Control of Growth Hormone Synthesis in Cultured GH_1 Cells by 3,5,3′-Triiodo-L-thyronine and Glucocorticoid Agonists and Antagonists: Studies on the Independent and Synergistic Regulation of the Growth Hormone Response†

Herbert H. Samuels,* Frederick Stanley, and Lawrence E. Shapiro

ABSTRACT: We have previously reported that the thyroid hormone induction of growth hormone synthesis and mRNA in cultured GH_1 cells, a rat pituitary cell line, is modulated by the thyroid hormone nuclear receptor. In addition, the induction of growth hormone synthesis and mRNA by glucocorticoid in GH_1 cells is highly dependent on thyroid hormone. In this study, using serum free defined medium and glucocorticoid agonists, antagonists, and partial agonist–antagonists, we have further defined the multihormonal control of the growth hormone response. Without glucocorticoid, 3,5,3′-triiodo-L-thyronine (L-triiodothyronine) induced a four- to sixfold increase in growth hormone synthesis within 24 h of incubation, and this increased to 10- to 12-fold by 48 h. Without L-triiodothyronine, cortisol or dexamethasone induced a small growth hormone response which was only observed after 24–48 h of incubation. In contrast, the growth hormone response of cells incubated with L-triiodothyronine plus cortisol was two- to fivefold greater than L-triiodothyronine alone and demonstrated induction kinetics which paralleled that of thyroid hormone. With L-triiodothyronine a half-maximal increase in growth hormone induction occurred at 3.5 nM dexamethasone and 12 nM cortisol. Progesterone alone induced no growth hormone response but inhibited the small glucocorticoid induced response observed after 48 h of incubation. With L-triiodothyronine, progesterone induced a partial submaximal response which was 1.5-fold greater than with L-triiodothyronine alone. In addition, increasing progesterone concentrations inhibited the growth hormone response induced by L-triiodothyronine plus cortisol to that observed for L-triiodothyronine plus progesterone. 17α-Methyltestosterone, a pure glucocorticoid antagonist, inhibited the growth hormone response of cells incubated with L-triiodothyronine plus cortisol to that of thyroid hormone alone. The relative response elicited by the glucocorticoid agonists and antagonists paralleled the relative affinity of these steroids for the glucocorticoid receptor. Quantitation of growth hormone mRNA indicated that 17α-methyltestosterone lowered the glucocorticoid component of the growth hormone response by controlling growth hormone mRNA levels. These results are compatible with a concerted pretranslational control mechanism in which the nuclear associated receptors for thyroid and glucocorticoid hormones act independently as well as synergistically to regulate the growth hormone response.

We have demonstrated that cultured GH_1 cells, a growth hormone and prolactin producing cell line, are a useful isolated cell system to study thyroid hormone action. In medium containing hypothyroid calf serum, 3,5,3′-triiodo-L-thyronine[1] stimulates a three- to tenfold increase in growth hormone synthesis and the induction appears to be controlled by the chromatin associated thyroid hormone nuclear receptor (Tsai & Samuels, 1974; Samuels & Tsai, 1973; Samuels & Shapiro, 1976; Samuels et al., 1976, 1977a). Glucocorticoids have also been shown to stimulate growth hormone production in GH_1 cells and several related cell lines (Kohler et al., 1969; Bancroft et al., 1969; Yu et al., 1977; Martial et al., 1977a). These studies, however, were carried out with cell culture medium containing thyroid hormone which may have influenced the glucocorticoid response. In short term studies, using medium supplemented with hypothyroid calf serum, we have demonstrated that thyroid hormone influences the induction of growth hormone synthesis by glucocorticoids in GH_1 cells (Samuels et al., 1977b; Shapiro et al., 1978). In the absence of added L-triiodothyronine, cortisol did not induce an increase in growth hormone synthesis (Samuels et al., 1977b). However, when growth hormone synthesis was induced by L-triiodothyronine, cortisol stimulated the response two- to fivefold further (Samuels et al., 1977b). We have recently shown that this multihormonal control of growth hormone synthesis appears to be explained by the accumulation of growth hormone mRNA (Shapiro et al., 1978) which suggests that both thyroid and glucocorticoid hormones act at a pretranslational level to regulate the growth hormone response.

In this study, using serum free defined conditions, we have examined the detailed characteristics of the regulation of growth hormone synthesis by L-triiodothyronine and several glucocorticoid agonists and antagonists. We demonstrate that with L-triiodothyronine the properties of the growth hormone response to glucocorticoid agonists, antagonists, and partial agonists–antagonists is identical with other glucocorticoid responsive systems and appears to be mediated by the association of these steroids with the glucocorticoid receptor. Antiglucocorticoids inhibit the glucocorticoid induction of growth hormone synthesis by decreasing the accumulation of growth hormone mRNA without significantly altering the thyroid induced growth hormone response. These results are compatible with a concerted pretranslational control mechanism involving the receptors for both thyroid and glucocorticoid hormones, which interact synergistically to regulate the accumulation of growth hormone mRNA in GH_1 cells.

† From the Endocrine Division and the Department of Medicine, New York University Medical Center, New York, New York 10016. *Received June 2, 1978.* This work was supported by grants from the National Institutes of Health (AM 16636 and AM 21566).

