Prolactin and Growth Hormone Stimulation of Lactation in Mice Requires Thyroid Hormones (44417)

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Abstract. **This experiment tested the hypothesis that thyroid hormones are essential for a milk production response to growth hormone (GH) and prolactin (PRL). Prior to breeding, female transgenic mice expressing the herpes simplex type-I thymidine kinase in the thyroid were treated with ganciclovir to ablate thyroid follicular cells. To provide for normal gestation, thyrocyte-ablated mice were supplied thyroxine (T_4) in drinking water (0.2 μg/ml) until 7 days before parturition. Litter size was adjusted to 9 pups, hormone administration began on Day 2 of lactation, and mice were sacrificed on Day 12. There were 5–6 mice in each of 7 treatments that included nonablated controls, thyrocyte-ablated controls, and thyrocyte-ablated mice treated with T_4, GH, PRL, GH + T_4, and PRL + T_4. Thyroxine was administered in drinking water, and GH and PRL (20 μg/d) were administered by subcutaneous injection. Compared with thyrocyte-ablated controls, litter weight gain was unaffected when dams were treated with GH, PRL, or T_4 alone. However, when dams were treated with GH or PRL in combination with T_4, litter weight gain increased 13% compared with thyrocyte-ablated controls and 18% compared with GH or PRL-treated mice. Concentration of T_4 in serum of pups averaged 62 ng/ml and did not differ among treatments. Concentration of T_4 in serum of dams averaged 76 ng/ml when T_4-treated. Thyroxine 5′-deiodinase (5′D), the enzyme that converts T_4 to triiodothyronine, was quantitated in liver, kidney, and mammary gland. Quantity of 5′D was lower in liver and kidney of thyrocyte-ablated dams without T_4 than in respective tissues of mice treated with T_4, and there was no effect of GH or PRL. However, in mammary gland, 5′D was increased by treatment with GH, PRL, or T_4. Data show that thyroid hormones are necessary for a galactopoietic response to GH and PRL and demonstrate a unique organ-specific regulation of 5′D by galactopoietic hormones.** [P.S.E.B.M. 1999, Vol 221]

Growth hormone (GH) and prolactin (PRL) are generally acknowledged as major galactopoietic hormones in ruminants and rodents, respectively (1). We hypothesized that galactopoietic effects of GH in lactating cows may be related to induction of organ-specific changes in thyroid hormone activity (2, 3). This hypothesis is consistent with the importance of thyroid hormones for maintaining normal lactation (4).

Although thyroxine (T_4) is the predominant thyroid hormone in the circulation, it has little if any inherent biological activity. The metabolically active thyroid hormone, triiodothyronine (T_3), is produced by enzymatic 5′-deiodination of T_4 within the thyroid and in extrathyroidal tissues (5–7). The extrathyroidal activity of thyroxine 5′-deiodinase (5′D) is an important control point for regulating the thyroid status of animal tissues in various physiological and pathological situations (8). During lactation of rats, 5′D activity decreases in liver and kidney (primary contributors of plasma T_3) and increases in mammary gland (9–11). These opposite organ-specific changes in 5′D activity are proportional to lactational intensity and are consistent with focusing metabolic priority on the mammary gland while maintaining the mammary gland in a euthyroid state.

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Similarly, increasing lactational intensity in dairy cows by GH administration alters 5′D activity in extrathyroidal tissues. During late lactation, GH increased 5′D activity in mammary tissue concurrent with increased milk production (2). During mid-lactation GH decreased 5′D in liver while maintaining 5′D activity in mammary gland (3). These data suggest an involvement of thyroid hormones and regulation of extrathyroidal 5′D activity in the galactopoietic response to GH.

The objective of the present study was to test the hypothesis that thyroid hormones are necessary to obtain milk production increases in response to galactopoietic hormones. Previous investigations have demonstrated the galactopoietic effects of GH and PRL in euthyroid mice (12, 13). Specifically, we examined effects of the galactopoietic hormones GH and PRL on litter weight gain and extrathyroidal 5′D activity, in thyrocyte-ablated mice with and without thyroxine replacement.

