Modulation of Thyroid Hormone Nuclear Receptors by Cholera Toxin in Cultured GH₁ Cells*

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The cellular actions of the thyroid hormones L-thyroxine and L-triiodothyronine are mediated by the association of hormone with a chromatin-associated receptor. In cultured GH₁ cells, a hormone-responsive rat pituitary cell line, the thyroid hormone decreases the concentration of its receptor at early incubation times by reducing the accumulation of newly synthesized receptor. In this study, we demonstrate that cholera toxin also reduces the amount of nuclear receptor in GH₁ cells in a time- and dose-dependent fashion, without altering the affinity of the receptor for hormone. The reduction of receptor mediated by cholera toxin is not secondary to a generalized inhibition of cell protein synthesis or cell replication rates and this effect can be abolished by pretreatment of the cholera toxin with soluble ganglioside IIα-N-acetylneuraminosylgangliotetraosylamide. This effect requires an intact cholera toxin molecule and does not occur at similar concentrations of the membrane-binding B subunit of cholera toxin. In order to study the influence of cholera toxin on thyroid hormone receptor turnover, we have used a dense amino acid-labeling technique. The results indicate that cholera toxin does not change the half-life of receptor, but decreases the rate of appearance of newly synthesized receptor. This decreased rate completely accounts for the lowered steady state receptor levels. The extent of cAMP stimulation by cholera toxin does not correlate with the extent of receptor reduction and forskolin, which stimulates cAMP levels 25- to 500-fold, does not decrease thyroid hormone receptor abundance. These studies suggest that cholera toxin modulates receptor levels by a mechanism(s) that is not mediated by cAMP in GH₁ cells.

Thyroid hormone action in mammalian cells appears to be initiated by a nuclear associated receptor. The receptor is an acidic non-histone chromatin-associated DNA-binding protein with an estimated M₀ ~ 50,000 and a sedimentation coefficient of 3.8 S (1–3). The level of receptor reflects a dynamic steady state that is determined by the rate of synthesis and degradation of receptor. Using a dense amino acid-labeling technique, we have shown that in GH₁ cells, a growth hormone-producing rat pituitary tumor cell line, the thyroid hormone receptor has a half-life of 4 to 5 h and a synthetic rate of approximately 1800 molecules/h/cell (4). A number of nutritional, pharmacological, or physiological factors may interact to modulate the levels of receptor in different tissues and cell types and may potentially influence the cell response to thyroid hormone (4–10). In GH₁ cells and in cultured normal pituitary cells, L-T₃ and various hormonal analogues elicit a dose-dependent decrease of receptor levels (4–7). During short incubation times (less than 15 h), L-T₃ elicits a reduction in receptor levels solely by decreasing the accumulation of newly synthesized receptor, while at longer incubation times (more than 24 h) the predominant effect is a reduction in the receptor half-life (4).

Thyroid hormone modulation of its receptor has not been reported to occur in rat liver, but other factors including administration of pharmacological doses of glucagon (8, 9) and β-adrenergic agonists elicit a decrease in receptor levels. Since these hormones can stimulate cAMP, an increase in endogenous cAMP levels may be responsible for the modulation of receptor. To examine this possibility, we studied the effect of cholera toxin in GH₁ cells. Cholera toxin ubiquitously stimulates adenylyl cyclase activity and mimics certain effects of compounds which stimulate cAMP levels without the side effects reported for cyclic nucleotides containing butyric acid or for inhibitors of cAMP phosphodiesterase (10, 11). The initial event in the action of cholera toxin involves the high affinity binding of the B protomer subunit to monoganglioside GMI on the cell membrane (12, 13). Subsequently the A protomer component is internalized and exerts its effect by catalyzing the ADP-ribosylation of a guanyl nucleotide regulatory protein which modulates adenylyl cyclase activity (14, 15).

Our results indicate that cholera toxin elicits a dose-dependent depletion of the thyroid hormone nuclear receptor. This effect occurs without inhibition of total protein synthetic rates and is due to a decrease in the appearance of newly synthesized receptor and not to an increase in its degradation rate. This action of cholera toxin appears to be unrelated to elevation of cAMP levels and suggests that cholera toxin modulates receptor levels by a mechanism(s) which is not mediated by cyclic nucleotide regulation.