[1] Abbreviations used: bisacrylamide, *N,N′*-methylenebis(acrylamide); L-triiodothyronine, 3,5,3′-triiodo-L-thyronine; L-thyroxine, 3,5,3′,5′-tetraiodo-L-thyronine; progesterone, 4-pregnene-3,20-dione; cortisol, 11β,17,21-trihydroxy-4-pregnene-3,20-dione; dexamethasone, 16α-methyl-9α-fluoro-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione; 17α-methyltestosterone, 17α-methyl-17β-hydroxy-4-androsten-3-one.

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Experimental Procedures

Hormones and Chemicals. L-[^{35}S]Methionine (900–1100 Ci/mmol) was obtained from Amersham/Searle. Aquasol liquid scintillation counting solution, L-[3′-^{125}I]triiodothyronine (700 Ci/mmol), [^{125}I]iodide (carrier free) for iodination, and [1,2,6,7-^{3}H]cortisol (100 Ci/mmol) were from New England Nuclear. Ham's F-10 culture medium, fetal calf serum, and horse serum were from Grand Island Biological Co. Hypothyroid calf serum was obtained from Rockland Farms, Gilbertsville, PA. Prior to thyroidectomy the serum L-thyroxine was 7 μg/100 mL and L-triiodothyronine was 130 ng/100 mL. After thyroidectomy the L-thyroxine was 0.05 μg/100 mL and the L-triiodothyronine level was below the sensitivity of the assay procedure (less than 5 ng/100 mL; Mitsuma et al., 1972). Cortisol levels determined by competitive binding (Murphy, 1967) were 0.75 μg/100 mL. Dexamethasone was obtained from Merck Sharp and Dohme, Rahway, NJ. All other steroid compounds were obtained from Schwarz/Mann. Anion-exchange resin (AG 1-X10) was from Bio-Rad, Richmond, CA. Oligo(dT)-cellulose, type 2, was from Collaborative Research. Goat anti-rhesus monkey antisera for use as a second antibody in the radioimmunoassay of growth hormone was obtained from Antibodies Incorporated, Davis, CA. Rat growth hormone for iodoination, reference standards, and rhesus anti-rat growth hormone antisera for quantitation of rat growth hormone by radioimmunoassay was obtained from Dr. Albert Parlow through the National Institutes of Health. All other chemicals were obtained from Sigma, St. Louis, MO.

Treatment of Hypothyroid Calf Serum with Resin and Charcoal. In certain experiments the hypothyroid calf serum was treated with anion-exchange resin (AG 1-X10) to further lower the already low endogenous thyroid hormone levels and/or activated charcoal to lower endogenous glucocorticoid levels. The hypothyroid calf serum was incubated for 2.5 h at 25 °C with 50 mg/mL of AG 1-X10 resin or 20 mg/mL of activated charcoal. The serum was first centrifuged at 27000*g* for 10 min and then sterilized by filtration with a 0.2-μm Millipore filter prior to the preparation of serum containing medium. To determine the effect of resin and charcoal treatment, hypothyroid calf serum was first incubated with L-[3′-^{125}I]triiodothyronine or [^{3}H]cortisol (each at 1×10^6 cpm per mL) at 25 °C for 15 h without resin or charcoal to achieve equilibration with serum binding proteins. Based on the recovery of L-[3′-^{125}I]triiodothyronine or [^{3}H]cortisol, the resin reduced the very low endogenous thyroid hormone levels 15- to 20-fold, and the charcoal treatment lowered the endogenous cortisol 12- to 15-fold. The endogenous cortisol level in the hypothyroid calf serum would, therefore, be reduced from 20 nM to less than 2 nM. The final cortisol concentration in the cell culture medium supplemented to 10% with serum would be less than 0.2 nM which does not stimulate growth hormone synthesis in cells incubated with L-triiodothyronine (Samuels et al., 1977b).

GH_1 Cell Culture Conditions. GH_1 cells were grown in monolayer culture using 25-cm^2 plastic flasks or 2 cm^2 multiwell plates (Falcon Plastics) as previously described (Samuels & Shapiro, 1976; Samuels et al., 1976, 1977a). The cells were inoculated at initial densities between 2 and 4×10^4 cells per cm^2 with growth medium (Ham's F-10 medium containing 2.5% fetal calf serum and 15% horse serum) and were incubated at 37 °C in an atmosphere of 95% air and 5% CO_2 for 48 to 72 h. The medium was then replaced with Ham's F-10 medium containing 10% hypothyroid calf serum and the cells were incubated for an additional 36–48 h to deplete the cells of hormone (Samuels et al., 1974). In most studies the serum containing medium was replaced with serum free Ham's F-10 medium with the hormone concentrations indicated in the text. The serum free medium containing hormone(s) was serially replaced at 24-h intervals up to 96 h, and the medium was saved for growth hormone quantitation by radioimmunoassay (Tsai & Samuels, 1974; Samuels et al., 1977b). At each 24-h period the cells were rinsed with 0.14 M NaCl at 4 °C and were analyzed for cell protein (Lowry et al., 1951) or DNA content (Burton, 1956). In two experiments the cells were initially incubated with medium containing 10% hypothyroid calf serum which had been pretreated with AG 1-X10 resin or activated charcoal. The growth hormone response was then examined as described above except that the respective serum medium component was maintained during the entire study.

