THYROID HORMONE ACTION: STIMULATION OF GROWTH HORMONE AND INHIBITION OF PROLACTIN SECRETION IN CULTURED GH_1 CELLS

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SUMMARY

Triiodothyronine induces a 3-fold increase in the rate of growth of cultured GH_1 cells, a growth hormone and prolactin producing pituitary cell line. Associated with the increased rate of cell replication is an increase in the rate of growth hormone secretion and a decrease in prolactin secretion. The Thyrotropin Releasing Hormone mediated increase in prolactin secretion was also inhibited by triiodothyronine. Half-maximal inhibition of prolactin secretion occurred at a free triiodothyronine concentration of 3×10^{-11}M. This value is identical to the equilibrium dissociation constant for binding of triiodothyronine with nuclear receptors for the thyroid hormones. This suggests that the thyroid hormone inhibition of prolactin secretion is mediated by control of regulatory events at the level of the cell nucleus.

Thyroid hormones have a profound effect on the growth and metabolism of virtually all tissues of higher organisms (1). In addition, thyroid hormones may play an important role in the secretion of growth hormone and prolactin from the adenohypophysis *in vivo* (2,3). In rats, two to three weeks after thyroidectomy the pituitary population of growth hormone producing cells decreases from 40% to less than 5% of the total cell population (4). Growth hormone secretion is markedly impaired in hypothyroidism in man and the rat and the secretion rate returns to normal after administration of thyroid hormone (2,3). In contrast, after thyroidectomy or in hypothyroidism in the rat and man the plasma prolactin levels have been reported to be unchanged or slightly increased (3,5).

Tashjain et al. first reported that Thyrotropin Releasing Hormone (TRH) which stimulates the rapid release of thyrotropin both *in vivo* and *in vitro* also enhanced the production of prolactin in cultured GH_3 and GH_1 cells, both

derived from a rat pituitary tumor (6). Subsequently it was reported that TRH can increase the plasma concentrations of prolactin after injection *in vivo* in man as well as several animal species (5,7,8,9). The prolactin response to TRH administration was enhanced by thyroidectomy or hypothyroidism, and the effect of TRH on prolactin secretion can be inhibited by the administration of thyroid hormone (5,8). Although thyroxine (T4) and triiodothyronine (T3) have been reported to inhibit the TRH induced release of prolactin *in vitro*, the free T3 and T4 concentrations were 1×10^{-6} M to 1×10^{-5} M (10). These free hormone concentrations are approximately 10^4 to 10^5-fold greater than the estimated biologically active free T3 or T4 concentrations *in vivo* (11,12) and are sufficiently high to interfere with oxidative metabolism and protein synthesis (13).

We have previously reported that T3 and T4 induced a three-fold increase in the rate of growth of GH_1 cells, a prolactin and growth hormone producing pituitary cell line, in culture (14). The concentrations of T3 and T4 which regulate cell growth are physiologic and the estimated free hormone concentrations in culture which induced a half-maximal biologic effect were 0.8×10^{-11} M for T3 and 1×10^{-10} M for T4 (14). Studies on the binding of [^{125}I] T3 and [^{125}I] T4 after incubation of hormone with intact GH_1 cells demonstrated, high-affinity, saturable, binding sites in the cell nucleus (15). The estimated equilibrium dissociation constants (Kd) were 2.9×10^{-11} M for T3 and 2.5×10^{-10} M for T4. These affinities were similar to the hormone concentrations which induced a half-maximal effect on cell growth and suggested that these nuclear binding activities functioned as cellular receptors for the thyroid hormones.

In this communication we report that T3 stimulates growth hormone secretion and inhibits the basal secretory rate as well as the TRH mediated increase of prolactin secretion of cultured GH_1 cells. The similarity of the free T3 concentration which results in half-maximal inhibition of prolactin secretion to the Kd for T3 nuclear binding suggests that the thyroid

hormone inhibition of prolactin secretion is regulated at the level of the cell nucleus.

MATERIALS AND METHODS

GH_1 cells were originally obtained from the American Type Culture Collection, Rockville Md. The cells were grown in 75 cm^2 T flasks (Falcon) with Ham's F-10 medium supplemented with horse serum (15%) and fetal calf serum (2.5%), (growth media), as previously described (14). In general, the media was replaced twice weekly, and the cells were subcultured after dispersion with an ethylenediaminetetraacetic acid (EDTA) salt solution (14). For studies of thyroid hormone on cell growth and hormone secretion, the cells were inoculated into 25 cm^2 T flasks (Falcon) at a cell density of 40,000 cells per cm^2.