Materials and Methods

Thyrocyte Ablation. A hypothyroid condition was induced, using a specific, nonsurgical ablation of thyroid follicular cells, in a line of susceptible transgenic mice (14). A hybrid gene, containing the Herpes Simplex Virus Type-I thymidine kinase gene attached to a 3.1-kb fragment of promoter and 5′-flanking region of the bovine thyroglobulin gene, was introduced into the germline of C57BL/6 × CBA/Ca mice. The transgenic females appear normal and display normal thyroid function. However, when they are treated with the anti-Herpes drug, ganciclovir (9–1,3-dihydroxy-2-propoxy (methyl) guanine), the drug is phosphorylated within the thyrocytes by the viral thymidine kinase expressed in these cells. This results in ablation of the thyrocytes, with no apparent damage to other cells except in the testes, which appear to express the viral gene constitutively. As the males are rendered sterile, the line is propagated by breeding homozygous transgenic females to heterozygous transgenic males. Offspring expressing the transgene were identified by polymerase chain reaction analysis of DNA isolated from tissue of tail biopsy (14). Using these transgenic mice, thyroid ablation can be accomplished while maintaining normal calcitonin and parathormone concentrations.

Transgenic female mice were randomly assigned to treatment groups. Beginning 15 days before mating to nontransgenic males, transgenic females assigned to the thyrocyte-ablated treatment groups were injected intraperitoneally with 2.25 mg of ganciclovir (16.7 mg/ml in Dulbecco's Phosphate Buffered Saline) twice per day, for 2 days. It was determined that 2-day treatment with ganciclovir was as effective for thyrocyte ablation as the 3-day injection protocol previously employed (14). Ganciclovir was generously provided by Syntex Corporation (Palo Alto, CA).

Use of animals for this investigation was approved by the Beltsville Agricultural Research Center's Animal Care and Use Committee.

Treatment Groups. Thyroxine was provided in the drinking water (0.2 μg/ml) of all thyrocyte-ablated mice from mating until Day 14 of gestation to provide T_4 for a normal gestation, yet permit sufficient time for clearance of T_4 by the initiation of lactation. After parturition, five to six lactating mice were randomly assigned to hormone treatment groups. Hormone treatments were initiated on Day 2 of lactation (i.e., ≈ 48 hr postpartum) and included daily subcutaneous injection of 20 μg of bovine GH or PRL, with and without T_4-supplemented drinking water (0.2 μg/ml). Thus, treatments included: 1) nonablated or intact control; 2) thyrocyte-ablated control; 3) thyrocyte ablation + GH; 4) thyrocyte ablation + PRL; 5) thyrocyte ablation + T_4; 6) thyrocyte ablation + T_4 + GH; and 7) thyrocyte ablation + T_4 + PRL. Litter size was adjusted to nine pups, and litter weight gain was used as an indicator of milk production. When adjusting litter size, donor dams were of the same thyroid status as the recipients (intact, thyrocyte-ablated, and thyrocyte-ablated with T_4-supplementation).

Bovine GH (USDA-bGH-B-1) and PRL (USDA-bPRL-B-1) were provided by the USDA Animal Hormone Program (Dr. D. Bolt, Building 200, BARC-East, Beltsville, MD; prepared and characterized by Dr. A.F. Parlow, Harbor-UCLA Medical Center, Torrance, CA). USDA-bGH-B-1 had a biological potency of 1.9 IU/mg protein, and UDSA-bPRL-B-1 had a biological potency of 13.0 IU/mg protein. Contamination with other pituitary hormones was less that 0.5% by radioimmunoassay. GH and PRL were solubilized in alkaline saline (50 m*M* $NaHCO_3$, 100 m*M* NaCl) at a concentration of 0.2 mg/ml for injection. Control mice received injections of alkaline saline.

Tissue Collection and Hormone Assay. On the day before mating, blood of ganciclovir-treated mice was collected from the tail for subsequent determination of circulating T_4 and confirmation of thyrocyte ablation. Dams and pups were euthanized on Day 12 of lactation. Blood was collected from severed neck vessels of CO_2-anesthetized mice. Blood from pups within a litter was pooled. Samples of maternal mammary gland, kidney, and liver were immediately removed, frozen in liquid nitrogen, and stored at –80°C until 5′D assay. Activity of 5′D was determined as described below. Concentrations of T_4 were determined in maternal and pup serum using commercially available radioimmunoassay kits (ICN Diagnostics, Costa Mesa, CA), validated for murine serum.