In one experiment the effect of L-triiodothyronine, cortisol, progesterone, and 17α-methyltestosterone in various combinations on the growth hormone response was determined by radioimmunoassay as well as by incorporation of L-[^{35}S]-methionine for 15 min followed by immunoprecipitation with anti-growth-hormone antibody as previously described (Samuels & Shapiro, 1976). All studies indicated above were carried out using triplicate cell cultures with less than 5% variation.

Estimation of Growth Hormone mRNA in GH_1 Cells. GH_1 cells were cultured in duplicate roller bottles containing either L-triiodothyronine (5 nM), 17α-methyltestosterone (10 μM), L-triiodothyronine (5 nM) plus 17α-methyltestosterone (10 μM), L-triiodothyronine (5 nM) plus cortisol (50 nM), or L-triiodothyronine (5 nM) plus cortisol (50 nM) plus 17α-methyltestosterone (10 μM). Roller bottles which received no hormone served as controls. The Ham's medium, containing the same hormone concentrations, was replaced every 24 h for 72 h. The medium obtained from the last 24-h period was saved for growth hormone quantitation by radioimmunoassay and the poly(A) (+) mRNA fraction derived from total cytoplasmic RNA was translated in the wheat germ system using L-[^{35}S]methionine to estimate growth hormone mRNA levels as previously described (Shapiro et al., 1978). The radiolabeled immunoreactive growth hormone was recovered using rhesus monkey anti-growth-hormone antisera as described (Shapiro et al., 1978), except that the antigen–antibody complex was recovered with 20 μL of the protein A bearing Cowan 1 strain of *Staphylococcus aureus* as described by Kessler (1975) instead of using double-antibody precipitation. The bacteria were then incubated with 1% sodium dodecyl sulfate, 10 mM sodium phosphate (pH 7.6) at 50 °C for 20 min to release the adsorbed radiolabeled protein and the ^{35}S-labeled growth hormone was quantitated after electrophoresis on 10-cm discontinuous sodium dodecyl sulfate–polyacrylamide gels composed of 12.5% acrylamide and 0.36% bisacrylamide (Laemmli, 1970; Samuels & Shapiro, 1976; Shapiro et al., 1978). Total protein synthesis was determined with trichloroacetic acid as described (Shapiro et al., 1978).

Steroid Receptor Binding Studies in GH_1 Cell Cytosol. GH_1 cells (150×10^6) were incubated at 37 °C in roller bottles with Ham's F-10 medium supplemented to 10% (v/v) with hypothyroid calf serum which was pretreated with activated charcoal as described above. L-Triiodothyronine was added to achieve a final concentration of 5 nM. After 20 h the medium was replaced with serum free Ham's F-10 medium containing 5 nM L-triiodothyronine, and the cells were incubated for an additional 4 h at 37 °C. The roller bottles were

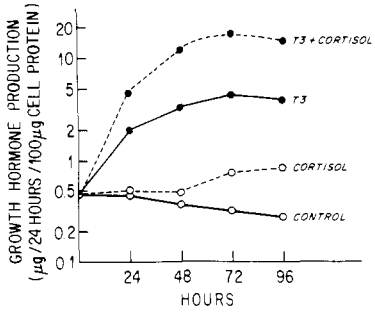

FIGURE 1: Kinetics of induction of growth hormone production by L-triiodothyronine (T3) and cortisol in serum free medium. The results reflect the growth hormone produced during each 24-h period and are expressed as a logarithmic function. Control (O—O); cortisol, 100 nM (O---O); L-triiodothyronine, 5 nM (●—●); L-triiodothyronine, 5 nM, plus cortisol, 100 nM (●---●).

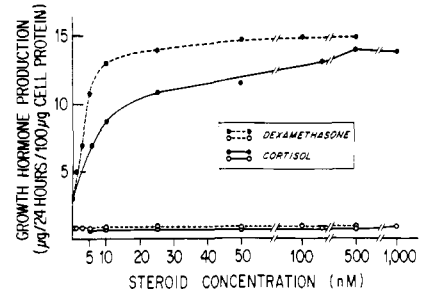

FIGURE 2: Influence of L-triiodothyronine on the dose–response induction of growth hormone by cortisol and dexamethasone. The cells were incubated with serum free Ham's F-10 medium with or without 5 nM L-triiodothyronine. The results reflect the growth hormone produced between 24 and 48 h of incubation. With L-triiodothyronine: cortisol (●—●); dexamethasone (●---●). Without L-triiodothyronine: cortisol (O—O); dexamethasone (O---O).

then chilled in an ice–water bath and all further procedures were carried out at 0–4 °C. The cell monolayers were rinsed four times with a solution consisting of 0.14 M NaCl and 10 mM sodium phosphate (pH 7.6), and any residual saline solution was removed by aspiration. The cells were harvested with a silicone rubber blade into buffer composed of 50 mM Tris (pH 7.6 at 25 °C), 1 mM $MgCl_2$, and 1 mM 2-mercaptoethanol. The cells were homogenized with a motorized Teflon pestle (Samuels et al., 1974), and the disrupted cells were first centrifuged at 800*g*, and the supernatant was then centrifuged at 120000*g* for 1.5 h to obtain the cytosol fraction. The total yield of cytosol protein determined by the method of Lowry et al. (1951) was approximately 10 mg and glucocorticoid receptor binding was performed with 160 μL of cytosol (500–800 μg of protein).