EXPERIMENTAL PROCEDURES

Hormones and Chemicals—All radioactive products were obtained from New England Nuclear (Boston, MA). Amino acids enriched in ³H, ¹³C, and ¹⁵N were obtained from Merck Sharp and Dohme. Cholera toxin has a chromatin-associated receptor. The receptor is an acidic non-histone chromatin-associated DNA-binding protein with an estimated M₀ ~ 50,000 and a sedimentation coefficient of 3.8 S (1–3). The level of receptor reflects a dynamic steady state that is determined by the rate of synthesis and degradation of receptor. Using a dense amino acid-labeling technique, we have shown that in GH₁ cells, a growth hormone-producing rat pituitary tumor cell line, the thyroid hormone receptor has a half-life of 4 to 5 h and a synthetic rate of approximately 1800 molecules/h/cell (4). A number of nutritional, pharmacological, or physiological factors may interact to modulate the levels of receptor in different tissues and cell types and may potentially influence the cell response to thyroid hormone (4–10). In GH₁ cells and in cultured normal pituitary cells, L-T₃ and various hormonal analogues elicit a dose-dependent decrease of receptor levels (4–7). During short incubation times (less than 15 h), L-T₃ elicits a reduction in receptor levels solely by decreasing the accumulation of newly synthesized receptor, while at longer incubation times (more than 24 h) the predominant effect is a reduction in the receptor half-life (4).

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1 The abbreviations used are: L-T₃, 3,5,3'-triiodo-L-thyronine; B₄, cAMP, N₅',O²'-dibutyryladenosine 3':5'-cyclic monophosphate; Kₑ, appearance rate of newly synthesized receptor; Kₑ, degradation rate constant; G₆0, IIα-N-acetylneuraminosylgangliotetraosylamide.

toxin was obtained from Schwarm/Mann or Calbiochem-Behring as were the B subunit of cholera toxin and forskolin. Cholera toxin and the B subunit were extensively dialyzed against 0.1 M sodium phosphate buffer, pH 7.6, before addition to cells. Ganglioside GM3 was from Supelco and concanavalin A was from Pharmacia. Cell culture products were from Gibco. All other reagents were obtained from E. Merck, Fisher Scientific, or Sigma and were of the highest purity available.

**GH, Cell Culture—**GH3 cells were grown in monolayer cultures as previously described (16). The cells were inoculated at initial densities of 2 to 4 × 10⁵ cells/cm² with Ham's F-10 medium containing 2.5% fetal calf serum and 15% horse serum and incubated at 37°C in an atmosphere of 95% air, 5% CO₂ for 96 h. The medium was replaced with Ham's F-10 medium containing 10% (v/v) calf serum depleted of thyroid hormone by treatment with AG 1X-8 resin and charcoal as previously described (17). The cells were incubated in thyroid hormone-depleted medium for an additional 48 h to ensure the complete cellular depletion of thyroid hormone (18).

**Quantitation of Thyroid Hormone Nuclear Receptor Levels—**Cell monolayers were incubated with cholera toxin or other compounds for the times indicated in the text and with 5 nM L-[¹²⁵I]T₃ (1250 µCi/µg) for 60-90 min prior to the time the cells were harvested. This condition was found to give an estimate of the total receptor levels since it binds to greater than 95% of the receptor population (5, 6). After incubation, the cells were chilled to 4°C and nuclear and extranuclear binding was determined as previously described (6, 7, 19). Nonspecific nuclear binding was determined by incubating cells with a 20-fold excess of nonradioactive L-T₃. The nonspecific nuclear binding fraction was less than 5% of the total nuclear bound L-[¹²⁵I]T₃, and was subtracted from the total to determine the receptor bound L-T₃. In some experiments, nuclear receptor levels were assayed simultaneously with rates of cell protein synthesis. The cells were incubated for 1 h with 5 nM L-[¹²⁵I]T₃ and 2 µCi/ml of L-[¹³C]leucine. After quantification of L-T₃ binding, the nuclear and extranuclear L-[¹²⁵I]T₃ was extracted with absolute ethanol after precipitation with 18% perchloric acid (w/v) as previously described (2). The incorporation of L-[¹³C]leucine into nuclear and extranuclear proteins was analyzed using a liquid scintillation counter.