5′D Assay. For assay of 5′D activity, fragments of frozen tissues were weighed and homogenized in 10 Vol (w/v) ice-cold buffer (0.01 *M* HEPES, pH 7.0, with 0.25 *M* sucrose and 1 m*M* EDTA) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). Crude homogenates of liver, kidney, and mammary gland were centrifuged at 2000*g* for 30 min at 4°C, and the supernatants were used for subsequent incubations. The protein content of supernatants was determined by the bicinchoninic acid assay (15).

Outer-ring deiodinating activity (5'D) was determined by quantitating the $^{125}I^-$ released from [5'-^{125}I]-rT_3 based upon the method of Leonard and Rosenberg (16) with modification as described previously (11). In brief, the [^{125}I]-rT_3 (SA > 1100 μCi/μg, New England Nuclear, Boston, MA), was purified by Sephadex LH-20 chromatography immediately before each assay. Enzyme assay mixture contained 0.1 *M* sodium phosphate buffer (pH 7.0), 1 m*M* EDTA, 5 m*M* (liver and kidney), or 10 m*M* (mammary gland) dithiothreitol, and ≈ 80,000 cpm [^{125}I]-rT_3 mixed with unlabeled rT_3 to a final concentration of 500 (liver) or 125 (mammary gland) n*M*. Incubations were for 5 min (liver and kidney) or 60 min (mammary tissue) at 37°C. Reactions were terminated by addition of 0.1 ml of an ice-cold solution containing equal volumes of bovine serum and 10 m*M* 6-*n*-propyl-2-thiouracil, followed by 0.3 ml ice-cold 10% (w/v) trichloroacetic acid. After 10 min at 0°C, mixtures were centrifuged at 1500*g* for 10 min, and radioactivity of free $^{125}I^-$ in the supernatant was counted. Reaction tubes (background) containing no enzyme were included in each assay. Net radioactivity was determined by subtracting supernatant radioactivity in background tubes from that in the sample tubes. Product formation was proportional to incubation time and protein content.

Statistical Analysis. Statistical analyses used the general linear models procedure of SAS (17). The least significant difference test was used for mean separation. Litter growth was analyzed based on net gain during the 10-day treatment period and based on the rate of gain determined by linear regression of individual litter growth curves. For all growth curves, the correlation coefficient (r^2) exceeded 0.985.

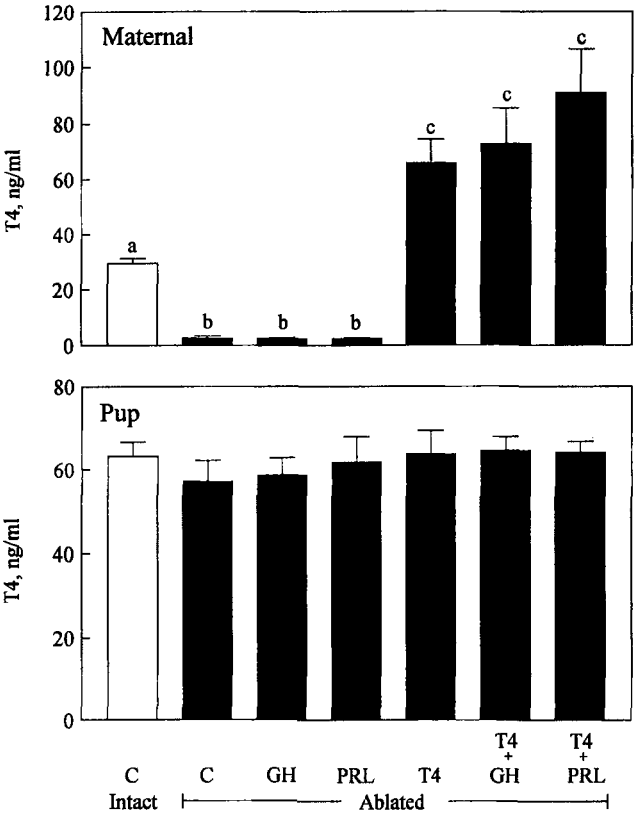

Figure 1. Concentrations of T_4 in maternal and pup serum. Each bar is the mean concentration for serum from five to six lactating mice and for serum from five to six litters, each of which was pooled from pups within the litter. Groups included: intact and thyrocyte-ablated control (C) mice, and ablated mice that were administered T_4, GH, PRL, or T_4 plus GH or PRL. Error bars are ± SEM. Bars bearing different letters differ ($P < 0.05$) by the least significant difference test.