Twenty microliters of [^{3}H]cortisol was first mixed with 20 μL of the appropriate concentration of nonradioactive steroid and the binding reaction was started by the addition of 160 μL of cytosol to each tube. The final concentration of [^{3}H]cortisol was 50 nM and the final concentrations of nonradioactive cortisol, dexamethasone, progesterone, and 17α-methyltestosterone were as indicated in the text. A set of parallel control tubes received 50 nM [^{3}H]cortisol plus nonradioactive steroids and 160 μL of homogenization buffer instead of cytosol. The samples were incubated for 15 h at 0–4 °C and the bound and free steroid was separated using the dextran–charcoal method (Rousseau et al., 1972; Samuels et al., 1977b). One milliliter of homogenization buffer containing 2 mg of activated charcoal and 0.2 mg of dextran (average molecular weight, 500 000) was added to each tube, and after 2 min the samples were centrifuged at 2000*g* for 5 min. The derived supernatant fractions were dissolved in Aquasol and quantitated in a liquid scintillation counter with 30% efficiency. The supernatant fractions from the control tubes, which did not receive cytosol, typically contained 4500 ± 300 cpm. This value was not altered by the nonradioactive steroid concentrations and was subtracted from the radioactivity in the supernatant of the tubes incubated with cytosol to determine the [^{3}H]cortisol bound to receptor in the cytosol fraction. Each point reflects the mean of triplicate determinations with variations within each triplicate of less than ±6% from the mean value.

Results

Kinetics of Induction of Growth Hormone Synthesis by L-Triiodothyronine and Cortisol. Figure 1 illustrates the kinetics of induction of growth hormone synthesis in cells incubated with cortisol (100 nM), with L-triiodothyronine (5 nM), and with L-triiodothyronine (5 nM) plus cortisol (100 nM) using serum free Ham's F-10 medium. The results are plotted as a logarithmic function to display all the results on the same graph. L-Triiodothyronine induced a rapid increase in the rate of growth hormone production to approximately 12-fold greater than the control after 72 h of incubation. The growth hormone response of the cells incubated with L-triiodothyronine plus cortisol was 40-fold greater than the control cells and at each time point is threefold greater than for L-triiodothyronine alone. Cortisol alone stimulated a two- to threefold increase in growth hormone synthesis which is only observed after 48 h of incubation and therefore deviates from the cortisol response observed with L-triiodothyronine in both the magnitude and the onset of the response. Identical kinetics of induction were obtained in parallel studies carried out with Ham's F-10 medium supplemented with either 10% hypothyroid calf serum or hypothyroid calf serum treated with AG 1-X10 resin which lowered any trace levels of thyroid hormone by a factor of 15- to 20-fold. Therefore, the delayed but small cortisol induced growth hormone response does not appear to be secondary to residual low thyroid hormone levels in the thyroidectomized calf serum. Furthermore, the kinetics of the growth hormone response was similar using medium supplemented with hypothyroid calf serum or charcoal treated hypothyroid calf serum indicating that the thyroid hormone induced response is not secondary to the low level of glucocorticoid (0.2 nM) in the hypothyroid calf serum.

Dose–Response Relationship of Growth Hormone Induction by Dexamethasone and Cortisol. Figure 2 illustrates a serum free dose–response study of growth hormone induction by dexamethasone and cortisol with and without 5 nM L-triiodothyronine. The response represents the growth hormone produced between 24 and 48 h of incubation with hormone. Without L-triiodothyronine neither steroid induced an increase in growth hormone production. Without added steroid, L-triiodothyronine induced a fourfold increase in growth hormone production and this was increased fivefold further with dexamethasone or cortisol. With L-triiodothyronine a half-maximal increase in growth hormone production occurred with 12 nM cortisol and 3.5 nM dexamethasone. These results indicate that the influence of thyroid hormone on glucocorticoid action in GH_1 cells is not restricted to cortisol, and the relative difference in the cortisol and dexamethasone induced response with L-triiodothyronine parallels the response of other isolated cell systems to these hormones (Samuels & Tomkins, 1970; Foster & Perkins, 1977; Young et al., 1975; Ringold et al., 1975; Shyamala & Dickson, 1976; Feldman & Loose, 1977; Rousseau et al., 1972).

Influence of Progesterone and 17α-Methyltestosterone on the Induction of Growth Hormone Synthesis by Cortisol and

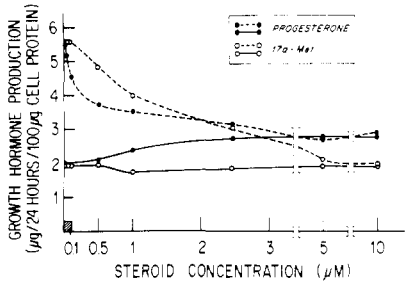

FIGURE 3: Influence of progesterone and 17α-methyltestosterone concentrations on growth hormone production in serum free media. The results reflect the growth hormone produced between 24 and 48 h of incubation. The steroid concentration refers to the level of progesterone or 17α-methyltestosterone (17α-Met) in the study. L-Triiodothyronine, 5 nM, plus 17α-methyltestosterone (O—O). L-Triiodothyronine, 5 nM, plus progesterone (●—●). L-Triiodothyronine, 5 nM, plus cortisol, 50 nM, plus 17α-methyltestosterone (O---O). L-Triiodothyronine, 5 nM, plus cortisol, 50 nM, plus progesterone (●---●). The shaded bar in the lower left represents the growth hormone produced in control cells which received no hormone.

