Selective stimulation of α-lactalbumin synthesis and its mRNA accumulation by thyroid hormone in the differentiation of the mouse mammary gland in vitro

Nobuyuki Terada and Takami Oka*

Laboratory of Biochemistry and Metabolism, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases (Building 10/Room 9B–15), National Institutes of Health, Bethesda, MD 20205, USA

Received 9 September 1982; revision received 21 September 1982

Lactalbumin synthesis *Thyroid hormone* *mRNA accumulation*
Mammary gland differentiation *Selective stimulation* *Lactogenesis modulation*

1. INTRODUCTION

α-Lactalbumin and casein are the major milk proteins produced by differentiated mammary epithelial cells. The synergistic actions of insulin, cortisol and prolactin stimulate the accumulation of these milk proteins in organ culture of the mouse mammary gland [1]. Several lines of evidence have been presented to indicate that the production of the 2 milk proteins are regulated differently by the interplay of hormones and some intracellular regulatory substances such as cyclic AMP and prostaglandins [2–4]. Thyroid hormones have been shown to stimulate the activity of α-lactalbumin in the lactose synthetase system but not the incorporation of inorganic phosphate into casein in cultured tissue [5]. However, it has not been determined whether the increase in the activity of α-lactalbumin is due to activation or enhanced synthesis of the protein. Moreover, the previous assay for casein does not allow one to assess the effect of hormone on the synthesis of casein, that may be different from its phosphorylation. Here, the effect of thyroid hormone on the synthesis of the two milk proteins and the acccumulation of their mRNA level has been investigated. The data demonstrate that thyroid hormone selectively augments the synthesis and the mRNA level of α-lactalbumin without affecting those of casein in cultured mammary gland.

* To whom correspondence should be addressed

2. MATERIALS AND METHODS

C3H/HeN mice in day 10–12 of pregnancy were obtained from the Animal Breeding Facility (National Institutes of Health). Bovine prolactin (NIH B5) was obtained from the Hormone Distribution Program (National Institutes of Health). Crystalline porcine zinc insulin was a generous gift from Lilly Research Laboratories. Cortisol and L-triiodothyronine were purchased from ICN Pharmaceuticals.

Mammary gland explants were prepared from abdominal glands and cultured as in [6]. The amount of α-lactalbumin in the cultured tissue was determined by a modified method of [7], using pure bovine α-lactalbumin as the standard [8]. The rate of α-lactalbumin synthesis and casein synthesis was measured by pulse-labeling cultured mammary explants with 100 μCi/ml [^{3}H]leucine (spec. act. 145 Ci/mmol) in medium which had the same composition except unlabeled leucine at the indicated time. The amount of isotopically labeled α-lactalbumin and casein was determined by immunoprecipitation with rabbit antibody against purified mouse milk α-lactalbumin and casein as in [2,9]. The rate of total protein synthesis was determined by measuring the acid-insoluble radioactivity of [^{3}H]leucine in the 105 000 × *g* supernatant fraction as in [2]. The amount of mRNA for α-lactalbumin and casein was determined by measuring the amount of [^{3}H]leucine (200 μCi/ml, spec. act. 52 Ci/mmol) incorporated into nascent

Published by Elsevier Biomedical Press
001457593/82/0000—0000/$2.75

polypeptides of the 2 proteins in a cell-free translation of poly (A)-rich RNA extracted from cultured tissue as in [9].

3. RESULTS

The level of α-lactalbumin was low in mammary explants cultured with insulin alone or insulin and cortisol [2], but was elevated 3–5-fold by the addition of prolactin with insulin. Addition of 0.01 μg cortisol/ml with insulin and prolactin caused a further increase in α-lactalbumin. However, addition of 1 μg cortisol/ml had an inhibitory effect [2]. The addition of L-triiodothyronine stimulated the accumulation of α-lactalbumin induced by insulin and prolactin or insulin, prolactin and the two concentrations of cortisol (fig. 1A). The addition of L-triiodothyronine with insulin caused virtually no increase in the amount of α-lactalbumin in cultured tissue. L-Triiodothyronine alone, or in combination with either cortisol or prolactin or both hormones were ineffective in augmenting the accumulation of α-lactalbumin (not shown). The time course study indicated that the stimulatory effect of thyroid hormone was apparent throughout 96 h culture and was maximal at 48 h (not shown).

