), insulin, or cortisol had no effect on iodide uptake into cultured mammary tissues taken from lactating rats. During the past year, however, we have discovered that PRL doubles the rate of iodide accumulation in cultured mammary tissues taken from 12- to 14-day pregnant mice (8). The failure of Maqsood and Reineke to observe hormone effects on iodide uptake likely relates to the tissues used (i.e., lactating vs. tissues from pregnant animals used in our studies). In any case, the present studies were designed to determine if the iodide uptake mechanism in mammary cells is similar to that reported for thyroid tissues (1, 9).

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 (PRL; National Institutes of Health PS-19) was a

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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); ^{3}HOH and [*carboxy*-^{14}C]inulin (405.8 mCi/g) from New England Nuclear (Boston, MA); ^{125}I from Amersham Life Sci., Inc. (Arlington Heights, IL); porcine insulin, penicillin, and streptomycin from Eli Lilly (Indianapolis, IN); and choline chloride, propylthiouracyl, aminotriazole, sodium perchlorate, and potassium thiocyanate from Sigma Chemical Co. (St. Louis, MO).

Explants of mouse mammary tissues were prepared and cultured as described earlier (10). The explants were cultured on siliconized lens paper floating on 6 ml of 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 where the effects of PRL on iodide transport were to be determined, the tissues were initially cultured for 24–36 hr in the absence of PRL or other substances; PRL or various inhibitors were then added, and incubations continued for the times specified for each experiment. For the final 2–4 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 homogenized in 2 ml 10% 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 washing the pellet with an additional 5 ml of 10% 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 (8, 10). For these calculations the total water content (51.0%) and extracellular space (24.6%) were determined by the volume of distribution of ^{3}HOH and [^{14}C]inulin (1 m*M*), respectively. In time course studies, equilibration was achieved with ^{3}HOH and [^{14}C]inulin 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 which 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 DPM/mg wet weight of tissues.

Statistical comparisons were made with Student's *t* test when two means were compared with an analysis of variance followed by Dunnet's test for multiple comparisons.

Results

Tables I and II show the effect of PRL on total iodide uptake (Table I) and iodide incorporation into a TCA precipitable fraction (Table II) in explants that were pulsed for 4 hr with iodide at concentrations ranging from 10 ng/ml to 5 μg/ml. Significant PRL effects are expressed with all iodide concentrations tested, and the magnitude of the PRL responses (% increase) were maximal at 100–500 ng/ml iodide. At higher iodide concentrations the PRL effects were attenuated. A peak 130% increase of iodide uptake was observed with 100 ng/ml iodide, whereas a 40% increase was observed with 5 μg/ml iodide. Similarly, a 10-fold increase in iodide incorporation was observed with 500 ng/ml iodide, whereas a 3-fold increase was observed with 5 μg/ml iodide. These effects may be caused by a higher intracellular iodide accumulation from uptake mechanisms not involving iodide transporters when the higher iodide concentrations were employed. As might be anticipated, total iodide uptake increases as the iodide concentration is increased, and radiolabeled iodide incorporation decreases as the specific activity of the ^{125}I in the pulse medium decreases.

Tables III and IV report the effects of two metabolic inhibitors on the PRL stimulation of iodide uptake and incorporation. A 24-hr treatment in the presence of actinomycin D or cyclohexamide abolishes both effects of PRL. Basal rates of iodide uptake and incorporation were also

Table I. I^- and PRL Stimulation of I^- Uptake

I^- (ng/ml)	Total cellular uptake of I^- (ng/ml intracellular water)		
	Control	PRL	*P*
10	56 ± 3	84 ± 5	<0.01
100	328 ± 25	756 ± 6	<0.01
500	1505 ± 30	3295 ± 175	<0.01
1000	2820 ± 120	4570 ± 140	<0.01
2000	3980 ± 160	6400 ± 340	<0.01
5000	7250 ± 300	10200 ± 151	<0.01

Note. Tissues were cultured for 1 day with insulin plus cortisol, then 1 day ± 1 μg/ml PRL, and finally 4 hr with ^{125}I (0.25 μCi/ml) at the concentrations indicated. The intracellular accumulation of iodide was then calculated. Values represent the mean ± SE of six observations.

