A ROLE OF THYROID HORMONES IN DIFFERENTIATION OF MOUSE MAMMARY GLAND IN VITRO

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SUMMARY: Addition of thyroxine or triiodothyronine to cultures of mammary gland explants in the presence of insulin, hydrocortisone and prolactin, results in a selective enhancement of the activity of the milk protein, α-lactalbumin. This effect, which is specific for the L-isomer of the thyroid hormones, is not mediated through diffusible activators of the enzyme activity.

INTRODUCTION

Prolactin and thyroid hormones interact in the regulation of growth and differentiation in several biological systems, producing effects which are either antagonistic [e.g. amphibian metamorphosis (1,2)] or synergistic [e.g. newt molting and limb regeneration (3,4)]. Previous studies on the relationship of these agents in mammary gland development in several species in vivo have resulted in conflicting reports. Thyroid hormones have been shown to either enhance (5-7) or inhibit (8-10) lobulo-alveolar development of these glands, depending on the concentrations of the hormones and the developmental stage or previous hormonal treatment of the animals. Since thyrotropin-releasing hormone enhances secretion of prolactin by the pituitary (11,12), some of these effects of thyroxine may be mediated through the hypothalamic-pituitary axis. However, some direct effects on mammary gland may also occur. Indeed, Singh and Bern (13) have reported that both synergism and antagonism between thyroxine and prolactin can be demonstrated during alveolar growth in vitro using mammary glands from estrogen-progesterone primed immature mice. The nature of the effect depended on the relative concentrations of these two agents. I have investigated the role of physiological levels of thyroid hormones in differentiation in vitro using glands from a later stage of development (i.e. pregnancy) and have found a selective enhancement of the activity of the milk protein, α-lactalbumin, one of the components of the lactose synthetase system (14).

MATERIALS AND METHODS

Porcine zinc insulin was a gift from the Eli Lilly Company. Bovine prolactin was a gift from the National Institutes of Health. Hydrocortisone and all thyroid hormones were purchased from ICN Pharmaceuticals, Inc.

Mammary gland explants were prepared from C3H/HeN mice 10-12 days into their first pregnancy, as described previously (15).

Activities of galactosyl transferase and α-lactalbumin were measured as described previously (16). The production of lactose in the latter case was confirmed by chromatography using either an ascending system of propan-2-ol:H_2O (4:1 v/v) on Whatman 1 paper (17), or NH_4OH:isopropanol (1:4 v/v) and silica gel thin layer plates (18). Epithelial DNA content (16) and the combined activities of glucose-6-phosphate dehydrogenase and gluconate-6-phosphate dehydrogenase (19) were determined as described previously.

RESULTS AND DISCUSSION

When explants of mammary glands from mice in the middle of their first pregnancy are cultured in media containing the appropriate hormones, the epithelial cells are

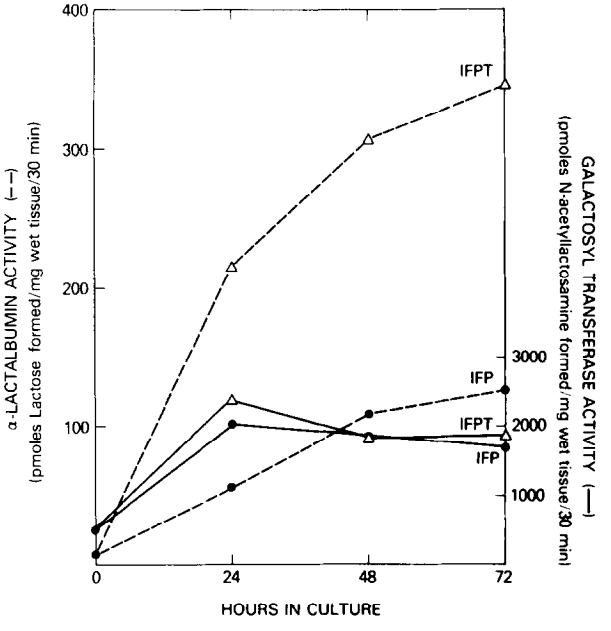

Figure 1. Time course of the development of the lactose synthetase system in the presence and absence of added thyroxine. Pooled explants from mammary glands of mid-pregnant mice were cultured in serum-free Medium 199 (GIBCO) containing 5 μg/ml each of insulin, hydrocortisone and prolactin (IFP;●) or insulin, hydrocortisone, prolactin and 10^{-7} M L-thyroxine (IFPT;Δ). Activities of galactosyl transferase(——) and α-lactalbumin (---) were measured as described in Materials and Methods. Each point represents the average of duplicate determinations. The data shown are representative of several similar experiments.