For T3 dose response studies and to examine the interrelationship of T3 and TRH on prolactin secretion, GH_1 cells were innoculated into 2 cm^2 wells of a micro tissue culture plate (Linbro Chemical Co.) at a cell density of 100,000 to 200,000 cells per cm^2.

After 24 to 72 hours of incubation at 37°C (95% air, 5% CO_2) the growth media was replaced with Ham's F-10 media supplemented to 10% with hypothyroid calf serum (hypo media)*. T3 was added to achieve the media concentrations indicated in the text. The free T3 concentration in the media was estimated by equilibrium dialysis as previously described (14,16). Synthetic TRH was obtained from Abbott Laboratories and the final concentration in all studies was 2×10^{-8}M.

After the various additions the cell cultures were then incubated at 37°C (95% air, 5% CO_2) and at the times indicated in the figures the media was aspirated and saved for growth hormone and prolactin determination. In studies on cell growth the cells were harvested with the aid of a rubber

*The hypothyroid calf serum was obtained from a thyroidectomized calf (Rockland Farms, Gilbertsville, Pa.). The concentrations of thyroid hormone prior to thyroidectomy were 2×10^{-9}M (130 ng/100 ml) for T3 and 1×10^{-7}M (7.7 ug/100 ml) for T4. After thyroidectomy the serum concentrations were 0.8×10^{-9}M (50 ng/100 ml) for T3 and 3×10^{-9}M (0.233 ug/100 ml) for T4 (14).

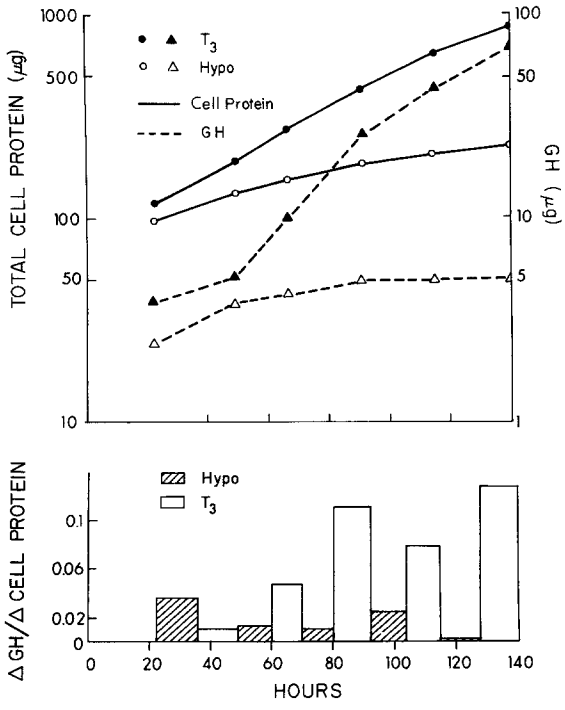


Figure 1: Effect of triiodothyronine on cell growth and growth hormone secretion. Each of 24 flasks (25 cm^2) was innoculated with 1 x 10^6 cells and the cultures were incubated with growth media for 24 hours. The media was then replaced with Ham's F-10 media containing 10% hypothyroid calf serum (hypo media). T3 was added to 12 of the flasks to achieve a final media concentration of 5 x 10^{-9}M (estimated free T3 concentration 1.0 x 10^{-10}M) and the cultures were incubated at 37°C. At the times indicated media was removed and saved for growth hormone and prolactin immunoassay. The cells were harvested as indicated in the methods section and quantitated by determination of total cell protein and DNA. Each point represents the average of a pair of flasks and the results of each pair agreed within 10%. The ratio of protein to DNA remained relatively constant (9.1 to 10.0) over the duration of the experiment. Only the total cell protein is illustrated.

policeman, and were washed three times with 0.9% saline by repeated dispersion and centrifugation at 800 x g.

Cell protein was quantitated by the method of Lowry et al (17) and DNA by the method of Burton (18). One million cells contain 13.0 ug of DNA and 130 ug of protein.

Growth hormone and prolactin content of the media were measured by specific radioimmunoassay using a double antibody immunoprecipitation method. Purified hormone, reference standards, and specific antisera were

kindly supplied by the Rat Pituitary Hormone Program of the National Institutes of Arthritis, Metabolism and Digestive Diseases (NIAMDD). The second antibody for immunoprecipation was obtained from Miles Laboratories, Elkhart, Ind. Radioiodination of the hormone standards and the radioimmunoassay methodology was that described by the Pituitary Hormone Program of the NIAMDD. No crossreaction was observed between growth hormone and prolactin and the culture media containing 10% hypothyroid calf serum demonstrated no immunoassayable material which crossreacted with either of the hormonal immunoassays.