Table II. I^- and PRL Stimulation of I^- Incorporation into TCA Precipitate

I^- (ng/ml)	^{125}I incorporated (DPM/mg tissue)		
	Control	PRL	*P*
10	116 ± 16	356 ± 11	<0.01
100	48 ± 6	282 ± 14	<0.01
500	24 ± 2	275 ± 27	<0.01
1000	17 ± 3	166 ± 10	<0.01
2000	15 ± 1	93 ± 7	<0.01
5000	10 ± 2	43 ± 2	<0.01

Note. Tissues were cultured for 1 day with insulin plus cortisol, then 1 day ± 1 μg/ml PRL, and finally 4 hr with ^{125}I (0.25 μCi/ml) at the I^-, concentrations indicated. Radioactivity in a 10% TCA precipitable fraction was then determined. Values represent the mean ± SE of six observations.

Table III. Effect of Metabolic Inhibitors on PRL Stimulation of I^- Uptake

Addition	I^- Uptake (distribution ratio)		
	Control	PRL	*P*
—	3.94 ± 0.22	8.34 ± 0.52	<0.01
1 μg/ml actinomycin D	2.15 ± 0.04	2.11 ± 0.08	NS
5 μg/ml cyclohexamide	2.02 ± 0.11	2.11 ± 0.02	NS

Note. Tissues were cultured for 1 day with insulin plus cortisol, then 1 day ± μg/ml PRL and/or the antibiotics, and finally with 0.25 μCi/ml ^{125}I for 2 hr. Values represent the mean ± SE of six observations.

Table IV. Effect of Metabolic Inhibitors on PRL Stimulation of I^- Incorporation

Addition	[^{125}I] incorporated (DPM/mg tissue)		
	Control	PRL	*P*
—	80 ± 2	236 ± 3	<0.01
1 μg/ml actinomycin D	26 ± 1	24 ± 1	NS
5 μg/ml cyclohexamide	26 ± 1	27 ± 1	NS

Note. Tissues were cultured for 1 day with insulin plus cortisol, then 1 day ± μg/ml PRL and/or the antibiotics, and finally with 0.25 μCi/ml ^{125}I for 2 hr. Values represent the mean ± SE of six observations.

reduced by 50% or more by the inhibitors of RNA (actinomycin D) and protein (cyclohexamide) biosynthesis. The efficacy of these antibiotics in inhibiting RNA and protein synthesis in cultured mammary tissues was established in earlier studies (10).

Perchlorate and thiocyanate have been used for decades to identify and characterize the iodide transporter in several tissues, especially the thyroid gland (1, 2, 9, 11). Tables V and VI show that both of these drugs suppress the rates of both iodide uptake and its incorporation into the TCA precipitable fraction. In addition, thiocyanate abolishes the effects of PRL on iodide uptake and incorporation, while perchlorate attenuates the magnitude of the PRL stimulation of these processes.

Propylthiouracil (PTU) and aminotriazole are peroxidase inhibitors and have been employed to characterize the regulation of iodide incorporation into proteins (1, 2, 9, 11). The dose-response effects of these drugs on iodide uptake and incorporation are presented in Tables VII and VIII. Both drugs reduced the basal rates of iodide uptake and incorporation. At the highest concentrations, these drugs abolished the PRL responses, while at the lower concentrations the drugs attenuated the magnitude of the PRL responses.

Since earlier studies with thyroid cells have suggested that the iodide transporter is sodium dependent (1, 2, 9, 11), studies were carried out to determine if this also maintains for the iodide uptake mechanism in cultured mammary cells. Tables IX and X clearly show that iodide uptake and incorporation are significantly decreased in the absence of sodium in the culture medium. Although PRL effects were still observed in the absence of sodium, the magnitude of the responses were significantly attenuated.