Results

One day before breeding (14 days after ganciclovir treatment), the effectiveness of thyrocyte ablation was confirmed by the fact that concentrations of T_4 in serum were below assay sensitivity (<0.7 ng/ml of serum; data not shown). On Day 12 of lactation, concentrations of T_4 in serum of intact control mice averaged 30 ng/ml (Fig. 1). However, during lactation, concentrations of T_4 in serum of thyrocyte-ablated controls and mice that were treated with GH or PRL alone, averaged 2–3 ng/ml. Supplying T_4 in drinking water effectively restored concentrations of T_4 in the serum of thyrocyte-ablated mice to the physiological range although concentrations were significantly higher ($P < 0.05$) than those for intact control mice. Thyrocyte-ablated mice that were administered T_4 averaged 66 ng of T_4/ml of serum. Additional treatment with GH or PRL did not affect ($P > 0.2$) the circulating concentration of T_4 in the serum.

Circulating concentrations of T_4 indicated that pups in all treatment groups were euthyroid (Fig. 1) and were capable of regulating their thyroid status independent of the thyroid status of the dam. Circulating concentrations of T_4 did not differ ($P > 0.2$) among pups in different maternal treatment groups. Regardless of the treatment group, pups averaged 62 ng of T_4/ml of serum.

Effects of hormone treatment on litter and maternal weight gains are summarized in Table I. Litters of intact control mice gained 38 g during the 10-day treatment period. Pups of thyrocyte-ablated controls gained 34 g during this period, and tended ($P = 0.068$) to gain at a slower rate than those of intact controls. Treatment of lactating mice with only GH or PRL did not alter ($P > 0.2$) litter weight gain compared with that for litters of thyrocyte-ablated controls. Similarly, administration of thyroxine did not increase ($P > 0.2$) litter weight gain compared with ablated controls. However, when thyrocyte-ablated mice were treated with T_4 in conjunction with GH or PRL, litter weight gain was increased compared with that of ablated controls ($P < 0.05$). Litter weights at initiation of hormone treatments did not differ ($P > 0.2$) among groups. Average litter size at birth was 7.7 pups, and did not differ among groups ($P > 0.2$).

Maternal body weight gain during the lactation treatment period was influenced by hormone status (Table I). Maternal body weight averaged 31.4 g at initiation of hormone treatment and did not differ between treatment groups ($P > 0.2$). However, thyrocyte-ablation increased maternal

Table I. Weight Gain of Litters and Dams During Lactation

Treatment[1]	*n*	Litter gain[2]		Dam gain[2]	Initial measures		
		g/day	g/10 days	g/10 days	#Pups[3]	Litter,[4] g	Dam,[4] g
Intact control	6	3.92^{ab}	38.0^{ab}	4.3^{bc}	8.0	16.3	30.8
Thyrocyte-ablated							
Control	6	3.58^{bc}	33.9^{bc}	6.4^{a}	8.8	14.6	32.4
GH	5	3.37^{c}	32.6^{c}	5.7^{ac}	6.6	14.9	31.5
PRL	5	3.37^{c}	32.6^{c}	5.4^{ac}	7.2	15.1	30.2
T_4	5	3.64^{abc}	35.1^{abc}	5.3^{ac}	7.6	15.6	31.2
T_4 + GH	5	3.95^{a}	38.6^{a}	4.6^{bc}	6.8	14.3	31.4
T_4 + PRL	6	3.97^{a}	38.3^{a}	6.2^{a}	8.7	13.0	32.1
SE[5]		0.14	1.7	0.7	0.8	0.9	1.2