RESULTS AND DISCUSSION

Figure 1 illustrates the effect of T3 at a concentration of 5×10^{-9}M in the total media (estimated free hormone concentration 1×10^{-10}M on cell growth and growth hormone secretion. T3 increased the rate of cell growth approximately 3-fold. The mean generation time of cells grown with hypo media was 110 hours compared to 39 hours in the presence of T3. In addition, the accumulation of immunoassayable growth hormone in the media was also enhanced in cell cultures containing thyroid hormone. To determine whether the effect of T3 on growth hormone secretion was selective or reflected a general increase in the rate of cell protein synthesis we compared the micrograms of growth hormone released to the increment of total cell protein during the same time interval. The results are illustrated in the bar graph at the bottom of figure 1. After 48 hours of incubation the increment of growth hormone released compared to the increment of total cell protein was significantly greater during each time interval with cells cultured with T3. These results suggest that although T3 stimulates the rate of protein synthesis as a result of cell growth, T3 appears to have a more selective effect on growth hormone production than total cell protein.

Figure 2 illustrates the effect of T3 on prolactin secretion into the media. The absolute rates of prolactin secretion were similar for GH_1 cells cultured with or without thyroid hormone. The bar graph at the bottom of

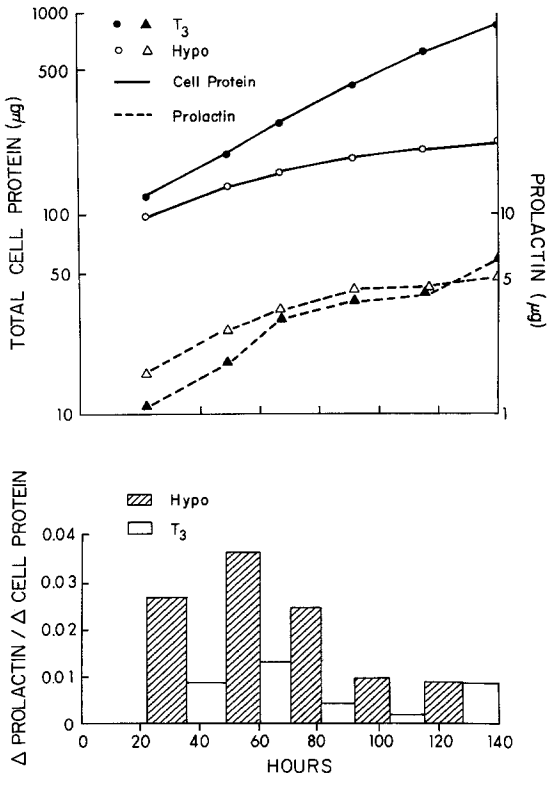
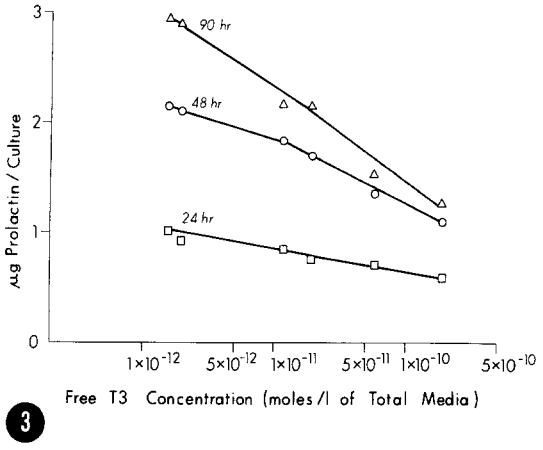


Figure 2: Effect of triiodothyronine on cell growth and prolactin secretion. The experiment was the same as in Figure 1.

figure 2 compares the micrograms of prolactin secreted to the increment of total cell protein during each time interval. Up to 133 hours of culture, prolactin secretion when related to the increment of total cell protein was significantly lower with cells cultured with T3. These results suggest that T3 has a selective effect on inhibiting prolactin production and/or secretion.

Figure 3 illustrates the effect of various concentrations of T3 on the prolactin secretory rate of GH_1 cells in the stationary phase of growth after 24, 48, and 90 hours of incubation. In each case the estimated free hormone concentration which inhibits prolactin secretion to $\frac{1}{2}$ of the maximal secretory rate was approximately 3×10^{-11}M. This concentration is virtually identical to the estimated Kd for T3 binding to putative nuclear receptors which we reported in GH_1 cells (15).