L-*Triiodothyronine.* Based on the induction of tyrosine aminotransferase in hepatoma tissue culture (HTC) cells, Samuels & Tomkins (1970) classified steroid compounds into four groups: (1) optimal inducers (full agonists), e.g., cortisol and dexamethasone, which can induce a maximal response; (2) suboptimal inducers (partial agonists), e.g., 17α-hydroxprogesterone, 11-deoxycortisol, and progesterone, which even at high concentrations only induce a submaximal response but also can inhibit induction by optimal inducers to the submaximal level at appropriate concentrations; (3) antiinducers (antagonists), e.g., 17α-methyltestosterone, which do not induce a response but at appropriate concentrations can inhibit induction by optimal and suboptimal inducers; (4) inactive steroids which neither induce nor inhibit a response.

Figure 3 illustrates the effect of progesterone and 17α-methyltestosterone on the growth hormone response induced by L-triiodothyronine (5 nM), and L-triiodothyronine (5 nM) plus cortisol (50 nM). L-Triiodothyronine induced a fivefold increase in growth hormone production and 17α-methyltestosterone did not alter the thyroid hormone induced response. In some experiments, not illustrated, 17α-methyltestosterone at 10 μM decreased the L-triiodothyronine induced growth hormone response by 10–15% which might be a result of a small amount of glucocorticoid remaining in the cell. The growth hormone response with L-triiodothyronine and cortisol was 2.5-fold greater than with L-triiodothyronine alone, and 17α-methyltestosterone inhibited the L-triiodothyronine plus cortisol response to that observed with L-triiodothyronine alone. Half-maximal inhibition of the response occurred at 1.8 μM 17α-methyltestosterone.

Progesterone partially stimulated the growth hormone response in the L-triiodothyronine incubated cells with a half-maximal induction at 1 μM progesterone. In cells incubated with L-triiodothyronine plus cortisol, progesterone progressively inhibited the growth hormone response to the final level observed for L-triiodothyronine plus progesterone. Half-maximal inhibition occurred at 250 nM progesterone. Without triiodothyronine, progesterone did not increase growth hormone production or decrease the control cell response (not illustrated).

Figure 4 illustrates a long term kinetic study of the effect of 17α-methyltestosterone (10 μM) on the response induced by L-triiodothyronine (5 nM), cortisol (50 nM), and L-triiodothyronine (5 nM) plus cortisol (50 nM). 17α-Methyltestosterone only minimally altered the L-triiodothyronine

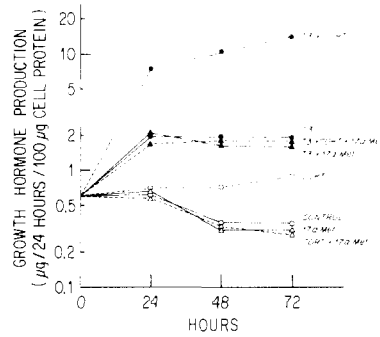

FIGURE 4: Influence of 17α-methyltestosterone on the kinetics of growth hormone production in serum free media. The results reflect the growth hormone produced during each 24-h period and are expressed as a logarithmic function. Hormone concentrations were: 17α-methyltestosterone (17α-Met), (10 μM); cortisol (cort.), 50 nM; L-triiodothyronine (T3), 5 nM. Incubation conditions: control (O—O); 17α-methyltestosterone (Δ—Δ); cortisol (O---O); cortisol plus 17α-methyltestosterone (Δ---Δ); L-triiodothyronine (●—●); L-triiodothyronine plus cortisol (●---●); L-triiodothyronine plus 17α-methyltestosterone (▲—▲); L-triiodothyronine plus cortisol, plus 17α-methyltestosterone (▲---▲).

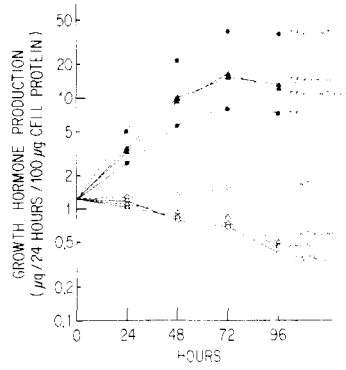

FIGURE 5: Influence of progesterone on the kinetics of growth hormone production in serum free media. The results reflect the growth hormone produced during each 24-h period and are expressed as a logarithmic function. Hormone concentrations were: progesterone (prog.), 10 μM; cortisol (cort.), 50 nM; L-triiodotyronine (T3), 5 nM. Incubation conditions: control (O—O); progesterone (Δ—Δ); cortisol (O---O); cortisol plus progesterone (Δ---Δ); L-triiodothyronine (●—●); L-triiodothyronine plus cortisol (●---●); L-triiodothyronine plus progesterone (▲—▲); L-triiodothyronine plus cortisol plus progesterone (▲---▲).

induced response at 48 and 72 h to 85% of that induced by L-triiodothyronine alone. 17α-Methyltestosterone at each time point reduced the L-triiodothyronine plus cortisol response to that observed for L-triiodothyronine plus 17α-methyltestosterone, and the small growth hormone response induced by cortisol alone was completely inhibited.