Table V. Effect of I^- Transport Inhibitors of PRL Stimulation of I^- Uptake

Inhibitor	I^- uptake (distribution ratio)		
	Control	PRL	*P*
—	11.6 ± 0.7	22.1 ± 1.4	<0.01
0.1 m*M* ClO_4^-	2.4 ± 0.1	3.3 ± 0.1	<0.01
0.5 m*M* ClO_4^-	2.3 ± 0.1	3.0 ± 0.1	<0.01
0.1 m*M* KSCN	1.8 ± 0.1	1.7 ± 0.1	NS

Note. Tissues were cultured for 1 day with insulin plus cortisol, then 1 day ± μg/ml PRL, and finally 4 hr with ^{125}I (0.25 μCi/ml, 0.3 ng/ml) ± the inhibitors as specified. The intracellular accumulation of iodide was then calculated. Values represent the mean ± SE of six observations.

Table VI. Effect of I^- Transport Inhibitors on PRL Stimulation of I^- Incorporation

Inhibitor	^{125}I incorporated (DPM/mg tissue)		
	Control	PRL	*P*
—	367 ± 19	842 ± 44	<0.01
0.1 m*M* ClO_4^-	62 ± 6	93 ± 5	<0.01
0.5 m*M* ClO_4^-	48 ± 2	80 ± 4	<0.01
0.1 m*M* KSCN	12 ± 2	15 ± 2	NS

Note. Tissues were cultured for 1 day with insulin plus cortisol, then 1 day ± μg/ml PRL, and finally 4 hr with ^{125}I (0.25 μCi/ml, 0.3 ng/ml) ± the inhibitors as specified. The intracellular accumulation of iodide was then calculated. Values represent the mean ± SE of six observations.

Table VII. Effect of Peroxidase Inhibitors on PRL Stimulation of Iodide Uptake

Inhibitor	^{125}I incorporated (distribution ratio)		
	Control	PRL	*P*
—	6.01 ± 0.28	9.28 ± 0.47	<0.01
2.5 μg/ml PTU	6.22 ± 0.40	8.50 ± 0.47	<0.01
5 μg/ml PTU	4.76 ± 0.25	7.23 ± 0.45	<0.01
10 μg/ml PTU	5.00 ± 0.15	4.87 ± 0.24	NS
500 μg/ml PTU	2.84 ± 0.08	3.15 ± 0.08	NS
0.1 m*M* aminotriazole	3.24 ± 0.16	3.94 ± 0.20	<0.05
0.5 m*M* aminotriazole	2.84 ± 0.08	3.08 ± 0.08	NS
1 m*M* aminotriazole	3.08 ± 0.07	2.89 ± 0.08	NS

Note. Tissues were cultured for 1 day with insulin plus cortisol, then 1 day ± μg/ml PRL, and finally 4 hr with ^{125}I (0.25 μCi/ml, 0.3 ng/ml) ± the inhibitors as specified. The intracellular accumulation of iodide was then calculated. Values represent the mean ± SE of six observations.

Discussion

The results of these experiments are compatible with the suggestion that an iodide transporter exists in the mammary gland which has characteristics similar to the iodide transporter in the thyroid gland. Iodide uptake is inhibited by perchlorate and thiocyanate in both cell types, and the

Table VIII. Effect of Peroxidase Inhibitors on PRL Stimulation of Iodide Incorporation

Inhibitor	^{125}I incorporated (DPM/mg tissue)		
	Control	PRL	*P*
—	168 ± 11	425 ± 29	<0.01
2.5 μg/ml PTU	72 ± 6	170 ± 20	<0.01
5 μg/ml PTU	46 ± 7	69 ± 6	<0.05
10 μg/ml PTU	20 ± 3	26 ± 1	NS
500 μg/ml PTU	11 ± 2	13 ± 2	NS
0.1 m*M* aminotriazole	134 ± 18	287 ± 22	<0.01
0.5 m*M* aminotriazole	72 ± 6	133 ± 7	<0.01
1 m*M* aminotriazole	76 ± 7	93 ± 3	NS