stimulated to differentiate and elaborate the milk proteins characteristic of this gland (15,16). This process has a minimal hormonal requirement for insulin (I), hydrocortisone (F) or another glucocorticoid (20), and prolactin (P) (15). Figure 1 shows that the activity of one of the milk proteins, α-lactalbumin, increases several-fold in such a culture system, reaching a maximum within 48 hr of explantation. However, the addition of L-thyroxine (L-T_4) to these cultures results in an even greater stimulation of α-lactalbumin activity. This effect, which occurs within 6 hr of explantation, usually reaches its peak between 48 and 72 hr. The lower activity of the α-lactalbumin in explants cultured in the presence of the three hormone combination (IFP) is not due to a limiting amount of the other component of the lactose synthetase system, UDP-galactosyl transferase (21). The activity of this enzyme, which is not a unique product of the mammary gland (22), is also stimulated by I, F and P in culture. However, it is not further increased by the addition of L-T_4.

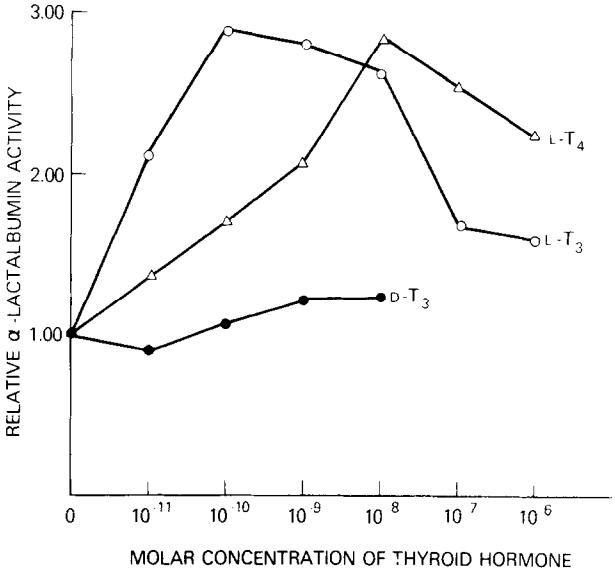

Figure 2. Effect of various concentrations of thyroid hormones on α-lactalbumin activity. Pooled explants from mammary glands of mid-pregnant mice were cultured for 48 hr in the presence of insulin, hydrocortisone and prolactin plus either L-thyroxine (L-T_4; △-△), 3,5,3'-triiodo-L-thyronine (L-T_3; o——o) or 3,5,3'-triiodo-D-thyronine (D-T_3; ●——●) at the indicated concentrations. Activity of α-lactalbumin was measured as described in Materials and Methods. Each point represents the average of from 2 to 6 separate experiments. Values for α-lactalbumin activity within any given experiment were based on closely agreeing duplicate determinations.

Figure 2 shows that at low concentrations (i.e. 10^{-11}M to 10^{-10}M), both L-T_4 and 3,5,3'-triiodo-L-thyronine (L-T_3) are able to stimulate α-lactalbumin activity in vitro. While addition of either of these thyroid hormones to cultures containing serum-free Medium 199 supplemented with I, F and P ultimately results in nearly the same maximal level of α-lactalbumin activity, this effect requires 10^{-8}M L-T_4, but only 10^{-10}M L-T_3.

Figure 2 also demonstrates the stereo specificity of this effect in that only the L-isomer of T_3 is active. Monoiodotyrosine (10^{-6}M), diiodotyrosine (10^{-6}M) and KI (10^{-6}M) do not substitute for L-T_3 or L-T_4. Furthermore, thyroxine does not substitute for prolactin in α-lactalbumin production (data not shown). Direct addition of L-T_4 (10^{-9}M to 10^{-6}M) to the lactose synthetase assay system does not increase the enzyme activity.

These data suggest that the thyroid hormones play an important role in the differentiation of the mouse mammary gland. However, it was conceivable that the increase in α-lactalbumin activity which occurs when these agents are added to cultures containing I, F and P merely reflects a general increase in metabolic activity. To explore this possibility, other hormonally-induced events which occur during the development of mammary gland in vitro were examined. Figure 3 shows that like the activity of the galactosyl transferase, the hormonally-induced increase in combined activities of glucose-6-phosphate dehydrogenase and gluconate-6-phosphate dehydrogenase are not affected by the addition of L-T_4 to the cultures. Similarly unaffected are the insulin-induced growth of the epithelial component of the mammary gland as measured by epithelial DNA content or ^{3}H-thymidine incorporation (data not shown), and the insulin-enhanced accumulation of α-aminoisobutyric acid (data not shown). Only the activity of α-lactalbumin, a unique product of the fully differentiated mammary gland, is enhanced.