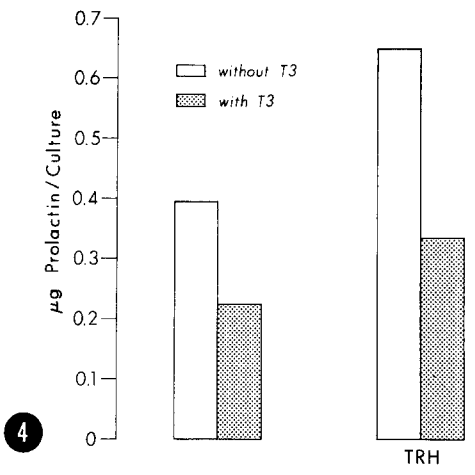


Figure 3: Dose response relationship of triiodothyronine concentration to prolactin secretion. GH_1 cells (2×10^5) were innoculated into each 2 cm^2 chamber of a Linbro cell culture plate. After 72 hours of incubation at 37°C the media of each well was replaced with 1.0 ml of Ham's F-10 medium containing 10% hypothyroid calf serum (hypo media). The endogenous T3 concentration in the hypo media was 0.8×10^{-11}M. Triiodothyronine was then added to achieve a final media concentration of 1.0×10^{-10}M, 8×10^{-10}M, 1×10^{-9}M, 4×10^{-9}M, and 1×10^{-8}M. The unbound, free T3 concentrations estimated by equilibrium dialysis was approximately 2% of the total T3 media concentration with 10% serum. The estimated free T3 concentrations are illustrated in the figure with the lowest T3 concentrations representing the free T3 level in the hypo media. At 24, 48, and 90 hours of incubation, 0.1 ml of media was removed at each hormone concentration for prolactin determination. Correction was made for serial media sampling. The results at each T3 concentration represents the average of a pair of cell cultures and the results of each pair agreed within 10%.

Figure 4: Effect of triiodothyronine on the TRH mediated stimulation of prolactin secretion. GH_1 cells (1×10^5) were innoculated into 2 cm^2 chambers of a Linbro cell culture plate. After 72 hours of incubation the media of each well was replaced with 0.5 ml of Ham's F-10 media containing 10% hypothyroid calf serum (hypo media). T3 (final concentration 5×10^{-9}M) or TRH (final concentration 2×10^{-8}M) as well as in combination were each added to pairs of GH_1 cell cultures. After 24 hours of incubation the media was removed and the prolactin content determined by radioimmunoassay. Each bar graph value represents the average of the prolactin content of the media of a pair of cell cultures and the results of each pair agreed within 10%.

Figure 4 illustrates the effect of T3 on the basal secretory rate and the TRH (2×10^{-8}M) mediated stimulation of prolactin secretion after a 24 hour incubation at 37°C. In stationary phase cell cultures without T3, TRH increased the rate of prolactin secretion by 64%. In cultures incubated with T3, the basal prolactin secretory rate as well as the TRH mediated increase of prolactin secretion decreased approximately 50%. Concentrations of TRH

up to 2×10^{-6}M did not reverse the inhibitory effect of T3, and higher concentrations of T3 did not further surpress the rate of prolactin secretion. Although T3 did not completely inhibit the TRH mediated increase of prolactin secretion of GH_1 cells, it should be noted that thyroid hormone diminishes, but does not completely inhibit the TRH mediated increase of prolactin secretion *in vivo* (5).

Tashjian et al. reported that the intracellular pools of growth hormone and prolactin in GH_3 and GH_1 cells were extremely small when compared to the amount of growth hormone or prolactin released into the culture media during the incubation (19). In addition these hormones did not appear to be degraded either intracellularly or after secretion into the media (19). This suggested that the accumulation of prolactin or growth hormone in the media is a measure of the rate of synthesis of these hormones. This was confirmed with GH_3 cells by Dannies and Tashjian in which the TRH mediated increase in prolactin release into the media appeared to be directly related to the estimated rate of synthesis of the hormone (20).

In this regard, although absolute synthetic and degradation rates would have to be determined, the decrease in the rate of prolactin secretion by T3 in GH_1 cell cultures is likely due to a decrease in the rate of synthesis of the hormone.

Of interest is the observation that the free T3 concentration which results in half-maximal inhibition of prolactin production (3×10^{-11}M) is virtually identical to the concentration of T3 which results in half-maximal association with putative nuclear receptors for the thyroid hormones. This suggests that the observed inhibitory effect of thyroid hormone on prolactin production is modulated by regulatory effects at the level of the cell nucleus.

The growth hormone and prolactin response of cultured GH_1 cells to T3 is similar to the response of growth hormone and prolactin producing pituitary cells to thyroid hormone *in vivo* (3,5,7,8,9). Although this

does not exclude a regulatory effect of thyroid hormone at the level of the hypothalmus it indicates that physiological concentrations of thyroid hormone can regulate hormone production and/or secretion at the level of the pituitary cell.

Our observations suggest that the GH_1 cell culture system has promise as a valid *in vitro* model to define the regulatory mechanisms of thyroid hormones on growth hormone and prolactin production *in vivo*.

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