Note. Tissues were cultured for 1 day with insulin plus cortisol, then 1 day ± μg/ml PRL, and finally 4 hr with ^{125}I (0.25 μCi/ml, 0.3 ng/ml) ± the inhibitors as specified. The intracellular accumulation of iodide was then calculated. Values represent the mean ± SE of six observations.

Table IX. Effect of Sodium on Iodide Uptake

Pulse media	Iodide uptake (distribution ratio)		
	Control	PRL	*P*
KRB-sodium	7.29 ± 0.28	11.5 ± 0.3	<0.01
KRB-choline	3.96 ± 0.09	5.08 ± 0.32	<0.01

Note. Explants were cultured for 1 day with insulin plus cortisol, then 1 day with or without 1 μg/ml prolactin and finally 4 hr with ^{125}I (0.25 μCi/ml, 0.3 ng/ml) in the media specified in the table. Values represent the mean ± SE of six observations.

uptake is sodium dependent. Our results thus confirm the data derived from earlier *in vivo* studies (6). The recent cloning of the thyroid iodide transporters in rats (12) and humans (13) should allow future experimental studies that will provide the unequivocal demonstration that the iodide transporters in the two cell types are the same.

The results from our studies also suggest that the PRL stimulation of iodide uptake in mammary tissues occurs *via* a hormonal stimulation of an iodide transporter. The PRL effect was impaired both by two iodide transport inhibitors (perchlorate and thiocyanate) and when a sodium-free medium was employed for uptake determinations. Since the effect of PRL on iodide uptake was abolished by inhibitors of RNA (actinomycin D) and protein (cyclohexamide) synthesis, the PRL effect on the iodide transporter likely occurs *via* an RNA-DNA dependent mechanism. Accordingly, when the iodide transporter cDNAs become available for future northern hybridization studies, we would expect to observe an effect of PRL on the accumulation of the mRNA for the iodide transporter. A similar effect of PRL on the accumulation of the iodide transporters in future Western blot studies is anticipated if antibodies to the iodide transporter proteins are developed.

The results generated from the peroxidase inhibitor experiments were somewhat surprising and more difficult to interpret. The inhibition of iodide incorporation and total

Table X. Effect of Sodium on Iodide Incorporation into a 10% TCA Precipitate

Pulse media	Iodide incorporated (DPM/mg tissue)		
	Control	PRL	*P*
KRB-sodium	400 ± 27	636 ± 13	<0.01
KRB-choline	172 ± 13	216 ± 13	<0.01

Note. Explants were cultured for 1 day with insulin plus cortisol, then 1 day with or without 1 μg/ml prolactin and finally 4 hr with ^{125}I (0.25 μCi/ml, 0.3 ng/ml) in the media specified in the table. Valves represent the mean ± SE of six observations.

iodide accumulation by the peroxidase inhibitors occurred as expected, however, the abolishment of the PRL stimulation of iodide accumulation is difficult to explain if the mechanism for the PRL effect on iodide accumulation entirely involves a stimulation of iodide transporter activity. An alternative possibility is that PRL only stimulates peroxidase activity and this is the driving mechanism for the PRL stimulation of iodide uptake in mammary cells. The studies with the iodide transporter inhibitors, however, would not support this possibility. A second possible explanation for the experimental observations is that the peroxidase inhibitors may have overlapping inhibiting effects on the iodide transporters; however, data in the literature do not support this thesis. Finally, it is possible that the iodide transporters and peroxidase enzymes may be coupled in some fashion such that when one is inhibited, the other is inhibited as well. At the present time, this latter possibility seems most compatible with the experimental data.

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