**Preincubation of Cholera Toxin with Ganglioside GM₃—**GM₃ was dissolved in 0.1 M sodium phosphate buffer (pH 7.6) containing 0.1% bovine serum albumin at a concentration of 0.25 mg/ml and was incubated for 45 min at 25°C with different concentrations of cholera toxin. The mixture was added to the cells to give the final concentrations of cholera toxin indicated in the text. The final concentration of GM₃ in the medium was 25 µg/ml. After 24 h of incubation, nuclear receptor levels were determined as described above. An aliquot of medium was removed just prior to addition of L-[¹²⁵I]T₃ for the quantitation of cAMP levels.

**cAMP Determination—**Medium was removed and saved and cells were washed with 0.85% NaCl at 4°C. The cAMP was extracted from the cells with 5% perchloric acid (w/v). To measure extracellular cAMP, the culture medium was adjusted to 5% perchloric acid (w/v). The acid extracts were neutralized with KHCO₃ using brom cresol purple as a pH indicator. After appropriate dilutions were made with 0.1 M sodium acetate buffer (pH 6.2), the samples were acetylated according to the procedure of Harper and Brooker (20) and the cAMP was determined by radioimmunoassay adapted from the procedure of Steiner et al. (21) using immunoreagents provided by New England Nuclear.

**Determination of Thyroid Hormone Receptor Half-life—**Receptor half-life was determined using dense amino acids highly enriched for "H, "C, and "N as previously described (4). In one study, the cells were incubated for 24 h with 100 nM cholera toxin in thyroid hormone-depleted medium. In another experiment, the cells were incubated with 50 nM cholera toxin for 48 h. At times ranging from 1 to 5 h prior to cell harvesting, the medium was replaced with medium containing dense amino acids. One h before harvesting, all cells received 5 nM L-[¹³C]T₃. The L-[¹³C]T₃-receptor complexes were extracted at 4°C with 0.15 ml of buffer composed of 20 mM Tris, pH 7.6, 1.1 mM MgCl₂, 1 mM EDTA, 5 mM dithiothreitol, and 0.4 M KCl (4). This procedure extracted 75% of the receptor. Normal and decapsulated receptors were isolated by velocity sedimentation in discontinuous 17-32% sucrose gradients constructed with D₂O (4). The gradients were centrifuged in a SW 60 Ti rotor at 5°C for 48 h at 57,500 rpm. After centrifugation the gradients were fractionated into about 40 tubes and the radioactivity of each fraction was determined.

**Extraction of Histones and Gel Electrophoresis—**Nuclei were washed at 0°C with buffer containing 0.14 M NaCl, 2.4 mM MgCl₂, and 5 mM Tris-HCl, pH 7.4. The histones were extracted twice with 0.4 M H₂SO₄ as described (22), and precipitated overnight at -20°C with 10 volumes of acetone. The histones were collected by centrifugation at 25,000 × g for 20 min, dissolved in 0.9 M acetic acid, and electrophoresed in 14-cm-7.5% acrylamide gels at pH 2.7 with 2.5 M urea as described by Panyim and Chalkley (23). Approximately 50 µg of histone were applied to the gels and after electrophoresis were stained with amido black, destained, and scanned at 615 nm using a Giford spectrophotometer.

**Other Determinations—**Cellular protein was measured by the method of Lowry et al. (24) using bovine serum albumin as a standard. DNA was determined by the method of Burton (25) using 2-deoxyribose as a standard. Each data point presented is the mean of triplicate determinations that did not vary more than ±5-10%. Results are presented as the mean or mean ± S.D. Analysis of covariance (26) was used to determine the statistical significance of the slopes and intercepts of the Scatchard analysis (27) of the binding data illustrated in Fig. 2.

**RESULTS**

**Reduction of Nuclear L-T₃ Binding by Cholera Toxin—**Fig. 1 illustrates thyroid hormone nuclear receptor levels after a 24-h incubation with cholera toxin ranging from 1 to 100 nM. Cholera toxin caused a dose-dependent reduction of L-T₃ nuclear binding and a half-maximal decrease occurred at 5 nM. The extranuclear L-T₃ bound was similar at all cholera toxin concentrations (430 ± 38 fmol/100 µg of DNA) and was the same as control cell cultures (415 ± 53 fmol/100 µg of DNA). This indicates that the decrease in nuclear binding is not due to a decrease in cell permeability to L-T₃. Cholera toxin did not alter cell growth rates since the total DNA and protein content per flask was the same as the control cells.