[1] Treatment of thyrocyte-ablated mice was initiated on Day 2 of lactation. Hormone treatments included daily subcutaneous injection of 20 μg of GH or PRL, and T_4 supplementation of drinking water, 0.2 μg/ml.
[2] Litter weight gain and weight gain of lactating dams are summarized for the 10-day treatment period. After birth, litter size was adjusted to nine pups per lactating mouse. Daily litter gains (g/day) are means for slopes derived from linear regression of individual litter growth curves. Net litter gain and dam gain (g/10 days) are means for the difference between initial and final litter and dam weights, respectively.
[3] Mean litter size at birth; groups did not differ ($P > 0.1$).
[4] Mean weight of litter and dam on the first day of the treatment period; groups did not differ ($P > 0.1$).
[5] Pooled standard error (for $n = 5$).
[abc] Means with different superscripts differ ($P < 0.05$).

weight gain during lactation by 49% ($P < 0.05$). This weight gain was prevented by the combined treatment of GH and T_4.

Effects of hormone treatments on 5′D activities in mammary, kidney, and liver tissues are summarized in Figure 2. Approximately one-half of the tissue samples were destroyed during a freezer malfunction; determinations of enzyme activity were limited to tissues from three or four mice per treatment group. Despite a reduction in statistical power, important treatment differences were discernible. In all tissues, 5′D activity was lowest for thyrocyte-ablated control mice. Compared with intact controls, thyrocyte ablation reduced ($P < 0.05$) activity of 5′D in liver and kidney ($P < 0.05$), and tended to reduce activity in mammary gland ($P = 0.17$). Response of 5′D to hormone administration was highly correlated in liver and kidney (Pearson correlation coefficient = 0.88; $P < 0.0001$); but response of mammary 5′D to GH and PRL differed from that of hepatic and renal 5′D. Noteworthy is the fact that treatment of thyrocyte-ablated mice with GH or PRL, in the absence of T_4, increased activity of 5′D in mammary gland ($P < 0.05$), but not in liver or kidney ($P > 0.2$). Administration of T_4 by itself numerically increased 5′D activity in all three tissues, but the effect was statistically demonstrable ($P < 0.05$) only in kidney. Administration of GH in conjunction with T_4 increased ($P < 0.05$) activity of 5′D in all tissues when compared with the tissues of thyrocyte-ablated controls. However, effects of GH and T_4 on 5′D activity were not additive. The combined administration of GH and T_4 did not increase ($P > 0.2$) 5′D activity in mammary gland beyond that achieved with GH alone; nor did the combined administration of GH and T_4 increase ($P > 0.2$) activity of 5′D in liver and kidney beyond the effect of T_4 alone. Similarly, 5′D activity in renal and mammary tissues from mice administered PRL and T_4 did not differ ($P > 0.2$) compared with that from mice administered only T_4. However, the combined treatment of PRL and T_4 increased ($P < 0.05$) the 5′D activity in liver beyond that of T_4 alone ($P < 0.05$), and the combined treatment tended to decrease ($P = 0.12$) 5′D in mammary gland compared with that of PRL alone.

Discussion

In the present experiment, litter weight gain was used as an index of milk production to assess the independent and combined effects of T_4, GH, and PRL on milk production. When administered separately to lactating thyrocyte-ablated mice, these hormones did not stimulate litter weight gain. However, when the mice were treated with GH or PRL in combination with T_4, litter weight gain was increased 13% in comparison with that for thyrocyte-ablated controls and 18% in comparison with that for mice that were treated with GH or PRL alone. Thyrocyte ablation was confirmed by undetectable concentrations of T_4 in the serum of all ganciclovir-treated mice prior to mating and the advent of T_4 replacement therapy. Restoration of physiological concentrations of T_4 to the serum of lactating mice was necessary for administration of GH or PRL to increase litter weight gain.