In contrast with the accumulation of α-lactalbumin, L-triiodothyronine had little stimulatory effect on the accumulation of casein induced by insulin and prolactin or insulin, prolactin and cortisol (fig. 1B). No increase in casein accumulation was observed in tissue incubated in the presence of insulin and thyroid hormone.

Addition of L-triiodothyronine increased the rate of synthesis of α-lactalbumin in explants cultured with insulin, prolactin and 0.01 μg cortisol/ml by ~2-fold over that in explants cultured with insulin, cortisol and prolactin (table 1). Thyroid hormone, however, did not increase the rate of casein synthesis in explants cultured with insulin, cortisol and prolactin. The rate of total protein synthesis was also not affected by thyroid hormone (not shown). These results indicate that thyroid hormone stimulates selectively the synthesis of α-lactalbumin in cultured mammary tissue.

Addition of L-triiodothyronine resulted in enhancement of the level of mRNA for α-lactalbumin in explants cultured with insulin, prolactin and cortisol (table 2). The extent of increase was ~2-fold, as in the case of the rate of α-lactalbumin synthesis. Thyroid hormone, however, did not augment the increase in casein mRNA that was in-

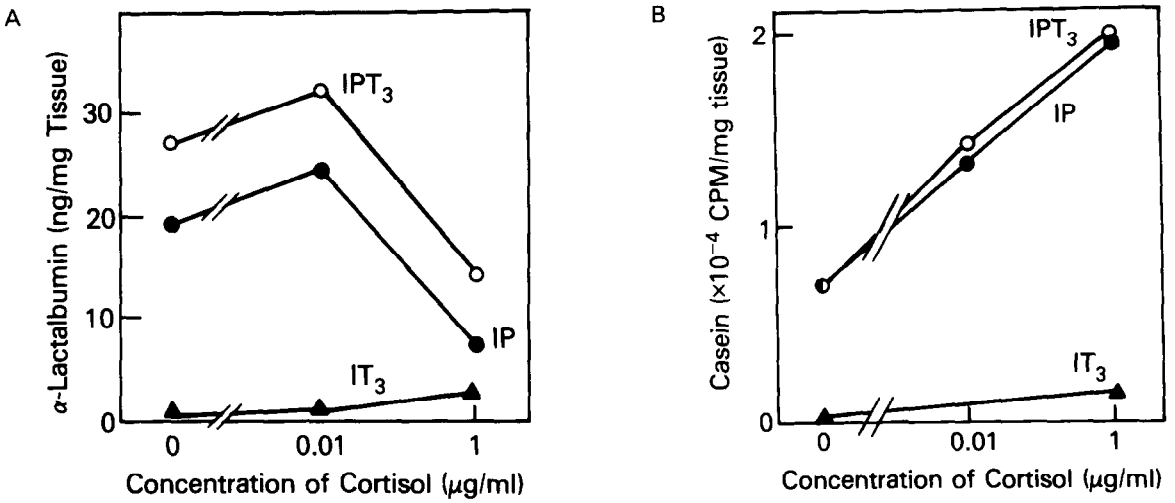

Fig. 1. The effect of L-triiodothyronine on the accumulation of (A) α-lactalbumin and (B) casein in mammary gland explants in culture. Mammary explants from midpregnant mice were cultured for 48 h in medium containing insulin and L-triiodothyronine (IT_3, ▲) or insulin and prolactin (IP, ●) or insulin, prolactin and L-triiodothyronine (IPT_3, ○) in the presence of the indicated concentrations of cortisol. Both insulin and prolactin were used at 5 μg/ml. L-Triiodothyronine was used at 10^{-9} M. The amount of α-lactalbumin and casein synthesized were determined as in section 2. Each point represents the average of 3 determinations with standard error that varied <6%.

Table 1

Effect of L-triiodothyronine on the rate of synthesis of α-lactalbumin and casein in cultured mammary gland

Culture condition	Rate of synthesis (cpm.g tissue^{-1}.h^{-1})	
	α-Lactalbumin	Casein
Uncultured tissue	350	283×10^2
Insulin + cortisol	120	117×10^2
+ prolactin	2459	1980×10^2
+ L-triiodothyronine	5085	2002×10^2

Mammary explants from midpregnant mice were cultured in medium containing the indicated combinations of hormones for 48 h. The rate of synthesis of α-lactalbumin and casein was determined at 46–48 h culture as in section 2. Cortisol was used at 0.01 μg/ml. Each value represents the average of 3 determinations that varied <9%. Other details are given in fig. 1

Table 2

Effect of L-triiodothyronine on the accumulation of mRNA for α-lactalbumin and casein in cultured mammary gland