Figure 5 illustrates a similar study using 10 μM progesterone. Progesterone alone does not stimulate growth hormone production but does inhibit the small response observed by cortisol alone to the same value observed with control cells. L-Triiodothyronine plus progesterone induced a response 1.5-fold that of L-triiodothyronine alone, and progesterone decreased the response induced by L-triiodothyronine plus cortisol to the identical value observed with L-triiodothyronine plus progesterone. Table I further extends the observations of Figures 3 to 5 and illustrates the results obtained with various combinations of L-triiodothyronine, cortisol, progesterone, and 17α-methyltestosterone. In this study the growth hormone response was quantitated both by radioimmunoassay and by synthetic rates measured by L-[^{35}S]methionine incorporation for 15 min followed by immunoprecipitation with anti-growth-hormone antibody (Samuels & Shapiro, 1976;

Table I: Influence of Different Hormone Combinations on the Induction of the Growth Hormone Response[a]

	GH production (radioimmunoassay)		GH synthesis		total protein synthesis
	μg/(24 h·100 μg of DNA)	hormone/ control	cpm/100 μg of DNA × 10^{-3}	hormone/ control	cpm/100 μg of DNA × 10^{-6}
control	5.5		21.5		5.8
cortisol	6.5	1.20	24.2	1.13	5.5
L-triiodothyronine	18.2	3.30	62.1	2.89	6.4
17α-methyltestosterone	5.8	1.05	23.1	1.07	6.7
progesterone	6.1	1.10	25.8	1.08	6.1
L-triiodothyronine plus 17α-methyltestosterone	16.5	3.00	53.2	2.47	6.9
L-triiodothyronine plus progesterone	23.5	4.27	83.2	3.86	6.8
L-triiodothyronine plus cortisol	49.1	8.92	159.6	7.42	6.2
L-triiodothyronine plus progesterone, plus 17α-methyltestosterone	17.8	3.24	58.7	2.73	7.1
L-triiodothyronine plus cortisol, plus 17α-methyltestosterone	17.1	3.11	55.5	2.58	7.3
L-triiodothyronine plus cortisol, plus progesterone	25.3	4.60	86.1	4.00	7.4
L-triiodothyronine plus cortisol, plus progesterone, plus 17α-methyltestosterone	17.2	3.15	60.0	2.79	7.2

[a] Six hundred thousand GH_1 cells were inoculated into 25-cm^2 flasks and cultured using the protocol indicated in Experimental Procedures. The cells were incubated with the hormone combinations indicated in the table and after 24 h the media were replaced with identical serum free media containing the same hormones for an additional 24 h. Growth hormone production was determined by the growth hormone which accumulated in the media between 24 and 48 h. At 48 h the instantaneous rate of growth hormone synthesis and total protein synthesis was determined with a 15-min incubation with L-[^{35}S]methionine followed by selective immunoprecipitation. The hormone concentrations were: L-triiodothyronine (5 nM); 17α-methyltestosterone (10 μM); progesterone (10 μM); and cortisol (50 nM).

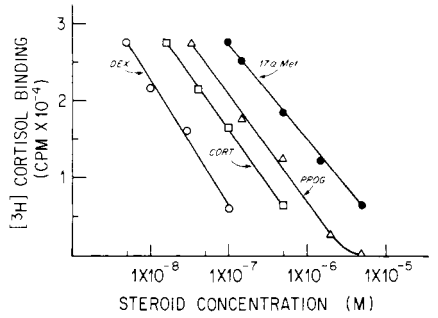

FIGURE 6: Association of glucocorticoid agonists and antagonists with cytosol glucocorticoid receptor. Cytosol from GH_1 cells equivalent to 800 μg of protein was incubated with 50 nM [^{3}H]cortisol and different concentrations of dexamethasone (dex.) (○); cortisol (cort.) (□); progesterone (prog.) (△); and 17α-methyltestosterone (17α-Met) (●) for 15 h at 0–4 °C. The [^{3}H]cortisol bound to cytosol glucocorticoid receptor was determined with dextran–charcoal as described under Experimental Procedures. Without nonradioactive steriod, cytosol bound 3.5 × 10^4 ± 800 cpm of [^{3}H]cortisol.

Samuels et al., 1976, 1977a). In each case the relative growth hormone response was similar whether measured by radioimmunoassay or incorporation synthetic rates. In addition, 17α-methyltestosterone also modulates the progesterone induced response in the presence of L-triiodothyronine and reduced it to that observed for L-triiodothyronine plus 17α-methyltestosterone.

Association of Glucocorticoid Agonists and Antagonists with Cytosol Glucocorticoid Receptors. Figure 6 illustrates the inhibition of binding of 50 nM [^{3}H]cortisol with cytosol glucocorticoid receptors by dexamethasone, cortisol, progesterone, and 17α-methyltestosterone. All inhibition curves are parallel, and half-maximal inhibition of [^{3}H]cortisol binding occurred at 35 nM dexamethasone, 120 nM cortisol, 300 nM progesterone, and 1500 nM 17α-methyltestosterone. The relative affinity of the steroids for the glucocorticoid receptor parallel their relative effects on the stimulation or inhibition of the growth hormone response illustrated in Figures 2 and 3 with L-triiodothyronine. Based on the steroid concentrations which induced a half-maximal increase in the growth hormone response (Figure 2), dexamethasone is 3.4-fold more active than cortisol, and this value is identical with the relative difference in affinity of the steroids for the receptor (calculated from the ratio of hormone concentrations which results in half-maximal inhibition of [^{3}H]cortisol binding, 120 nM/35 nM). In addition, the relative affinity of the receptor for progesterone and 17α-methyltestosterone parallels the relative influence of these compounds on inhibiting the growth hormone response observed with 50 nM cortisol and 5 nM L-triiodothyronine (Figure 3).