During the first 12 days of lactation, milk is the only source of nutrients and fluids to suckling pups. Nevertheless, use of litter weight gain to evaluate the galactopoietic effects of hormone administration necessitates distinguishing effects of the hormones on the dam from those on the suckling pups. Others have demonstrated that hypothyroidism prolongs gestation and results in smaller litters and increased fetal death (18). In the current study, thyroxine-supplemented water was provided to all thyrocyte-ablated mice until Day 14 of gestation to minimize effects of maternal hypothyroidism during gestation, yet provide ample time for thyroxine clearance before lactation. Litter size at parturition and gestation length did not differ between intact (nonablated) control mice and thyrocyte-ablated mice. The

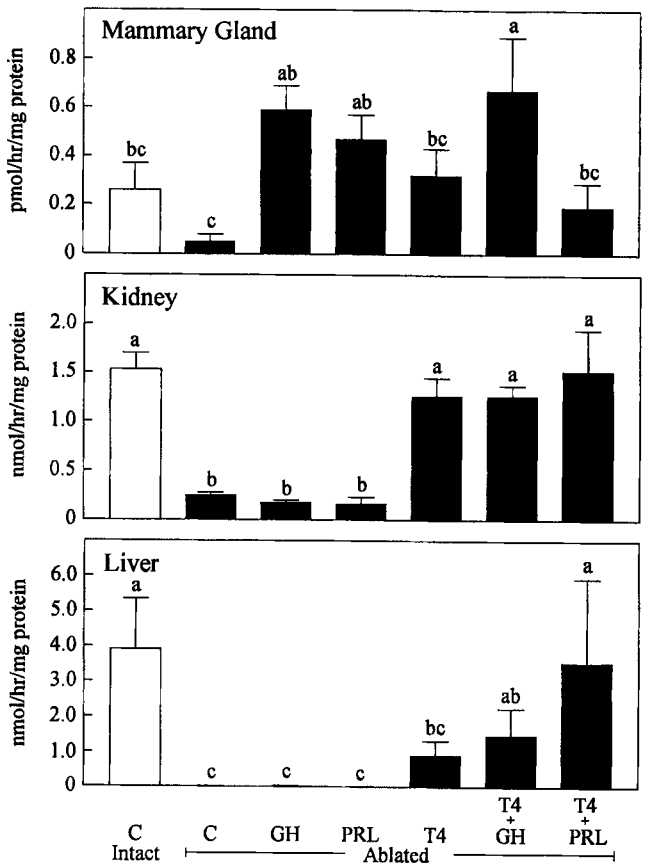

Figure 2. Activity of thyroxine 5′-deiodinase in liver, kidney, and mammary gland. Each bar is the mean activity for tissue from three to four lactating mice. Groups included: intact and thyrocyte-ablated control (C) mice, and ablated mice that were administered T_4, GH, PRL, or T_4 plus GH or PRL. Error bars are ± SEM. Bars bearing different letters differ ($P < 0.05$) by the least significant difference test.

subsequent random assignment of mice to hormone treatments resulted in groups that did not differ with regard to initial litter size or weight. Nor did differences in mortality arise among suckling pups in the different treatment groups. With the exception of intact control mice, all dams were similarly treated during gestation, and the results indicate that thyrocyte ablation, coupled with T_4 treatment, did not grossly affect fetal development.

With onset of lactation, hormone-induced differences in litter weight gain did not appear to be due to transfer of hormones from the dams to their suckling pups *via* milk. Although Fukuda *et al.* (19) reported that such transfer of T_4 occurs in rats, others have failed to demonstrate transfer of thyroid hormones or TSH in milk and failed to demonstrate the physiological importance of such transfer to litter growth of rodents (20–22). Furthermore, by Day 12 of lactation, all pups were clearly euthyroid, and serum concentrations of T_4 did not differ among pups in different treatment groups. Although thyroxine synergizes with glucocorticoids to induce postnatal maturation of the rodent small intestine (23), these changes occur near the time of weaning. Because this is after the experimental timeframe, and the circulating T_4 did not differ among the pups, changes in gut maturation can be ruled out as contributing factors in the present investigation. As expected, litters of thyrocyte-ablated mice grew less than those of intact controls. However, administration of T_4 was unable to restore litter weight gain to that of intact controls. Perhaps the dose of T_4, although resulting in circulating concentrations within physiological range, was not stimulatory to milk production. Indeed, chronic administration of physiological doses of T_4 during gestation accelerated lactogenesis, and inhibited maternal nursing behavior and the milk ejection reflex in rats (24). Furthermore, biphasic effects of thyroid hormones on various physiological processes have commonly been reported (25). Because GH and PRL failed to enhance litter weight gain when administered in the absence of T_4, it seems unlikely that stimulation of litter growth rate in the presence of T_4 was due to transfer of these hormones, or other inducible factors such as the insulin-like growth factors, to suckling pups. Most likely, effects on litter weight gain were due to effects on maternal milk production, rather than metabolic effects on the suckling pups.