That the cholera toxin-mediated decrease in nuclear receptor levels does not reflect a generalized effect on inhibition of protein synthesis is illustrated at the bottom of Fig. 1. The incorporation of L-[¹³C]leucine into nuclear or cytoplasmic proteins was not decreased in the cells incubated with 1-100 nM cholera toxin.

Fig. 2 compares a Scatchard plot (27) of L-[¹²⁵I]T₃ nuclear binding versus the concentration of cholera toxin (ChT) as determined by Scatchard analysis (27) of the binding data illustrated in Fig. 2.
Regulation of Thyroid Hormone Receptors

bated cells

and cells incubated only with cholera toxin received 5 nM L-[\alpha^32P]I-T3 and receptor-bound L-[\alpha^32P]I-T3 was determined as previously shown that L-T3 elicits a dose-dependent depletion coefficient: control cells which did not receive cholera toxin for 24 h. The plots are linear (correlation coefficient: control cells (O); cholera toxin (ChT) -incubated cells (●)).

Effect of Cholera Toxin on Receptor Half-life and Appearance of its Nuclear Receptor in GH1 cells cultured for 24 h. The receptor of normal density began to decrease after an estimated lag time of 0.3 h in control cells and 0.6 h in cholera toxin-treated cells. These lag times were discounted for the construction of the plot and the calculation of the receptor half-life. Hormone-receptor complexes were extracted from isolated nuclei and normal and density labeled receptors were separated by velocity sedimentation in sucrose-D2O gradients. Fig. 4 illustrates the velocity sedimentation of receptor from control cells incubated with dense amino acids for 1 and 5 h. Two radioactive peaks were resolved. The faster sedimenting peak represents the newly synthesized dense receptor and the slower sedimenting peak represents the pre-existing receptor of normal density. At 1 h, almost all of the receptor was of normal density and only a small peak of dense receptor is present. At 5 h in dense amino acid medium, 53% of the receptor was of high density.

Measurement of thyroid hormone receptor half-life in cells cultured with or without cholera toxin. GH1 cells were incubated with or without cholera toxin (ChT) for 24 h. The experimental protocol is described in Fig. 4. The receptor of normal density began to decrease after an estimated lag time of 0.3 h in control cells and 0.6 h in cholera toxin-treated cells. These lag times were discounted for the construction of the plot and the calculation of the receptor half-life.
for the control cells and 24.4 ± 0.7 fmol/h/100 μg of DNA in the cholera toxin-incubated cells. 

In another experiment, the $t_\alpha$ and $K_r$ values were determined in cells incubated with 50 nM cholera toxin for 48 h and compared to control cell cultures (not illustrated). A 48-h incubation with this concentration of cholera toxin would be expected to elicit a 50% reduction of receptor (see Fig. 7A). Receptor levels were 86 ± 7 fmol/100 μg of DNA in the cholera toxin-incubated cells and 176 ± 6 fmol/100 μg of DNA in the control cell cultures. The cholera toxin-incubated cells demonstrated a receptor $t_\alpha$ of 4.2 h and a receptor $K_r$ of 14.1 ± 1.4 fmol/h/100 μg of DNA, whereas the control cells showed a receptor $t_\alpha$ of 4.5 h and a $K_r$ of 26.9 ± 1.2 fmol/h/100 μg of DNA. Therefore, at both 24 and 48 h of cholera toxin incubation, the decrease in steady-state receptor levels results from a decrease in the nuclear accumulation of newly synthesized receptor without any alteration of receptor half-life.