Influence of 17α-Methyltestosterone on the Induction of Growth Hormone mRNA Levels by L-Triiodothyronine and Cortisol. Glucocorticoid antagonists, which inhibit the nuclear translocation of the glucocorticoid receptor (Rousseau et al., 1973; Shyamala, 1975), have been shown to inhibit the glucocorticoid mediated induction of mouse mammary tumor viral specific RNA in cultured mouse mammary carcinoma cells (Young et al., 1975; Ringold et al., 1975; Shyamala & Dickson, 1976). It has not been documented, however, that antiglucocorticoids act by inhibiting the glucocorticoid mediated accumulation of specific eukaryotic mRNA.

Figure 7 compares the influence of 17α-methyltestosterone on the rate of growth hormone production in intact cells to the level of cytoplasmic growth hormone mRNA estimated by translation using the wheat germ system. The validity of this quantitation has been demonstrated by Martial et al. (1977a) using cell free translation and cDNA hybridization to quantitate growth hormone mRNA. As previously reported (Shapiro et al., 1978), L-triiodothyronine increases growth hormone mRNA in GH_1 cells, and this level is further increased in cells incubated with L-triiodothyronine plus cortisol. 17α-Methyltestosterone slightly decreased the level of growth hormone mRNA in the cells also incubated with L-triiodothyronine by 10% and reduced the level in the cells incubated with L-triiodothyronine plus cortisol to the growth hormone mRNA level determined in the cells incubated with L-triiodothyronine plus 17α-methyltestosterone.

In each case, there is a good relationship between growth hormone mRNA levels and the rate of growth hormone

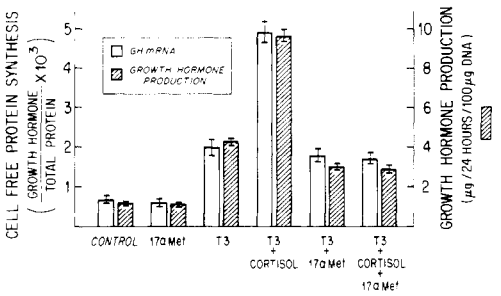

FIGURE 7: Influence of 17α-methyltestosterone on the level of cytoplasmic growth hormone mRNA. GH_1 cells (150×10^6) were incubated in roller bottles using serum free Ham's F-10 media with hormone as indicated in the text. The growth hormone production rate was determined from the accumulation of growth hormone in the medium between 48 and 72 h of incubation (hatched bars). Each roller bottle of cells yielded approximately 2 mg of DNA and 40 μg of poly(A) (+) mRNA based on 41 μg of RNA/A unit at 260 nm, and the mRNA was translated in the wheat germ system at a concentration of 20 μg/mL. Growth hormone mRNA, determined by cell free synthesis, is expressed relative to the total stimulated protein synthesis (open bars). Immunoprecipitation was performed with 0.25-mL fractions of the 120000g wheat germ supernatant. The total stimulated incorporation of L-[^{35}S]methionine into the released peptide fraction was 2×10^6 cpm ± 15%. The results are expressed as the mean ± range of triplicate assays. Hormone concentrations were: 17α-methyltestosterone (17αMet), 10 μM; L-triiodothyronine (T3), 5 nM; cortisol, 50 nM.

production, demonstrating that 17α-methyltestosterone modulates the glucocorticoid induced growth hormone response in cells incubated with L-triiodothyronine by decreasing the accumulation of cytoplasmic growth hormone mRNA in the cell.

Discussion

In this study we demonstrated that thyroid and glucocorticoid hormones can act independently as well as synergistically in controlling the growth hormone response in cultured GH_1 cells. The kinetics and magnitude of the responses, however, differ significantly. Cortisol alone induced a delayed response which is only observed after 24 to 48 h and is two- to threefold greater than the control at 72–96 h of incubation. In the experiments illustrated in Figures 1, 4, and 5, cortisol did not stimulate cell growth and did not increase cell viability as estimated by protein synthetic rates (Table I). This is in agreement with previous studies (Shapiro et al., 1978). Since the glucocorticoid induced response occurs in the apparent absence of thyroid hormone, without a change in protein synthetic rates, the delayed induction of the growth hormone response likely reflects a selective action of glucocorticoid. In contrast, thyroid hormone induces a rapid response of greater magnitude equal to three- to fourfold within 24 h and 10- to 12-fold by 48 h. This thyroid hormone induced growth hormone response appears to be independent of glucocorticoid since it occurs with charcoalized serum medium and is not significantly modified by 17α-methyltestosterone which acts as an anti-glucocorticoid in this system (Figures 3 and 4).

In addition to controlling the growth hormone response independently, thyroid and glucocorticoid hormones appear to act synergistically to increase the rate of growth hormone production and growth hormone RNA levels which are three- to fivefold greater than with L-triiodothyronine alone. Since both hormones can act independently to regulate the growth hormone response, it is not possible to determine whether one hormone regulates a rate-limiting step and the other hormone amplifies the initiated response or whether the synergistic response represents a multiplied function of the independent responses stimulated by each hormone.