These data are consistent with those of previous investigations which demonstrated that, although thyroid hormones are not essential for lactation (4), administration of T_4 augments the galactopoietic effects of exogenous GH and PRL (26, 27). More recently, investigations of the regulation of thyroid hormone metabolism during lactation led to the hypothesis that organ-specific changes in 5′D activity play a role in mediating the galactopoietic response of dairy cattle to bovine GH (2, 3). Consistent with this hypothesis, present data indicate that thyroid hormones are essential for eliciting a galactopoietic response of mice to GH or PRL, and provide information regarding regulation of 5′D in liver, kidney, and mammary tissues of lactating mice.

Two isozymes of 5′D have been identified. Both require thiol reducing agents for activity and are inhibited by iopanoic acid (28, 29), but may be distinguished on the basis of their K_m values for substrates, kinetic patterns for activation by thiols, and susceptibility to inhibition by propylthiouracil (PTU), iodoacetate (30), and gold-thioglucose (31). Type-I 5′D activity is found in high concentrations in rat kidney, liver, and thyroid (6). In euthyroid animals, virtually all of the plasma T_3 is produced by 5′-deiodination of T_4 in the thyroid, liver, and kidney (5–7) by type-I 5′D. Type-II 5′D is found mainly in brain, anterior pituitary, and brown adipose tissue (6). Although the 5′D in mammary glands of rats and mice possesses enzymatic characteristics of a type I 5′D such as that found in liver, kidney, and thyroid (10, 11), the mammary enzyme is encoded by an mRNA that differs from that in other tissues (11), being an mRNA with a truncated 3′-untranslated region (32). This is consistent with the unique regulation of mammary 5′D by galactopoietic hormones documented in the current communication.

Consistent with classical regulation of type-I 5′deiodinases, activity of 5′D in homogenates of liver, kidney, and mammary tissues was markedly reduced by thyrocyte abla-

tion. Conversely, administration of T_4 to thyrocyte-ablated mice appeared to restore 5′D activity in all three tissues partially although the effect was statistically demonstrable only in kidney. However, when thyrocyte-ablated mice were treated with GH or PRL alone, expression of 5′D increased in mammary tissue only. Response to GH and PRL in liver and kidney necessitated concomitant treatment with T_4. Thus, mammary 5′D appears to be uniquely responsive to galactopoietic hormones, consistent with the apparent uniqueness of its mRNA and consistent with a proposed role for the enzyme in mediating a milk production increase in response to GH or PRL. However, in the absence of substrate (T_4) for 5′D, generation of T_3 in the mammary gland does not occur. In the absence of T_4, GH and PRL are not galactopoietic. The function of T_3 in stimulating milk synthesis and secretion remains to be elucidated. Within the mammary gland, thyroid hormones are known to potentiate the effects of PRL (13, 33–36) and may be rate-limiting to milk production. Administration of PRL and GH to mice that were T_4 sufficient did not increase 5′D beyond that observed with T_4 alone, with the possible exception of an influence of PRL on hepatic 5′D.

A low level of T_3 generation may occur in lactating thyrocyte-ablated mice if mammary tissue is capable of generating modest quantities of T_4 as suggested by Michajlovskij *et al.* (37). This may explain the low concentrations of T_4 in serum of thyrocyte-ablated mice during lactation and its absence in serum at the beginning of gestation.

Results of this experiment support our hypothesis that thyroid hormones are necessary to obtain milk production increases in response to galactopoietic hormones. PRL and GH increased 5′D activity in mammary gland but not in liver or kidney. The effects of GH administration on tissue 5′D activities in mice were analogous to those obtained for lactating dairy cows (2, 3). Organ-specific changes in 5′D activity may serve to target thyroid hormone action and thus focus metabolic activity and nutrient flux to the mammary gland. Without thyroid hormones this process may be inoperative and incapable of supporting increased milk production in response to GH or PRL.

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