Relation between Receptor Depletion and cAMP Levels—Fig. 7A shows the time course of the cholera toxin effect on nuclear receptor levels. Receptor depletion was examined over a 72-h period with 1 and 100 nM cholera toxin. T3 receptor levels decreased in a time-dependent fashion, which was maximal for each concentration at 48 h. Cells incubated with 100 nM cholera toxin for the 48-h period showed a 54% reduction in L-T3 nuclear binding whereas 1 nM cholera toxin caused a 38% decrease. Cholera toxin did not alter the DNA content during the first 48 h. Longer incubation times did not elicit further reduction of receptor and the DNA content decreased by 15–20% only with 100 nM cholera toxin. Fig. 7B shows the cAMP released into the culture medium by cells in the same experiment. One and 100 nM cholera toxin stimulated similar cAMP accumulation during the first 4 h of incubation. With 1 nM cholera toxin, the cAMP remained constant for 50 h. With 100 nM cholera toxin, cAMP levels progressively decreased after 24 h, and at 48 h, when cAMP levels approached that of control cells, the level of the receptor (Fig. 7A) was maximally decreased.

The cAMP released into the medium parallels the intracellular cAMP (Fig. 8). This study illustrates the effect of a 24-h incubation with cholera toxin ranging from 0.05 to 100 nM on cAMP levels in the medium and the cells. After 24 h, intracellular cAMP represented only 3–5% of the cAMP released by the cells into the medium. Both values, however, show very good concordance at all cholera toxin concentrations. Maximal stimulation of cAMP was observed between 0.6 and 3 nM cholera toxin. With higher concentrations of cholera toxin, cAMP levels progressively decreased. As with the study in Fig. 7, the reduction of receptor does not correlate with the stimulation of cAMP; maximal increases of cAMP were obtained at low concentrations of cholera toxin which only elicited a small or no decrease of receptor. Fig. 9 shows the results of a similar experiment in which the effect of even lower concentrations of cholera toxin on receptor and cAMP levels were examined. Concentrations of cholera toxin ranging from 0.001 to 1 nM did not produce a significant depletion of receptor, whereas 10 or 100 nM elicited a 33% reduction of L-T3 binding. The highest cAMP levels were elicited by 0.1 nM cholera toxin which did not result in reduction of receptor. In hepatoma cells it has been reported that cAMP is degraded in the culture medium and its metabolites can inhibit cell growth or tyrosine aminotransferase induction (28). These effects of adenosine or 5'-AMP were reversed by uridine or cytidine. Fig. 7 suggests that high concentrations of cholera toxin may result in cAMP degradation by cells. Therefore, adenosine formed from cAMP degradation may be the

![Fig. 6. Accumulation of newly synthesized dense receptor in nuclei of cells incubated with or without cholera toxin.](http://www.jbc.org/)

![Fig. 7. Time course of thyroid hormone nuclear receptor reduction by cholera toxin.](http://www.jbc.org/)

![Fig. 8. Influence of cholera toxin concentrations on receptor (A) and cAMP levels (B).](http://www.jbc.org/)
The effects of cholera toxin on cells can be inhibited by preincubating the cholera toxin with soluble ganglioside GM1. Fig. 11A demonstrates that preincubation of cholera toxin with ganglioside GM1 completely prevents the decrease of receptor mediated by 1 to 30 nM cholera toxin. With 100 nM, only partial inhibition of the effect was observed. Fig. 11B indicates that ganglioside GM1 inhibits the action of cholera toxin since it prevents the stimulation of cAMP by cholera toxin. This study indicated that cholera toxin depletion of receptor is mediated via the known interaction of the cholera toxin B subunit with receptors on the cell membrane (14, 15). We considered the possibility, however, that a lectin-like action of the B subunit may be responsible for the observed cholera toxin effect on receptor levels. A global lectin-like action of the B subunit is not likely since a 24-h incubation of concanavalin A (200 nM) with GHI cells did not alter receptor levels (not illustrated).

Furthermore, a comparison of the effect of different con-
Influence of cholera toxin and forskolin on thyroid hormone nuclear receptor levels and cAMP production

A stock solution of forskolin (250 mM) was prepared in ethanol/H2O (1:1, v/v) and diluted into the medium to achieve the concentrations indicated. GH1 cells were incubated for 40 h with the above concentrations of cholera toxin and forskolin. An aliquot of the medium was removed (0.5 ml) and saved for determination of cAMP as described under "Experimental Procedures." L-[35S]T2 (5 nM) was then added and the cells were incubated for 1.5 h to estimate total nuclear bound L-[35S]T2. Parallel flasks received 5 μM nonradioactive L-T2 to determine the extent of nonspecific binding. The cAMP results represent the total cAMP which accumulated in the medium during the 40-h incubation. The results reflect the mean ± range of triplicate flasks. The total DNA content per flask ranged from 33 to 57 μg and was not significantly altered by the different incubation conditions.