In an examination of the relation of steroid structure to the induction of tyrosine aminotransferase in HTC cells, Samuels & Tomkins (1970) identified steroid compounds which functioned as complete agonists, e.g., cortisol and dexamethasone; complete antagonists, e.g., 17α-methyltestosterone; and partial agonists–antagonists, e.g., progesterone. In GH_1 cells 17α-methyltestosterone functions as a glucocorticoid antagonist and completely inhibits the growth hormone response induced by cortisol alone and the glucocorticoid induced component of the growth hormone response in cells cultured with L-triiodothyronine plus cortisol (Figures 3 and 4). This occurs in spite of the fact that cell protein synthesis is increased 10–15%. In some systems, progesterone has been reported to act as a weak partial glucocorticoid agonist and at appropriate concentrations can partially inhibit the biologic action of the complete agonist (Samuels & Tomkins, 1970; Foster & Perkins, 1977). In other systems progesterone has been reported to act as a pure glucocorticoid antagonist (Young et al., 1975; Ringold et al., 1975; Shyamala & Dickson, 1976; Feldman & Loose, 1977). In GH_1 cells progesterone alone does not appear to induce growth hormone synthesis but does fully inhibit the response induced by cortisol alone. With L-triiodothyronine, progesterone induces a partial increase in growth hormone production with a half-maximal response occurring at 1 μM. When incubated with L-triiodothyronine plus cortisol, increasing progesterone concentrations resulted in an inhibition of the growth hormone response to that observed for L-triiodothyronine plus progesterone (Figure 3).

In this study, using GH_1 cell cytosol, we demonstrated that the relative affinity of agonist or antagonist for the cytosol glucocorticoid receptor is directly related to the concentration dependent influence of these steroids on regulating the growth hormone response. The difference in relative affinity for cortisol and dexamethasone for the receptor (Figure 6) is directly related to the difference in agonist activity (Figure 2). Furthermore, the concentration-dependent inhibition of the cortisol-induced response (Figure 3) is also a direct function of the relative affinity of antagonist for the receptor. Therefore, the glucocorticoid induction of growth hormone synthesis, which is influenced by L-triiodothyronine, appears to be mediated by a similar putative receptor mechanism as reported in other glucocorticoid responsive systems (Samuels & Tomkins, 1970; Foster & Perkins, 1977; Young et al., 1975; Shyamala & Dickson, 1976; Feldman & Loose, 1977; Rousseau et al., 1972).

Several lines of evidence indicate that the multihormonal control of the growth hormone response involves the nuclear associated receptors for both thyroid and glucocorticoid hormones. Firstly this is implied by the observation that the multihormonal control of growth hormone synthesis in GH_1 cells parallels the total cytoplasmic growth hormone mRNA levels (Figure 7 and Shapiro et al. (1978)). Studies of thyroid and glucocorticoid hormone induction of growth hormone synthesis in GC cells by Martial et al. (1977b) also support pretranslational control by both hormones. Secondly, anti-glucocorticoid, which inhibits the nuclear translocation of the glucocorticoid receptor (Shyamala, 1975; Rousseau et al., 1973), appears to decrease the glucocorticoid component of growth hormone response by decreasing growth hormone mRNA levels (Figure 7). Furthermore, the concentration of dexamethasone (4 nM) which results in a half-maximal level of nuclear associated glucocorticoid receptor (Samuels et al., 1978) is in excellent agreement with the dexamethasone concentration (3.5 nM) which results in a half-maximal in-

duction of growth hormone production in the presence of L-triiodothyronine (Figure 2).

In addition the thyroid hormone nuclear receptor appears to play an important role in regulating the growth hormone response. This is based on the excellent agreement between receptor occupancy and the induction of the growth hormone response by L-triiodothyronine (Samuels & Shapiro, 1976) and a number of hormonal analogues (Samuels, 1978). In addition, we have shown that a reduction in thyroid hormone nuclear receptor levels in GH_1 cells appears to selectively reduce the magnitude of the growth hormone response induced by L-triiodothyronine (Samuels et al., 1977a). Furthermore, thyroid hormone does not modulate the cellular level or the cellular distribution of the glucocorticoid receptor (Samuels et al., 1977b, 1978), and glucocorticoid does not influence total level of the thyroid hormone nuclear receptor (Samuels et al., 1977b). Therefore, it appears that both the nuclear associated thyroid hormone and glucocorticoid hormone receptors act in a concerted fashion to regulate the growth hormone response.

Unlike the growth hormone response, glucocorticoid rapidly induces glutamine synthetase in GH_1 cells to a maximal level in 24–48 h and this induction is not influenced by thyroid hormone (Samuels et al., 1978). Half-maximal glutamine synthetase induction occurred at 3 nM dexamethasone which is essentially identical with that observed for growth hormone induction with L-triiodothyronine (3.5 nM) (Figure 2). Since it has been shown in other cell systems that glucocorticoid stimulates the synthesis of glutamine synthetase by enhancing the accumulation of the mRNA for the enzyme (Sarkar & Griffith, 1976), it appears that glucocorticoids can rapidly act at the pretranslational level in GH_1 cells independent of thyroid hormone. Therefore, the influence of thyroid hormone on the glucocorticoid control of the growth hormone response may be relatively specific for this function. Whether this concerted control mechanism involves the transcription of the growth hormone gene, the nuclear processing of the growth hormone transcript or other pretranslational control mechanisms remains to be defined.

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