Culture condition	mRNA accumulation (cpm.g tissue^{-1})	
	α-Lactalbumin	Casein
Uncultured tissue	1.33×10^4	1.97×10^6
Insulin + cortisol	1.20×10^4	1.83×10^6
+ prolactin	9.23×10^4	21.29×10^6
+ L-triiodothyronine	19.37×10^4	22.53×10^6

Mammary explants from midpregnant mice were cultured in medium containing the indicated combinations of hormones for 48 h. The amount of mRNA for α-lactalbumin and casein was determined as in section 2. Cortisol was used at 0.01 μg/ml. Each value represents the average of 3 determinations. Standard error was <7% for each value. Other details are given in fig. 1

duced by insulin, prolactin and cortisol.

When the effect of thyroid hormone on the incorporation of [^{3}H]uridine into total RNA was measured, there was no apparent stimulation of total RNA synthesis. Thus it appears that thyroid hormone exerts selective action on the accumulation of mRNA for α-lactalbumin in the mammary tissue.

4. DISCUSSION

This study has demonstrated that L-triiodothyronine augments the synthesis of α-lactalbumin induced by synergistic action of insulin, prolactin and cortisol in cultured mammary gland. This effect of thyroid hormone is apparently mediated by the increase in the level of mRNA for α-lactalbumin and also is selective in the sense that it exerts no appreciable effect on the synthesis and mRNA accumulation of casein. In [5], the stimulatory effect of thyroid hormone on the activity of α-lactalbumin was inhibited by cycloheximide and actinomycin in cultured mammary tissue. It is not yet known whether thyroid hormone stimulates selectively the transcription of mRNA for α-lactalbumin or enhances its half-life.

In [2], cortisol at 0.01 μg/ml stimulated the accumulation of α-lactalbumin induced by insulin and prolactin but the steroid at higher concentrations inhibits this process. Prostaglandins have been shown to reverse the inhibitory effect of cortisol on α-lactalbumin accumulation without affecting casein synthesis [4]. However, prostaglandins do not augment the accumulation of α-lactalbumin induced by insulin and prolactin or insulin, prolactin and 0.01 μg cortisol/ml [2]. These effects of prostaglandins are thus clearly different from those of thyroid hormone, indicating that the 2 agents enhance the production of α-lactalbumin by different mechanisms.

Here we have shown that thyroid hormone does not affect the synthesis of casein. This indicates that the stimulatory action of the hormone on α-lactalbumin accumulation is not due to a general increase in metabolic activity of cultured tissue, as suggested in [5].

In contrast with this report and [5], thyroid hormone has been shown to stimulate casein synthesis in cultured mammary gland from pseudopregnant rabbits [10]. However, the rabbit system is different from the mouse system such that the former does not require glucocorticoid for induction of casein synthesis [10]. Thus it is conceivable that the action of thyroid hormone on the production of milk proteins may differ among various species.

The finding that thyroid hormone can selectively augment the synthesis and mRNA accumulation of α-lactalbumin lends further support for the view that the hormone plays a modulatory role in the

control of lactogenesis [5]. The data are also important for delineating the complex regulatory mechanism involved in functional differentiation of the mammary gland.

REFERENCES

[1] Topper, Y.J. and Oka, T. (1974) Lactation (Larson, B.L. and Smith, V.R. eds) vol. 1, pp. 327–348 Academic Press, London, New York.
[2] Ono, M. and Oka, T. (1980) Cell 19, 473–480.
[3] Perry, J.W. and Oka, T. (1980) Proc. Natl. Acad. Sci. USA 77, 2093–2097.
[4] Terada, N., Ono, M., Nagamatsu, Y. and Oka, T. (1982) J. Biol. Chem. 257, 11199–11202.
[5] Vonderhaar, B.K. (1979) Endocrinology, 100, 1423–1431.
[6] Topper, Y.J., Oka, T. and Vonderhaar, B.K. (1975) Methods Enzymol. 39, 443–454.
[7] Fitzgerald, D.K., Covin, B., Mawal, R. and Ebner, K.E. (1970) Anal. Biochem. 36, 43–61.
[8] Nagamatsu, Y. and Oka, T. (1980) Biochem. J. 185, 227–237.
[9] Takemoto, T., Nagamatsu, Y. and Oka, T. (1980) Dev. Biol. 78, 247–257.
[10] Houdebeine, L.M., Delouis, G. and Devinoy, E. (1978) Biochemie 60, 809–812.