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>L-T2 bound (fmol/100 μg DNA)</th>
<th>Medium cAMP (pmol/100 μg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>95 ± 4</td>
<td>77 ± 15</td>
</tr>
<tr>
<td>Cholera toxin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 nM</td>
<td>65 ± 3</td>
<td>1,833 ± 105</td>
</tr>
<tr>
<td>Forskolin</td>
<td></td>
<td></td>
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<tr>
<td>1 μM</td>
<td>90 ± 5</td>
<td>1,931 ± 122</td>
</tr>
<tr>
<td>10 μM</td>
<td>97 ± 6</td>
<td>27,413 ± 899</td>
</tr>
<tr>
<td>100 μM</td>
<td>102 ± 8</td>
<td>40,180 ± 975</td>
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</table>

FIG. 11. Effect of preincubation of cholera toxin with soluble ganglioside GM1 on nuclear receptor (A) and cAMP (B) levels. Different concentrations of cholera toxin (ChT) were preincubated for 45 min at room temperature with 0.25 mg/ml of ganglioside GM1. GH1 cells were then cultured with cholera toxin alone (●) or with the ganglioside-incubated cholera toxin (○) for 24 h at the concentrations indicated. One h prior to cell harvesting, a sample of medium was saved for determination of cAMP levels and 5 nM L-[35S]T2 was added for nuclear receptor determination.

centrations of cholera toxin and the B subunit indicates that the cholera toxin reduction of receptor does not reflect an intrinsic action of the B subunit (Table II). After 48 h, 1 nM cholera toxin resulted in a 32% decrease of receptor which decreased to 49% at 50 nM cholera toxin in keeping with the results illustrated in Fig. 7. In contrast, between 1 and 10 nM, the B subunit caused no alteration in receptor levels. At B subunit concentrations of 25 and 50 nM, a small decrease of receptor occurred (13 and 15%, respectively). However, these changes were less than at 1 nM cholera toxin (32%), indicating that the cholera toxin reduction of receptor does not reflect an intrinsic effect of the B subunit but requires the intact toxin molecule.

DISCUSSION

Although the thyroid hormone receptor appears to be present at relatively constant levels in individual tissues, a number of different factors appear to modulate receptor levels in different cell types (2-9). In this study, we present evidence that cholera toxin modulates thyroid hormone receptor levels in GH1 cells. Cholera toxin elicits a time- and dose-dependent decrease in the binding of L-T2 to its nuclear receptor, which is due to a reduction in receptor levels and not to a change of the affinity of the receptor for hormone (Fig. 2). Although cholera toxin can inhibit DNA synthesis in several cell types (31), the reduction of thyroid hormone receptors in GH1 cells cannot be attributed to inhibition of cell replication. During the first 48 h of incubation when the receptor is maximally reduced, cholera toxin did not alter cellular DNA content and at longer incubation times DNA was only slightly decreased. Furthermore, the reduction of receptor is not due to a generalized effect on inhibiting protein synthesis since cytoplasmic and nuclear protein synthetic rates were not inhibited by cholera toxin in GH1 cells (Fig. 1).

The possibility that a contaminant of the cholera toxin preparation is responsible for decreasing the receptor is unlikely since receptor depletion can be abolished by preincubation of the cholera toxin with soluble ganglioside GM1. In addition, this decrease in receptor levels was observed with a wide variety of different lots of cholera toxin. The cholera toxin effect on receptor levels does not appear to reflect an intrinsic action of the B subunit (Table II). At 10 nM, cholera toxin elicited a 45% reduction of receptor while the B subunit at 10 nM caused no significant decrease in receptor levels. A small decrease in receptor was elicited by the B subunit at 25 (13%) and 50 nM (15%) but was less than observed at 1 nM cholera toxin (32%). The small decrease of receptor observed at 25 and 50 nM B subunit may reflect an intrinsic action of the B subunit or a small degree of cholera toxin contamination. However, the marked difference in the 48-h dose-response inhibition of receptor between cholera toxin and the B subunit supports the notion that the decrease in steady state receptor levels is mediated by the intact toxin molecule.