This selective effect on α-lactalbumin activity is not mediated through diffusible activators. In experiments in which tissue cultured in the presence of I, F and P is homogenized with explants cultured in the presence of the three hormones plus L-T_4 the resulting α-lactalbumin activity is additive. Whether the effect of the

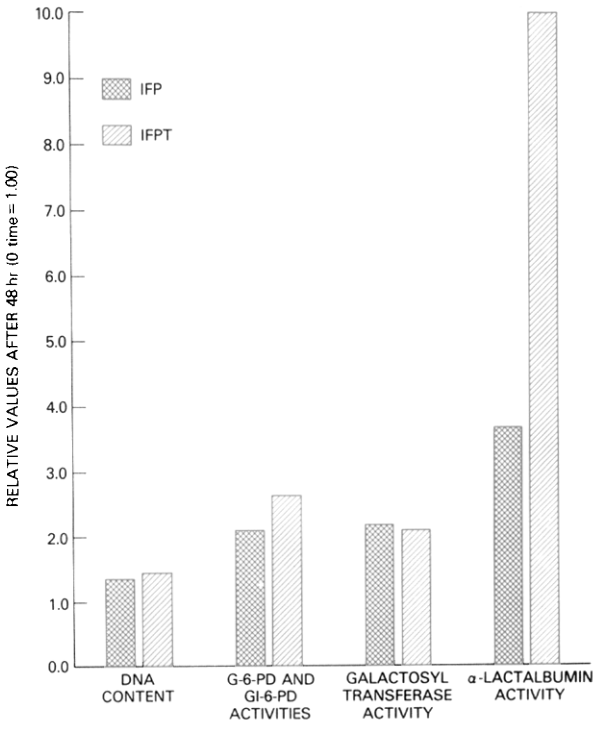

Figure 3. The effect of L-thyroxine on various hormonally-induced functions in explants from mammary glands of mid-pregnant mice. Pooled explants from 4 mice were cultured for 48 hr in the presence of 5 μg per ml each of insulin, hydrocortisone and prolactin (IFP) or insulin, hydrocortisone, prolactin and 10^{-8}M L-thyroxine (IFPT). At the time of explantation and at the end of the 48 hr of culture, tissue was examined for epithelial DNA content, the combined activities of glucose-6-phoshate dehydrogenase (G-6-PD) and gluconate-6-phosphate dehydrogenase (Gl-6-PD), galactosyl transferase activity and α-lactalbumin activity, as described in Materials and Methods All data are expressed as relative to the uncultured controls. Actual values at 0 hr were: epithelial DNA content, 230 μg DNA/mg wet tissue; combined activities of G-6-PD and Gl-6-PD, 2.45 x 10^{-4} units/mg wet tissue; galactosyl transferase 1330 pmoles n-acetyllactosamine formed/mg wet tissue/30 min; α-lactalbumin activity, 44 pmoles lactose formed/mg wet tissue/30 min. Data shown represent the average of duplicate determinations and are representative of several similar experiments.

thyroid hormone is on the activity of, or on synthesis of the protein is currently under investigation.

These data suggest that the thyroid hormones play a key role in terminal differentiation of the mouse mammary gland. Whether these agents are an absolute requirement for the elaboration of the milk proteins *in vivo* is not yet known. The action of thyroid hormones in this system appears to be multiphasic since they also may play a role in the control of growth of the epithelial component

of the mammary gland. Previous work by Singh and Bern (13) using whole gland cultures of tissue from estrogen-progesterone primed immature mice has shown both inhibition and stimulation of lobulo-alveolar growth by thyroxine. In contrast to this report, we were unable to demonstrate effects of thyroid hormones on growth in explants from glands of mid-pregnant mice. These diverse findings may reflect a different level of prolactin involvement in epithelial growth during various stages of development (23,24). Clarification of the nature of the thyroid hormones' multiple contributions to mammary gland differentiation awaits the further examination of tissue from animals in several developmental and hormonal states. In addition, this system may prove to be a useful tool for investigating the mechanism of action of thyroid hormones.

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