The cholera toxin-mediated decrease in receptor levels could be due to an increase in receptor degradation, to a decrease of receptor synthesis, or to both effects. Using a dense amino acid-labeling technique, the present study shows that cholera toxin does not alter receptor degradation since the half-life was 4.2 h in both control and cholera toxin-treated cells (Fig. 5). This value is in good agreement with that previously reported in our laboratory (4). However, the rate of appearance of newly synthesized receptor in the nucleus decreased from 34.1 ± 1.3 fmol/h/100 μg of DNA in the control cells to 24.4 ± 0.7 fmol/h/100 μg of DNA in cells incubated with cholera toxin for 24 h. This suggests that cholera toxin acts by decreasing receptor synthesis. A dense

### Table I

Influence of cholera toxin and forskolin on thyroid hormone nuclear receptor levels

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Nuclear L-T2 bound</th>
</tr>
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<tbody>
<tr>
<td>Cholera toxin</td>
<td>1.0 68 ± 103 ± 5</td>
</tr>
<tr>
<td>2.5</td>
<td>63 ± 102 ± 4</td>
</tr>
<tr>
<td>5.0</td>
<td>68 ± 97 ± 6</td>
</tr>
<tr>
<td>10</td>
<td>55 ± 93 ± 4</td>
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<tr>
<td>25</td>
<td>55 ± 97 ± 6</td>
</tr>
<tr>
<td>50</td>
<td>51 ± 85 ± 5</td>
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### Table II

Influence of cholera toxin and the B subunit on thyroid hormone nuclear receptor levels

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Cholera toxin B subunit % control</th>
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<tbody>
<tr>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td></td>
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<tr>
<td>5.0</td>
<td></td>
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<tr>
<td>10</td>
<td></td>
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<td>25</td>
<td></td>
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<td>50</td>
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a amino acid-labeling study performed with cells incubated with cholera toxin for 48 h also supports this conclusion. As discussed in a previous study (4), the rate of appearance of receptor in the nucleus probably represents the rate of synthesis of receptor. However, the dense amino acid-labeling method does not exclude the possibility that a posttranslational event may alter the appearance or detection of newly synthesized receptor in the nucleus. For example, cholera toxin might increase the cytoplasmic degradation of receptor or inhibit the transfer of receptor to the nucleus or the association of receptor with chromatin.

In previous studies, we have shown that thyroid hormone can reduce the concentration of its receptor in GH1 cells (4–7). During the first 10–15 h of incubation, t-rt. decreases the receptor solely by decreasing the appearance rate of newly synthesized receptor (4). At longer times (e.g., 24 h), t-rt. also decreases receptor half-life. Cholera toxin with t-rt. (Fig. 3) demonstrated an additive effect on receptor reduction during a 24-h incubation. This additive effect is expected since 24 h t-rt. can also shorten the receptor half-life while cholera toxin decreases the receptor only by reducing the nuclear accumulation of newly synthesized receptor. The effect of cholera toxin on thyroid hormone receptor depletion occurs less rapidly than that produced by other agents which have been reported to cause a decrease of receptor. For example, the reduction of receptor mediated by t-rt. on n-butryrate in GH1 cells can be detected within 3 h after addition to cells (2, 4–7). Administration of pharmacological doses of glucagon in vivo produced a maximal depression of receptor in liver in about 6 h (8). However, 6 h after addition of cholera toxin to GH1 cells, the effect on the receptor is minimal (Fig. 7) and does not become maximal until 48 h.

Since cholera toxin is believed to exert its action on cells through cAMP production, we considered the possibility that the cholera toxin effect on receptor abundance. Recent studies have shown that cholera toxin can stimulate the ADP-ribosylation of a wide variety of soluble and membrane proteins which suggests that the toxin may modify cellular functions other than the adenylate cyclase system (32–36). This is supported by a study in 3T3 cells which reported that cholera toxin stimulated mitogenesis by a mechanism which is not explained by the enhancement of cAMP production (37). In recent studies, we have found that incubation of GH1 cells with cholera toxin or t-rt. stimulated the ADP-ribosylation of intracellular proteins. Whether this effect is responsible for the reduction of receptor levels is currently under investigation.

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Modulation of thyroid hormone nuclear receptors by cholera toxin in cultured GH1 cells.

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